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Review

Mechanisms of initiation and propagation of the calcium wave during fertilization in deuterostomes

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Introduction

During fertilization, the interaction between male and female gametes provokes a cascade of events resulting in the activation of the female gamete (Epel, 1990; Bement, 1992 for review) (Fig. 1). The two main physiological results of activation are prevention of polyspermy (Jaffe, 1976; Ginsburg, 1987 for review) and the onset of the cell cycle (Guerrier *et al.*, 1990; Whitaker and Patel, 1990 for reviews and Kline and Kline, 1992; Tombes *et al.*, 1992) leading to the first division of the zygote. In general, several groups of often loosely overlapping events can be defined according to their temporal sequence.

The first detectable fertilization event is a small depolarization step interpreted as the signature of the fusion between sperm and egg (Dale *et al.*, 1978; Chambers and McCulloh, 1990 for review). It is followed by the latent period (Allen and Griffin, 1958; for review Whitaker *et al.*, 1989). Then, a small number of events initiate the beginning of the cascade. These events include depolarization of the plasma membrane and an increase in the concentration of free cytoplasmic calcium ($[Ca^{2+}]c$) (e.g. in the sea urchin egg, Eisen *et al.*, 1984). In deuterostomes this is brought about by a progressive temporally and spatially controlled liberation of Ca^{2+} from internal stores (Gillot *et al.*, 1990; Jaffe, L.F., 1990). The increase starts at one pole of the egg and propagates as a wave of high $[Ca^{2+}]c$ to the opposite pole. This phenomenon is referred to as the calcium wave. Since its first description in the Medaka fish egg (Gilkey *et al.*, 1978), the calcium wave and the cascade of events linked to it have been described in eggs of various echinoderms (Eisen and Reynolds, 1985; Yoshimoto *et al.*, 1986; Hamaguchi and Hamaguchi, 1990), *Xenopus* (Kubota *et al.*, 1987), ascidians (Speksnijder *et al.*, 1989; Brownlee and Dale, 1990) and hamster (Miyasaki *et al.*, 1986), and in other cell types (for review, Jaffe, 1991). Variations occur in the pattern and duration of the calcium wave. This is especially so for oocytes blocked at various stages of meiosis. These oocytes undergo a shorter calcium wave followed until the end of meiosis by several small Ca^{2+} spikes (Guerrier *et al.*, 1990; Whitaker and Patel, 1990).

Apart from an early detected increase in tyrosine kinase activity (see below), the calcium wave is believed to be the major and seminal step in activation by the sperm. However, the origin and mechanism of propagation of the calcium wave remain puzzling. It has been proposed that a conserved mechanism initiates and propagates the Ca^{2+} wave in deuterostome eggs (Jaffe, L.F., 1990, 1991). However, later fertilization events such as protein Kinase C activation (Bement, 1992), cortical reaction (Stewart-Savage and Bavister, 1992) and cell cycle onset involve relatively diverse mechanisms and are difficult to explain in terms of a single initial signal. Cross-fertilization experiments (Iwao and Jaffe, 1989; Kane, 1990) have shown that the mechanism by which the calcium wave *originates* is intimately linked with the role of the sperm (apart from the gift of a haploid nucleus). Recent advances have furthered our understanding of the mechanism of *propagation* of the calcium wave. This review will concentrate on these two points and will also discuss the biological significance of the calcium wave.

How is the calcium wave triggered?

Attempts to understand the role of the sperm as a trigger for the calcium wave have led to four main hypotheses summarized in Fig. 2. These hypotheses can be divided into two groups. The first group proposes that the a sperm component migrates into the egg. Either the sperm is supposed to create a localized leak for Ca^{2+} in the egg membrane (Fig. 2A) (Jaffe, L.F., 1980, 1983, 1985, 1990) or the sperm is assumed to contain a sperm factor (Dale *et al.*, 1985) which, once delivered into the egg during gamete fusion, initiates the calcium wave (Fig. 2B) (for review see Whitaker *et al.*, 1989; Dale and De Felice, 1990; Whitaker and Crossley, 1990). The second group assumes that the sperm gives a signal through a sperm receptor linked to a membrane transduction pathway. This pathway could be a G protein linked to inositol(1,4,5)-trisphosphate (Ins(1,4,5)P3) and diacylglycerol (DAG) production by a phospholipase C (PLC) (Fig. 2C) (for review see Jaffe, L.A., 1989, 1990; Whitaker and Crossley, 1990 for sea urchin eggs; Nuccitelli *et al.*, 1989; Kline *et al.*, 1990; Busa, 1988, 1990 for *Xenopus* eggs; Miyasaki, 1989, 1990 for mammalian eggs). Recent results showing a modification of the tyrosine phosphorylation pattern of proteins after fertilization of sea urchin eggs (Ciapa and Epel, 1991) raise the possibility of a tyrosine kinase receptor for the sperm (Fig. 2D).

The calcium leak theory

This hypothesis is based on four main assumptions (Jaffe, L.F., 1990): (1) the acrosome reaction which activates the sperm causes an increase of its cytoplasmic Ca^{2+} level through the activation of Ca^{2+} carriers (probably Ca^{2+} channels); upon fusion with the egg, the sperm membrane enriched in activated Ca^{2+} carriers incorporates them into the egg membrane and creates a localized leak (2) through which a Ca^{2+} influx takes place throughout fertilization; (3) cortical cisternae of the endoplasmic reticulum (ER) are gradually loaded with Ca^{2+} originating from the leak; (4) this accounts for the latent period which ends when luminal $[Ca^{2+}]$ exceeds a threshold. Then the cisternae «detonate» Ca^{2+} and, in doing so, initiate the

Abbreviations used in this paper: Fab, antigen-binding fragment; Ins(1,4,5)P3, inositol 1,4,5-trisphosphate; Ins(1,3,4,5)P4, inositol 1,3,4,5-tetrakisphosphate; PtdIns(4,5)P2, phosphatidylinositol 4,5-bisphosphate; DAG, diacyglycerol; PLC, phospholipase C; PKC, protein kinase C; cADPR, cyclic adenosine dinucleotide phosphate ribose; GTP, guanosine trisphosphate; cGMP, cyclic guanosine monophosphate; cAMP, cyclic adenosine monophosphate; GTP-γ-S, guanosine 5'o(3-thiotrisphosphate); GDP-ß-S, guanosine 5'o(2-thiodiphosphate); NAD^+, nicotinamide adenine dinucleotide; $NADPH+H^+$, nicotinamide adenine dinucleotide phosphate; EGTA, ethylene glycol bis(ß-aminoethyl ether)-N,N,N',N',-tetraacetate; CICR, calcium induced calcium release. IICR, Ins(1,4,5)P3-induced Ca^{2+} release; ER, endoplasmic reticulum; RR, ryanodine receptor; IR, Ins(1,4,5)P3 receptor.

Ca^{2+} wave which propagates by Ca^{2+}-induced Ca^{2+} release. This model is proposed to be valid for all deuterostome species with modifications for the eggs where multiple waves occur (ascidians, Speksnijder *et al.*, 1989 and Brownlee and Dale, 1990; hamster, Miyasaki *et al.*, 1986). In these cases, the leak remains active and acts as a pacemaker initiating multiple waves.

In sea urchin (for review see Schackmann, 1989) and mammalian eggs (Kopf, 1990) Ca^{2+} influx does indeed occur during the acrosome reaction triggered by small egg jelly peptides. Ca^{2+} channels have been characterized in sea urchin and mammalian sperm (Guerrero and Darszon, 1989; Florman *et al.*, 1992) and are probably responsible for Ca^{2+} influx during acrosome reaction. In sea urchin, distinct voltage-activated Ca^{2+} channels (Guerrero and Darszon, 1989) are thought to be activated by a depolarization involving a K^+ channel and a Na^+/H^+ exchanger (Gonzalez-Martinez *et al.*, 1992). However, if, as suggested by the leak theory, sperm Ca^{2+} channels are inserted into the egg membrane, they must remain in their activated state, which requires a depolarized membrane. The unfertilized sea urchin egg membrane potential is around -70mV (see for review Hagiwara and Jaffe, 1979) and in other deuterostome eggs generally more negative than -20mV. Membrane potential remains below -60mV during the latent period (Dale *et al.*, 1978); thus sperm Ca^{2+} channels identified in sperm should be inactivated as soon as sperm fusion establishes electrical continuity between the gamete membranes. Whether sperm Ca^{2+} channels are covalently modified (e.g. by phosphorylation) during acrosome reaction has not been tested.

An increase of Ca^{2+} influx occurs in two phases during fertilization in the sea urchin egg (Paul and Johnston, 1978; Schmidt *et al.*, 1982; Gillot *et al.*, 1990 for review; Walter *et al.*, 1989): a sustained large influx after the calcium wave is preceded by a transient increase in Ca^{2+} influx which could start as early as 3 sec after sperm-egg fusion (Paul and Johnston, 1978). However, $[Ca^{2+}]$ is reported to increase only after the latent period (Swann *et al.*, 1992; Whalley *et al.*, 1992), which lasts at least 7 sec (Shen and Steinhardt, 1984). In fact an early localized $[Ca^{2+}]$ rise may have been occluded by Ca^{2+} imaging of the whole egg. And a very early Ca^{2+} influx was suggested by the dependence of the early depolarization step on extracellular Ca^{2+} (Dale *et al.*, 1978). Indeed, it has been recently demonstrated that there is a small transient increase in $[Ca^{2+}]$ coincident with the sperm egg fusion only detectable when it is measured locally in the cortex (S.S. Shen, personal communication). This transient is abolished by the Ca^{2+} channel blocker nifedipine (Fleckenstein, 1977) and thus can be interpreted as the result of an early Ca^{2+} influx through Ca^{2+} channels as assumed by the leak theory. Coincident with this newly demonstrated phenomenon, a small inward current preceding the fertilization potential (Lynn *et al.*, 1988) appears as soon as electrical continuity is established between sperm and egg membrane (McCulloh and Chambers, 1992). This current, characteristic of the latent period, appears to originate from the sperm for the following reasons: a) it is recorded through an isolated membrane patch only when sperm is injected inside the patch pipette (McCulloh and Chambers, 1991); b) the intensity of the current flowing through the patch is equivalent to the intensity of the current recorded for the whole egg (McCulloh and Chambers, 1992); c) cross fertilization between two sea urchin species, characterized by distinct changes in conductance pattern during the latent period, indicates that these changes are dependent on the sperm but not on the egg species (Kane 1990). Thus, sperm induces a specific inward current which is distinct from the large

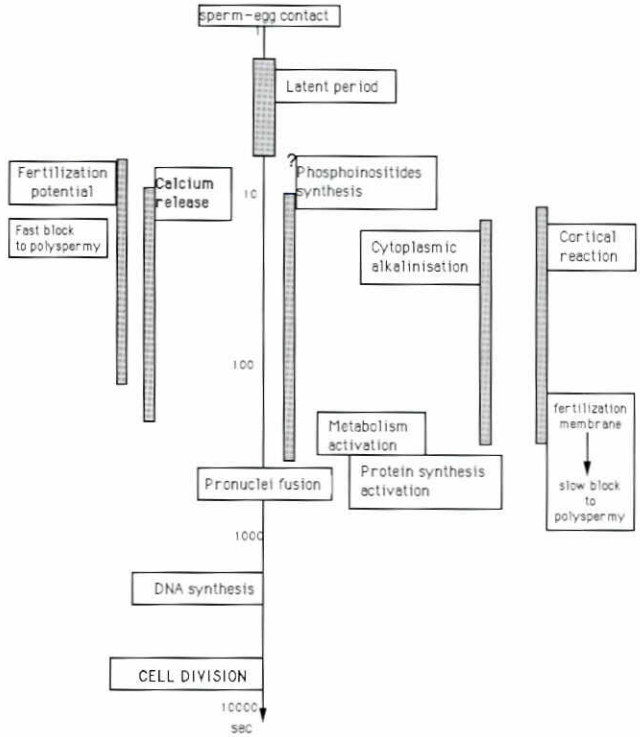

Fig. 1. The sequence of events during fertilization of the sea urchin egg. *The time scale is logarithmic. Grey columns span approximate durations of each event cited in the box linked to.*

fertilization current that reflects Ca^{2+} activation of egg channels (David *et al.*, 1988) and is subsequently inhibited by Ca^{2+} chelators (Zucker and Steinhardt, 1978; Swann and Whitaker, 1986; Swann *et al.*, 1992). As previously estimated (Dale *et al.*, 1978), current/voltage (I/V) relationships established in eggs injected with Ca^{2+} chelators (in which sperm-induced currents are the only currents occurring during fertilization) show a reversal potential of -4 mV (Swann *et al.*, 1992), which indicates a lack of ionic specificity. However, in this case it is not clear which compartment (the external medium or the sperm cytoplasm) should be taken into account for the calculation of the equilibrium potential for the main candidate ions. However in both cases, the reversal potential for Ca^{2+} would be positive since $[Ca^{2+}]$ in the egg cytoplasm is clamped at 0.2 μM by chelators, $[Ca^{2+}]$ in the extracellular medium is 10 mM and probably of micromolar order in sperm head (Jaffe, L.F., 1990).

Whatever the nature of the sperm-induced current, it must be remembered that sea urchin eggs can be activated by sperm in the absence of external Ca^{2+} (Schmidt *et al.*, 1982), although higher sperm concentrations are required and the fertilization envelope develops more slowly and is weaker. Recent results (S.S. Shen, personal communication) show that the small Ca^{2+} transient probably originates from Ca^{2+} channel activity. However this treatment does not inhibit the calcium wave and the initial Ca^{2+} transient may not have any fundamental role in the initiation of the wave. Although this subject is still a matter of controversy, it is proposed that sperm induces a non-specific current and that a small Ca^{2+}

influx resulting from this current contributes to but does not appear to be the main trigger for the calcium wave. The sperm-induced current does not necessarily occur through insertion of sperm ion channels in the egg plasma membrane and participation of sperm Ca^{2+} channels activated during the acrosome reaction appears unlikely.

There are no reports of a very early calcium influx preceding the actual calcium wave in other deuterostome species. Such a small and localized increase may be overlooked for reasons stated above but also because as soon as Ca^{2+} enters the egg it might be removed by the endoplasmic reticulum (ER) (see below) (Ca^{2+} injections in Medaka fish eggs induce a small transient Ca^{2+} signal followed after 20 sec by a calcium wave (Iwamatsu *et al.*, 1988)). It is also interesting to note that, in the ascidian egg, the successive Ca^{2+} waves originate from a pacemaker (Speksnijder, 1992) but are independent of external Ca^{2+} (Speksnijder *et al.*, 1989). The pacemaker is probably linked to the ER, not to the plasma membrane (Speksnijder, 1992). This assumption rules out a consistent leak created in the plasma membrane by sperm channels. Rather, the creation of a specialized structure with the ER is proposed (see below and Fig. 4).

The structure of the ER of sea urchin eggs has been studied *in vivo* and in cortical preparations with dicarbocyanine which diffuses into membranes (Terasaki *et al.*, 1991). Dicarbocyanine staining has a pattern similar to the pattern revealed by immunostaining with an antibody against a calsequestrin-like protein (Henson *et al.*, 1989; Terasaki *et al.*, 1991). Dicarbocyanine, which thus appears to reveal the ER, stains living sea urchin eggs and shows the ER organized as a central area of connected sheet surrounded by a cortical network of tubules (Terasaki and Jaffe, 1991). Early studies have shown that ER is the main source of Ca^{2+} release during the calcium wave (see review by Gillot *et al.*, 1990). Isolated cortices where the Ca^{2+}-sensitive dye fluo-3 has been compartmentalized have shown spontaneous wave of fluorescence (Terasaki and Sardet, 1991). Moreover, the cortical ER network undergoes a transient disorganization during the calcium wave which does not affect the inner part of the network (Terasaki and Jaffe, 1991). So, cortical ER appears to be the site of Ca^{2+} release triggered by sperm. Immunolocalization with antibodies directed against the ryanodine receptor also confirms this statement and the dual nature of the ER in sea urchin eggs (McPherson *et al.*, 1992). Anti-ryanodine receptor antibodies stain only the cortical region whereas anti-calsequestrin antibodies stain the whole ER (Henson *et al.*, 1989). In sea urchin eggs, ER thus appears to be a potential target for Ca^{2+}-induced Ca^{2+} release (CICR) (see Fabiato, 1983 for definition and Tsien and Tsien, 1990 for review). In contrast, *Xenopus* oocytes do not seem to possess ryanodine receptors but Ins(1,4,5)P3 receptors have been purified and immunolocalized in the cortex (Parys *et al.*, 1992). This indicates the occurrence of different pathways for the calcium wave production among various deuterostome species (and see below).

Electron-dense structures have been detected between regions putatively rich in ryanodine receptors and plasma membrane, cortical granules or yolk bodies in sea urchin eggs (McPherson *et al.*, 1992). Similar structures have also been identified in the cortex of *Xenopus* (Charbonneau and Grey, 1984) and ascidian eggs between the endoplasmic reticulum and the plasma membrane (Sardet *et al.*, 1992b). In ascidian eggs, the cortical endoplasmic reticulum is also composed of a tubular network which appears in video images recorded with differential interference contrast to be organized with anchoring sites around which tubules pivot (Sardet *et al.*, 1992b). These electron-dense and anchoring structures are reminiscent of the organization of electron-dense structures known in skeletal muscle triads (Fleischer and Inui, 1989 for review). It is now accepted that the dihydropyridine receptor (DHPR which is also an L type Ca^{2+} channel) of the sarcolemma is linked mechanically to the sarcoplasmic reticulum ryanodine receptor and constitutes structures previously described as «feet». The DHPR acts as a voltage sensor and transduces directly sarcolemma depolarization to the ryanodine receptor which opens and releases Ca^{2+} responsible for muscle contraction (see reviews by Fleischer and Inui, 1989; Catterall, 1991). In the sea urchin egg, since depolarization does not trigger egg activation, the mechanism which operates in skeletal muscle probably does not exist but a similar organization based on an exchange of messages between a plasma membrane voltage sensor and ryanodine receptors of the cortical ER could explain the fast polyspermy block and the effects of voltage-clamping the egg on sperm fusion (Lynn *et al.*, 1988; McCulloh and Chambers, 1992). It has been proposed that a voltage-sensitive sperm protein interacts with amphibian egg plasma membrane (Jaffe, L.A., 1990) to account for the sperm-dependence of the fast polyspermy block (Iwao and Jaffe, 1989). Thereafter a slightly different version of this model is proposed: a specific plasma membrane sperm protein interacts with an egg plasma membrane voltage sensor only for a window of egg plasma-membrane potential (e.g. for the sea urchin *Strongylocentrotus purpuratus* the egg membrane potential must be more negative than +20mV (Jaffe, 1976)) and allows further interactions between the two membranes to proceed (Fig. 4A).

An indirect approach for defining the relationships between sperm fusion mechanism and the structure of the ER is to consider potential specialized structures of egg plasma membrane and ER. Specialized domains have been detected in the ascidian *Phallusia mammillata* (Sardet *et al.*, 1992b). The vegetal pole richer in ER than the animal pole is a preferential site for sperm entry (Speksnijder *et al.*, 1989) and is likely to contain a pacemaker initiating successive Ca^{2+} wave during fertilization (Speksnijder, 1992). Among amphibians, *Discoglossus pictus* eggs possess a unique structure where sperm entry takes place, the dimple, particularly rich in endoplasmic reticulum (Campanella *et al.*, 1988; Talevi and Campanella, 1988). Putative Ca^{2+} gradients detected by K pyroantimonate could originate from this structure during fertilization (Gualtieri *et al.*, 1992). In *Xenopus* eggs the animal pole which is a preferential domain for sperm entry is richer in «feet» (Charbonneau and Grey, 1984) and more sensitive to Ins(1,4,5)P3 injections (Berridge, 1988) than the vegetal pole. These studies show that there is a possible link between endoplasmic reticulum organization and sperm entry site and action. However, neither a specialized membrane domain for interaction with sperm (McCulloh and Chambers, 1992) nor a specialized structure in the cortical endoplasmic reticulum (Sardet *et al.*, 1992b) has been detected in sea urchin eggs.

In conclusion, eggs of several species possess a particular cortical endoplasmic reticulum which is a potential site for Ca^{2+} uptake and release as required by the calcium leak theory. There are, however, only a few examples providing evidence of its function *in vivo*. Cortical injections of Ca^{2+} in Medaka fish eggs show that Ca^{2+} is rapidly taken up by a store, followed after 20 sec by a calcium wave (Iwamatsu *et al.*, 1988). Moreover, when overloaded, both Ins(1,4,5)P3-sensitive and insensitive Ca^{2+} stores release Ca^{2+} spontaneously (Nelson and Nelson, 1990; Missiaen *et al.*, 1991).

This provides evidence for CICR triggered by a sudden Ca^{2+} release when luminal [Ca^{2+}] exceeds a threshold. The last assumption of the calcium leak theory, the propagation of the calcium wave by CICR, is discussed below. In brief, CICR is very likely to occur in sea urchin and in modified forms in vertebrates.

The calcium leak theory is supported by certain observations only: an early sperm-activated [Ca^{2+}] rise loads some specialized compartments of the ER during the latent period. This period ends with a localized massive Ca^{2+} release which triggers CICR. These events are not specific to this theory and involvement of sperm Ca^{2+} channels seems unlikely. During fertilization in vertebrate and ascidian eggs, the persistence of a sperm induced leak is unlikely but some sperm-dependent modifications of the cortical ER could create pacemakers.

The sperm factor theory

The sperm factor theory states that an activating factor is injected by sperm during its fusion with egg. This theory originates from electrophysiological studies which verified the nature of the latent period (see reviews by Whitaker *et al.*, 1989; Dale and De Felice, 1990). To summarize, it can be outlined that in sea urchin, ascidian and the anura *Discoglossus pictus*, a step depolarization of a couple of mV precedes the fertilization potential. In the sea urchin, 7-30 sec separate sperm attachment from the onset of the cortical reaction: the so-called latent period (Allen and Griffin, 1958; Shen and Steinhardt, 1984). This was the original definition of the latent period, which has gradually been modified with characterization of more physiological early events. The original definition states that the latent period begins with the first communication between gamete membranes and ends with the first detectable sign relevant to egg activation. Electrical continuity establishment between gametes seems to be a reasonable starting point. Since the Ca^{2+} wave is still the first event characterized which is necessary and sufficient for egg activation, it could be used to define the end of the latent period (the fertilization current starts before the actual Ca^{2+} rise but nevertheless appears to be more a consequence than a source for egg activation and thus a less suitable boundary). This definition (see Swann *et al.*, 1992 Fig. 2 for an illustration) clarifies both the kinetics and the function of the latent period which then reflects the latency necessary to the sperm signal transduction by the egg. However, it does not greatly affect the interpretation of early results and the latent period duration would be sufficient to allow a sperm factor to diffuse into the egg. Recent electrophysiological studies have clarified the way sperm fuses with eggs and some characteristics of the latent period (McCulloh and Chambers, 1992; Swann *et al.*, 1992). These studies derive from voltage clamp procedure which allowed determination of the length of the latent period (Shen and Steinhardt, 1984). Current pattern during fertilization has been studied in voltage-clamped sea urchin eggs (Lynn *et al.*, 1988 and see review by Chambers and McCulloh, 1990). Depending on the potential at which eggs are clamped, various currents are associated with different sperm behavior. Depolarization above +20mV mimics the fast polyspermy block (Jaffe, 1976). Sperm does not attach and no current or depolarization is detected. Activation always happens when eggs are clamped between -15 mV and +15 mV and sperm causes the «type I» activation current and enters. Three phases can be distinguished in the type I response. Phase I is characterized by the abrupt onset of an inward current which subsequently increases slowly and corresponds roughly to the latent period. Phase 2 then starts with the onset of the

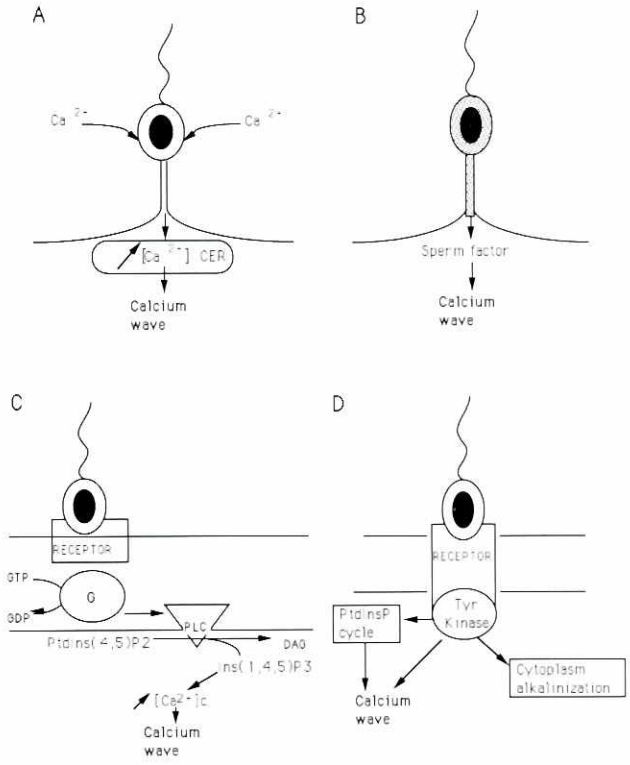

Fig. 2. Theories concerning the way the sperm triggers the calcium wave in the egg (for explanations see text). (A) *The leak theory. CER: cortical endoplasmic reticulum;* **(B)** *the sperm factor theory.* **(C)** *The sperm receptor/Ins(1,4,5)P3 transduction;* **(D)** *a new model; a signaling pathway similar to TH cell activation. It involves a sperm receptor complex and activation of a tyrosine kinase (Tyr Kinase).*

fertilization potential and a large increase in current culminates in a peak and decreases rapidly (phase 3). Phase 2 is coincident with the Ca^{2+} rise and is cancelled by injection of EGTA (Swann *et al.*, 1992). It originates from a wave of current which progresses over the entire egg surface in parallel with the Ca^{2+} wave (McCulloh and Chambers, 1991). Phase 2 current is therefore clearly accounted for by opening of Ca^{2+}-activated channels detected in single electrode voltage clamp studies (David *et al.*, 1988). Phase 3 current is probably due to inactivation of Ca^{2+}-activated channels since the Ca^{2+} transient is sustained during this phase (Swann *et al.*, 1992). In contrast, phase 1 current is insensitive to EGTA injection (Swann *et al.*, 1992) and originates from a sperm component. This component may be a putative diffusible factor. In this case a cytoplasmic connection must be established between sperm and egg. Indeed, phase 1 current starts concomitantly with an increase in membrane capacitance which indicates establishment of electrical continuity between membranes (McCulloh and Chambers, 1992). When eggs are clamped at potentials more negative than -30mV, sperm tend to attach to the egg surface for various durations (1 to tens of sec) and then detach. In these cases, type II and type III sperm-induced currents (see below) are recorded and eggs seldom activate (Lynn *et al.*, 1988). These facts are surprising since the unfertilized sea

urchin egg membrane potential is around -70 mV (Hagiwara and Jaffe, 1979 for review) and sperm-induced depolarization has an amplitude of a few mV (Dale *et al.*, 1978).

Why are eggs clamped at physiological potential not fertilized by sperm? Anomalous current responses consist in transient inward current (type II) which are either very short (couple of sec) or similar in duration and amplitude to phase 1 current of type I response. In type III response, phase 1-like transient current is followed after a variable lag phase by a larger inward current, similar to phases 2 and 3 of type I response. Type III responses are more frequent when the potential is more negative and are usually followed by an incomplete cortical reaction, though parthenogenetic development never occurs. Initial transient current of both type II and type III responses is also coincident with an increase in capacitance of the patch (McCulloh and Chambers, 1992). Type II responses show that even with a prolonged and intimate contact between sperm and egg activation can fail. This reveals a *second step* responsible for the actual sperm egg *fusion* which is dependent on the rapid and large depolarization occurring during phase II. This would explain the failure of sperm entry after attachment to eggs clamped at potentials more negative than -30 mV.

Since in type II responses phase 1-like transient currents last for tens of sec but are not followed by egg activation, an initiation of egg activation through an interaction between a receptor and an agonist appears to be very unlikely. Triggering the response by a receptor agonist interaction is likely to be irreversible and shorter than the duration of phase 1-like currents. However, a more complex relationship between multiple sperm ligands and multiple egg receptors can be considered (see below).

In contrast, during type III responses, partial egg activation takes place after a transient sperm-egg membrane electrical connection followed by sperm detachment. This favours diffusion of a sperm factor which needs to reach a threshold to initiate egg activation (however, no connection has been established between the length of phase 1 current and the occurrence of egg activation).

What could be the pathway for diffusion of the putative sperm-factor? Increase of capacitance can be accounted for by addition of the sperm membrane to the egg membrane but does not necessarily mean that cytoplasmic continuity between gametes is established (McCulloh and Chambers, 1992). Since sperm can detach after the transient capacitance increase, a simple fusion with cytoplasmic communication is unlikely. However, the acrosome tubule created during gamete fusion is a fine structure which could break easily, resulting in reversible cytoplasmic continuity. Calculations of the time required for an activator to diffuse across the acrosome could explain results reported above in terms of timing (Whitaker *et al.*, 1989).

In the loose patch recording configuration (McCulloh and Chambers, 1991, 1992) current can originate from egg or sperm membrane. The former hypothesis is compatible with egg channels activated either by a sperm activator (Fig. 3C) or through a pathway involving a sperm receptor (Fig. 3D). Sperm membrane-associated current could result from either (1) cytoplasmic continuity established during phase 1 (e.g by the acrosome tubule) giving rise to current flowing through sperm channels (Fig. 3A) (similar to the leak theory); or (2) from a more complex unstable state of membrane fusion with formation of gap junction-like pores which could account for establishment of electrical connection without total cytoplasmic continuity and diffusion of an activator accompanied by a non-selective current (McCulloh and Chambers, 1992). In this case, the sperm-induced current flows into the egg from the close compartment constituted by the sperm head and will be detectable in loose patch recording only if the resistance of the sperm membrane is very low, which was not determined.

Ultimately, the biological significance of phase 1 current can be questioned. Phase 1 current is neither necessary nor sufficient for sea urchin egg activation and has even appeared to be simply concomitant with it. There is no relationship between its duration and the occurrence or amplitude of the fertilization current (phases 2 and 3) (McCulloh and Chambers, 1992). Similarly, the small Ca^{2+} increase recently detected during the latent period (Buck and Shen, 1993, personal communication) can be abolished by nifedipine treatment without affecting the calcium wave. Electrical events during latent period may simply reflect sperm-egg fusion and electrical perturbation associated with it but not the mechanism responsible for egg activation.

Whatever interpretations are given for electrical events during the latent period, its duration can largely account for the diffusion of a sperm cytoplasmic factor (Whitaker *et al.*, 1989). Injections of cytosolic sperm extract activate the egg cortical reaction in sea urchin (Dale *et al.*, 1985), rabbit and mouse (Stice and Robl, 1990) and activate a fertilization-like current in ascidian eggs (Dale, 1988) (see also for review Dale and De Felice, 1990; Swann and Whitaker, 1990; Whitaker and Crossley, 1990). Injection of sperm extracts in heterologous eggs also triggers activation, albeit with a lower efficiency (Dale *et al.*, 1985; Dale 1988; Stice and Robl, 1990). Similarly, polyethyleneglycol-induced fusion between sea urchin egg and starfish sperm leads to egg activation (Kyozuka and Osanai, 1989). Thus sperm contains an unknown cytoplasmic egg activator with a low species specificity. Interestingly, a high (but not low) molecular weight (Mr>100,000) cytosolic fraction from both hamster and boar sperm triggers repetitive hyperpolarizing responses and Ca^{2+} transients after injection in hamster oocyte (Swann, 1990). These responses are typical of fertilization responses in mammalian oocytes (Swann, 1990, 1992). Similarities between fertilization current and current activated by homologous sperm extract injection have also been reported in ascidian eggs (Dale, 1988).

Although identity of the putative sperm factors is not known, injection of classical second messengers have been performed and their content in sperm estimated (see review by Whitaker and Crossley, 1990). During the acrosome reaction Ins(1,4,5)P3 concentration increases in sea urchin and mammalian sperm (Iwasa *et al.*, 1989; Harrison and Roldan, 1990). Cyclic nucleotides, cAMP and cGMP are also actively produced during the acrosome reaction (Kopf and Garbers, 1979; Hansbrough and Garbers, 1981). Increased cGMP could result from guanylate cyclase activation by a jelly peptide and would be involved in sperm motility (for review see Shapiro *et al.*, 1990). Although cGMP injections are three orders of magnitude less potent than Ins(1,4,5)P3 injections to activate sea urchin eggs (Whitaker and Crossley, 1990; Whalley *et al.*, 1992), cGMP induces a Ca^{2+} transient similar to that induced by sperm in sea urchin egg (Whalley *et al.*, 1992) and in Medaka fish egg (Iwamatsu *et al.*, 1988). This second messenger is the only one to reproduce the fertilization Ca^{2+} transient. However, cGMP does not increase pH in sea urchin eggs (Whalley *et al.*, 1992). Moreover, cGMP injections in sea urchin eggs (Whalley *et al.*, 1992) and in Medaka fish egg (Iwamatsu *et al.*, 1988) trigger Ca^{2+} increase only after a latency whose duration is inversely related to the final cytoplasmic [cGMP] in sea urchin eggs (Whalley *et al.*, 1992). This

may reflect the time necessary to convert cGMP into an active metabolite which could actually be the sea urchin sperm factor (see also the discussion on GTPγS). Thus, though cGMP appears by its mimicking of the sperm-induced Ca^{2+} transient to be a favorite candidate for a sperm factor, it would need to be present at very high concentration in sperm, which is unlikely (Whitaker and Crossley, 1990) (activation of an egg guanylate cyclase by the sperm factor is an alternative model) and only partially triggers egg activation (it is interesting to note that Ca^{2+} rise and pH increase can be uncoupled, suggesting a more complex relationship between these two fertilization events than originally proposed (Swann and Whitaker, 1985)).

Studies have been made of Ca^{2+} release by NAD^+ and $NADPH+H^+$ in sea urchin homogenates (Clapper *et al.*, 1987) and these observations were the initiation of the discovery of a potential new second messenger, the cyclic adenosine diphosphate ribose (cADPR). It was first noticed that nicotinamide cofactors are as potent as Ins(1,4,5)P3 in activating Ca^{2+} release *in vitro* in sea urchin egg homogenates, though they do so only after a lag of a few minutes (Clapper *et al.*, 1987). A cytosolic factor was found to create an NAD^+-derived activity which triggers Ca^{2+} release and cortical reaction immediately after its injection in intact sea urchin eggs. The delay characteristic of nicotinamide cofactor-induced Ca^{2+} release by homogenates therefore reflected the duration required for the synthesis of the real activator which was characterized as a new cyclic nucleotide, cADPR (Lee *et al.*, 1989). This product has been characterized in various tissues at concentrations in the nanomolar range (Walseth *et al.*, 1990) sufficient to trigger CICR in sea urchin homogenates (Galione *et al.*, 1991). Sperm cADPR concentration needs to be determined together with its *in vivo* mode of action before conclusions can be drawn concerning its role as a sperm factor.

The Ins(1,4,5)P3 content in sperm (4.10^{-19}-10^{-18}moles/sperm) has been claimed to be sufficient for sea urchin egg activation (Iwasa *et al.*, 1989) because microinjection of similar amounts of Ins(1,4,5)P3 activate sea urchin eggs (Irvine and Moor, 1986; Crossley *et al.*, 1988). In fact, the localized concentration at the injection site and not the total amount appears to be the limiting factor for calcium wave initiation (Whalley *et al.*, 1992). This means that the sperm should create in the egg a localized high [Ins(1,4,5)P3] at its binding site which is denied by diffusion kinetics (Whitaker *et al.*, 1989). Thus, Ins(1,4,5)P3 does not appear to be a possible sperm factor in sea urchin.

Possible involvement of a sperm receptor/G protein/PLC pathway in transduction of the sperm signal

The problem of the identity of sperm receptors

In deuterostomes, despite intensive investigation, the questions of existence and identity of a putative sperm receptor remain controversial. In mammals, three glycoproteins of the zona pellucida of the oocyte, ZP1, ZP2, ZP3, have been characterized (for review see, Garbers 1989; Jones, 1990; Wassarman, 1990; Sidhu and Guraya, 1991). Long filaments constituted by ZP2-ZP3 dimers are interconnected by ZP1. ZP3 is considered to be the primary sperm receptor and to trigger the acrosome reaction. ZP2 could act as a secondary receptor specific for the acrosome-reacted sperm. Recognition would involve sugar residues. Similarly, a sulfate rich fucose polymer from sea urchin egg jelly coat induces the acrosome reaction (Garbers *et al.*, 1983), and recently, a potential 350 kDa

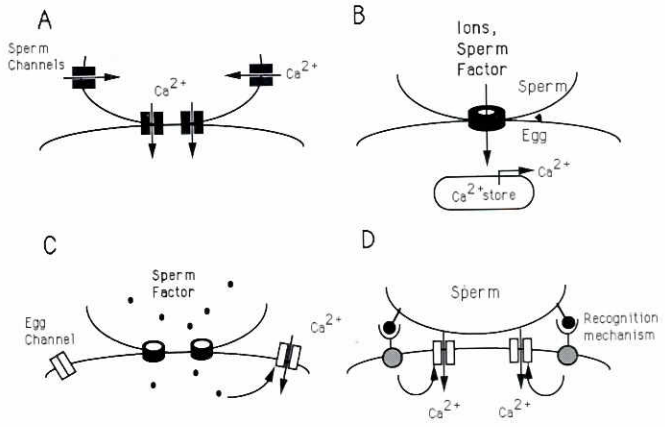

Fig. 3. Possible mechanisms responsible for the early current recorded during the latent period. (A) *Sperm channels are inserted in the egg membrane and are responsible for a Ca^{2+} influx;* **(B)** *a pore structure is created between the sperm and egg membranes; various ions flowing through the pore create the current; a sperm factor diffuses through the pore and activates Ca^{2+} from internal Ca^{2+} stores;* **(C)** *same as B but the sperm factor activates egg Ca^{2+} channels responsible for the current;* **(D)** *recognition between sperm and egg occurs through an egg sperm receptor and activates egg Ca^{2+} channels. This recognition strengthens adpression between sperm and egg membrane which is responsible for electrical continuity (not shown in the Figure).*

receptor has been characterized in sea urchin egg vitelline layer (Foltz and Lennartz, 1992). There is recent evidence of sperm proteins binding to specific sites of the egg membrane (Blobel *et al.*, 1992; Rochwerger *et al.*, 1992) but, to our knowledge, a sperm receptor has never been characterized in sea urchin or in mammalian egg plasma membrane. Interestingly, Fabs directed against the putative 350 kDa sperm receptor inhibit sperm binding to sea urchin eggs and cause cortical granule exocytosis in a small percentage of eggs. The putative sperm receptor could thus be linked through an unknown intermediate to a plasma membrane transduction pathway. However evidence for such a hypothesis remains elusive. Sperm-egg recognition appears to result from multiple steps. This would imply existence of several recognition steps characteristic of self and non-self recognition by immunological systems (see below).

Involvement of Ins(1,4,5)P3 and Phospholipase C

Ins(1,4,5)P3 synthesis occurs in various cell types by activation of a phospholipase C (PLC) which cleaves PtdIns(4,5)P2 into diacylglycerol (DAG) and Ins(1,4,5)P3 (for review see Berridge and Irvine, 1984, 1989; Cockroft and Thomas, 1992). PLCs are activated by different paths. The best known includes G-proteins activated by receptor-agonist interaction. To test possible involvement of this pathway we will examine first the putative involvement of Ins(1,4,5)P3 as a trigger for egg activation and then possible relationships between G-proteins and activation.

Involvement of Ins(1,4,5)P3 as a trigger of the Ca^{2+} wave has been studied by various approaches since the first evidence of egg activation by its injection (Whitaker and Irvine, 1984). Presence of an Ins(1,4,5)P3 production pathway in eggs, phosphoinositides synthesis after fertilization, effects of injections and recently action

of Ins(1,4,5)P3 inhibitors and localization of Ins(1,4,5)P3 receptor (IR) have been tested. The results of these studies present a rather complex and uncertain picture of Ins(1,4,5)P3 involvement as the egg activation trigger and reveal discrepancies between species.

Xenopus eggs respond to acetylcholine by a depolarization (Kusano *et al.*, 1977) and serotonin triggers (probably via a G-protein), Ca^{2+} transients in hamster eggs (Miyasaki *et al.*, 1990). No clear biological significance has been attributed to these potential Ins(1,4,5)P3 production pathways. Expression of mRNA coding for hormone receptors linked to Ins(1,4,5)P3 production and evocation of egg activation by relevant hormone treatments have demonstrated that functional membrane transduction pathways leading to Ins(1,4,5)P3 production exist in *Xenopus* and mouse eggs.

In contrast, sea urchin has been the main species where Ins(1,4,5)P3 production has been studied after fertilization. Early reports (Schmell and Lennartz, 1974; Turner *et al.*, 1984; Kamel *et al.*, 1985) showed a transient increase in polyphosphoinositol turnover after fertilization with an actual increase of 40% of a trisphosphoinositol-containing fraction within 15 sec (Turner *et al.*, 1984). Further studies (Ciapa and Whitaker, 1986) revealed that a transient increase of egg trisphosphoinositol content starts 10 sec after fertilization and peaks after 20 sec, parallel with the Ca^{2+} wave. It is followed by a larger rise which persists for at least 30 min. PtdInsP2, PtdInsP and PIns concentrations remain constant or increase within 40 sec after fertilization (Ciapa *et al.*, 1992) while a massive increase of phosphoinositide turnover explains Ins(1,4,5)P3 production. The former increase can be explained by Ca^{2+}-sensitivity of phospholipaseC and PtdInsP kinase (Whitaker and Aitchison, 1985; Oberdorf *et al.*, 1989; Ciapa *et al.*, 1992), which appears localized preferentially in the egg cortex (Oberdorf *et al.*, 1989), the site of Ca^{2+} release. This Ca^{2+} sensitivity could also account for the initial increase of InsP3 content after fertilization, which is associated with, but does not seem to precede, the fertilization Ca^{2+} transient.

Ins(1,4,5)P3 injections (about 10 μM final cytoplasmic concentration) trigger activation of eggs of various sea urchin species (for review see Whitaker and Crossley, 1990). Activation is indicated by cortical reaction (Whitaker and Irvine, 1984; Irvine and Moor, 1986; Turner *et al.*, 1986; Crossley *et al.*, 1988) fertilization potential (Slack *et al.*, 1986) and [Ca^{2+}] rise (Crossley *et al.*, 1988; Whalley *et al.*, 1992). Similar results have been obtained in Medaka fish eggs (Nuccitelli, 1987; I,wamatsu *et al.*, 1988), starfish oocytes (Picard *et al.*, 1985), *Xenopus* egg (Busa *et al.*, 1985; Oron *et al.*, 1985; Parker and Miledi, 1986; Kubota *et al.*, 1987; Ferguson *et al.*, 1990 and for review see Busa, 1990) and mammalian oocytes (Cran *et al.*, 1988; Miyasaki, 1988; Peres *et al.*, 1990; for review see Miyasaki, 1989, 1990). However, injection procedure bypasses membrane transduction steps, which may explain an abrupt onset of transient without any lag phase after injection and moreover Ins(1,4,5)P3-induced Ca^{2+} transients do not usually reproduce those induced by sperm.

In sea urchin (Whitaker and Crossley, 1990; Whalley *et al.*, 1992), the sperm-induced Ca^{2+} transient develops more progressively and lasts longer than the Ins(1,4,5)P3-induced one. The former also shows a plateau during the decrease whereas the latter decreases exponentially. Abrupt increase of Ins(1,4,5)P3-induced Ca^{2+} transient could also be explained together with the results reported above by a missing event not related to membrane transduction. Similar results have been obtained both in ascidians where Ins(1,4,5)P3 induces a fertilization current distinct from the fertilization current (Dale, 1988) and in Medaka fish egg (Iwamatsu *et al.*, 1988). Heparin blocks Ins(1,4,5)P3-induced Ca^{2+} release in sea urchin (Rakow and Shen, 1990) but does not block sperm-induction of the Ca^{2+} wave (Rakow and Shen 1990; Buck *et al.*, 1992; Whalley *et al.*, 1992). Heparin reduces only slightly the transient peak level and lengthens the latent period (Whalley *et al.*, 1992; S.S. Shen personal communication). These data indicate that Ins(1,4,5)P3 takes part in, but might not be necessary to initiate, the fertilization Ca^{2+} wave in sea urchin eggs.

In vertebrate eggs (at least in *Xenopus* mouse and hamster eggs), comparisons between Ins(1,4,5)P3 and sperm-induced Ca^{2+} responses are more difficult to achieve since Ins(1,4,5)P3 is involved in regenerative Ca^{2+} release mechanisms which could hide the initial fertilization step. In *Xenopus* eggs, Ins(1,4,5)P3 can mimic the sperm-induced transient (Busa and Nuccitelli 1985; Busa *et al.*, 1985) but can also initiate abrupt localized transients which do not propagate and are followed by oscillations (Berridge, 1988; Ferguson *et al.*, 1991; Parker and Ivorra, 1991). Interestingly, antibodies to PtdIns(4,5)P2 injected prior to fertilization inhibit egg activation by sperm (Larabell and Nuccitelli, 1992). This would indicate that there is an initial requirement for Ins(1,4,5)P3 synthesis. This interpretation is reinforced by immunodetection experiments where Ins(1,4,5)P3 receptors but not ryanodine receptors were detected (Parys *et al.*, 1992). There may be only Ins(1,4,5)P3-sensitive Ca^{2+} stores in *Xenopus* eggs and Ins(1,4,5)P3 would be part of Ca^{2+} wave-triggering mechanism.

In mouse and hamster eggs, Ins(1,4,5)P3 injection or photorelease from caged Ins(1,4,5)P3 can trigger Ca^{2+} oscillations similar to those induced by sperm (Peres *et al.*, 1991) but also other types of transients (Peres *et al.*, 1990; Miyasaki, 1989, 1990; Swann, 1992). A monoclonal antibody to mouse IR abolishes sperm-induced Ca^{2+} oscillations completely in a dose-dependent manner (Miyasaki *et al.*, 1992) and, as in *Xenopus*, Ins(1,4,5)P3 receptors only have been immunolocalized. This indicates that Ins(1,4,5)P3 Ca^{2+} release is necessary for egg activation by sperm but it can not be concluded that Ins(1,4,5)P3 is the trigger since Ca^{2+} injections also trigger regenerative Ca^{2+} release in hamster eggs (Miyasaki *et al.*, 1992).

It can be concluded that Ins(1,4,5)P3 is probably involved in early initiation of Ca^{2+} waves in many deuterostome eggs. However, there are major discrepancies between species. In sea urchin, and perhaps also in Medaka fish and ascidian, Ins(1,4,5)P3 would play a minor role, whereas it would represent a major step in mammalian and *Xenopus* egg activation.

Involvement of G-protein during sperm signal transduction

Expression of mRNAs coding for muscarinic acetylcholine receptor (AchR) and serotonin respectively, injected in *Xenopus* eggs confers responsiveness for these hormones to the egg (see review by Jaffe, L.A., 1989), which then undergoes a fertilization-like potential, cortical reaction and Ca^{2+} wave (Kline *et al.*, 1988; Lechleiter *et al.*, 1991). Similar experiments leading to similar results have also been conducted in mouse eggs (Miyasaki *et al.*, 1990; Williams *et al.*, 1992). Thus *Xenopus* and mouse or hamster eggs possess functional G-protein/PLC pathway. No such direct evidence of the existence of an Ins(1,4,5)P3 production pathway linked to G-protein has been obtained in sea urchin (but see below experiments with G-protein inhibitors).

Some putative G-proteins have been detected by ADPribosylation by cholera toxin and pertussis toxin in sea urchin and *Xenopus* eggs,

but not in hamster eggs (Turner *et al.*, 1897; Miyasaki, 1988; Kline *et al.*, 1990). Given the large diversity of G-proteins involved in various pathways which are differentially sensitive to toxins (Cockroft and Stutchfield, 1988; Hepler and Gilman, 1992 for review), these results are not necessarily relevant to fertilization.

GTPγS injections evoke late egg activation responses in sea urchin (Turner *et al.*, 1987 and for review Jaffe, L.A., 1989, 1990; Whitaker and Crossley, 1990), *Xenopus* (Kline *et al.*, 1990) and mouse (Miyasaki, 1988 and for review Miyasaki, 1989, 1990). In sea urchin eggs, GTPγS injection produces a Ca^{2+} transient (Crossley *et al.*, 1991) and an increase in pH (Jaffe, L.A., 1989; Crossley *et al.*, 1991). These seem to result from GTPγS-induced production of Ins(1,4,5)P3 and DAG (Crossley *et al.*, 1991) and the Ca^{2+} transient is inhibited by heparin. However, there is a delay of several minutes between GTPγS injections and first signs of egg activation in sea urchin (Crossley *et al.*, 1991), *Xenopus* and mouse eggs. Latency durations are variable in each model and in sea urchin eggs are inversely correlated to GTPγS amounts injected (Crossley *et al.*, 1991). Moreover, in sea urchin, the GTPγS-induced Ca^{2+} transient does not directly originate from intracellular Ca^{2+} stores and appears to have kinetics distinct from the Ins(1,4,5)P3-induced one. It presents a plateau during decrease very similar to cGMP and sperm-induced Ca^{2+} transients (Whalley *et al.*, 1992). Similarly, in mouse eggs, in contrast with hyperpolarizing responses (HR) produced by continuous Ins(1,4,5)P3 injections, GTPγS-induced HRs are abolished gradually by phorbol ester treatment (Swann *et al.*, 1989). Thus, GTPγS probably activates sea urchin and mouse eggs through a mechanism distinct from Ins(1,4,5)P3 pathway and does not act through G-proteins.

GDPßS inhibits sperm-activated cortical reaction in sea urchin eggs (Turner *et al.*, 1987) and, surprisingly, induction of cortical reaction by Ins(1,4,5)P3 (Jaffe, L.A., 1990; Crossley *et al.*, 1991). It *does not inhibit* induction of Ca^{2+} transient by sperm (though eggs do not present cortical reaction) (Crossley *et al.*, 1991), but instead elicits a very brief Ca^{2+} transient. GDPßS thus inhibits one or multiple steps leading to cortical reaction which are downstream from the Ca^{2+} wave. In conclusion, neither GTPγS nor GDPßS interfere with sea urchin egg fertilization through a sperm-linked G-protein. In *Xenopus* and hamster eggs, GDPßS inhibition of the induction of the fertilization Ca^{2+} wave has not been established (Kline *et al.*, 1990; Miyasaki, 1990), but in those eggs, GTPγS does not seem to act through the same pathways used by sperm, and G-protein involvement is therefore unlikely.

In sea urchin eggs, after a latency, GTPγS induces a fertilization-like Ca^{2+} transient by an unknown mechanism distinct from a G-protein/PLC/Ins(1,4,5)P3 pathway. Since this latency is heavily dose-dependent and usually lasts longer than 60 sec, it is possible that GTPγS does not act directly but is metabolized into an active product such as cGMP. Evidence for this is summarized as follows. In contrast to Ins(1,4,5)P3, neither cGMP nor GTPγS release Ca^{2+} from eggs permeabilized with digitonin (Whalley and Whitaker, 1988). cGMP and GTPγS induce similar Ca^{2+} transients which share the same shape, are preceded by a latency and are sensitive to extracellular Ca^{2+} (compare Crossley *et al.*, 1991 with Whalley *et al.*, 1992). However, unlike cGMP, GTPγS produces Ins(1,4,5)P3, DAG and consequently a cytoplasmic pH increase (Swann and Whitaker, 1985) and a heparin-sensitive Ca^{2+} transient, though cGMP action also involves an active Ins(1,4,5)P3 sensitive Ca^{2+} store. The latency between the injection and the Ca^{2+} transient is shorter for cGMP (20 sec on average) than for GTPγS (order of minutes). These observations lead us to hypothesize that GTPγS is converted in cGMP. Enzymes responsible for this conversion are guanylate cyclases (E.C. 4.6.1.2) (Thompson and Garbers, 1990 for review). Many guanylate cyclases are integral membrane protein and are receptors. There are also cytoplasmic guanylate cyclases. Isolated guanylate cyclase from rat lung has a higher affinity for GTPγS than for GTP (Km are 3 μM and 28 μM respectively) for [GTPγS] higher than 5 μM (for [GTPγS] <4 μM, Km is 70μM) (Brandwein *et al.*, 1982). Guanylate cyclase characteristics and the range of nucleotide concentration required for submaximal responses ([GTPγS], (100 μM) and [cGMP], (10 μM)) make this hypothesis worthy of consideration. The metabolic pathway is probably more complicated since cGMP itself could act by a metabolite (see above) and it would be important to dissect GTP metabolization in sea urchin eggs.

Tyrosine kinase activities: a very early step of fertilization?

Recent results support the possibility of an alternative transduction pathway of the sperm signal. A tyrosine kinase activity has been characterized before the onset of the calcium wave (Ciapa *et al.*, 1991). Tyrosine kinase receptors form a complex family of proteins to which belong various growth factor receptors such as the epidermal growth factor EGF and the platelet derived growth factor PDGF (Yarden and Ullrich, 1988; Ullrich and Schlessinger, 1990). An increasing number of oncogenes also belong to this family. The coupling of the ligand to its receptor activates a complex network of events by as yet ill-defined ways, leading to cell division. An early signature of the coupling is the autophosphorylation of the receptor molecule on specific tyrosine residues and the tyr-phosphorylation of certain proteins involved in cell activation. This pathway includes the phospholipase C (PLC) responsible for Ins(1,4,5)P3 and diacylglycerol (DAG) synthesis. Ins(1,4,5)P3 elicits calcium release from internal calcium stores and DAG coupled with high $[Ca^{2+}]c$ activates protein kinase C (PKC). PKC acts on multiple targets, in particular on the Na^+/H^+ exchanger leading to the cytoplasmic alkalinization (Fig. 2C). This activation cascade echoes the fertilization events and could explain the possibility that an Ins(1,4,5)P3 synthesis occurs without G-protein activation. Indeed, newly tyrosine-phosphorylated proteins have been detected after fertilization in the sea urchin egg. A 350 kDa protein is phosphorylated after cytoplasmic alkalinization (Jiang *et al.*, 1990). Even though this phosphorylation has not been detected before the calcium wave, it is interesting to note that the sperm receptor identified on the surface of the egg has the same molecular weight as this potential tyrosine kinase substrate (Foltz and Lennartz, 1992). Two other proteins respectively of 91 kDa and 138 kDa are phosphorylated very early during fertilization before the $[Ca^{2+}]c$ rise (Ciapa and Epel, 1991). The identity of these proteins remains unknown but this result may reveal the existence of a tyrosine kinase activity (or the inhibition of a specific phosphatase) as the first detectable sign of fertilization. The sperm binding capacity of these proteins has not been tested and these results are still preliminary.

It has been suggested above that gametes could interact through multiple receptors as is the case in interactions between cells of the immune system. Indeed, there might be parallels with helper T (T_H) cell activation, since the sperm may be recognized as a nonself element by the female gamete. The T cell receptor (TCR) (Abraham *et al.*, 1992; Izquierdo *et al.*, 1992 for reviews) is a heterodimer associated in a stable complex with CD3 that consists of five other chains. On transcellular binding to the antigen-presenting B cell, TCR tends to associate with a CD4 chain of the same membrane in

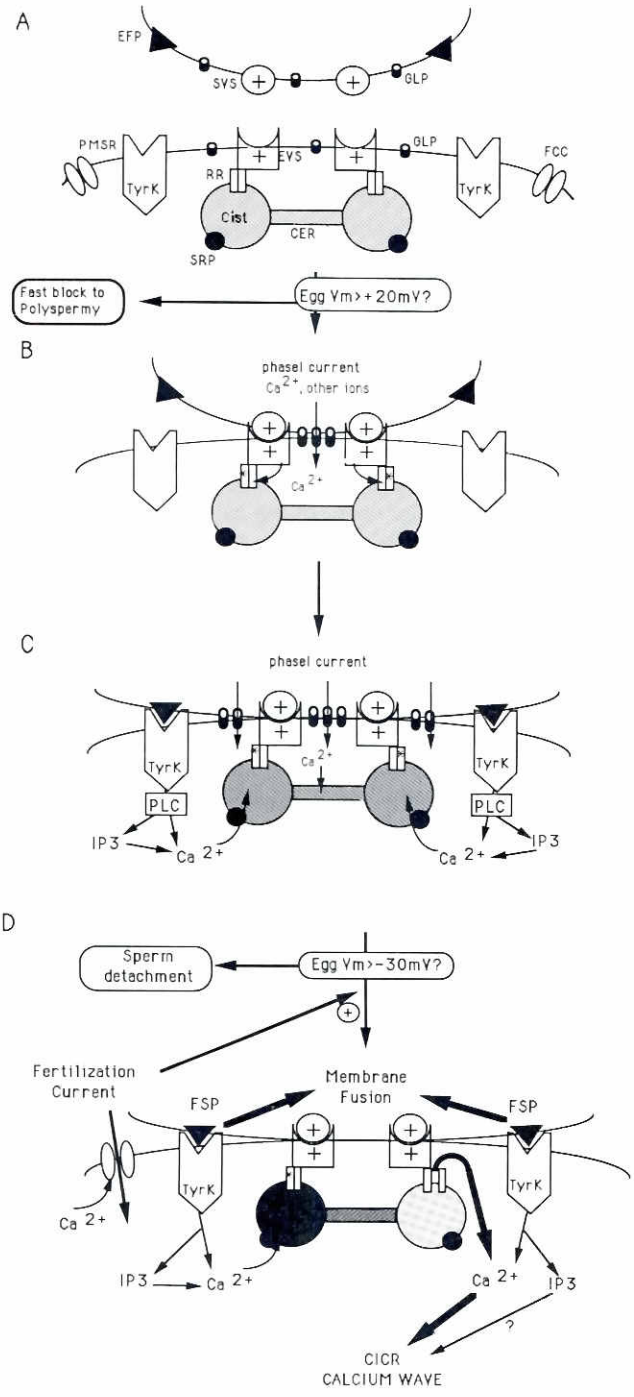

Fig. 4. A model proposed for the sperm signal transduction during the latent period (refer to text for the details). *The following abbreviations are used: EVS, egg voltage-sensitive protein; SVS, sperm voltage-sensitive protein; RR, ryanodine receptor; * indicates the sensitized state; Cist, cisternae; CER, cortical endoplasmic reticulum; GLP, gap junction-like pore; EFP, egg fertilization protein which possesses an integrin recognition domain and also acts as a sperm fusion protein (FSP); PMSR, plasma membrane receptor for sperm; TyrK, tyrosine kinase domain; PLC, phospholipase C; SRP, endoplasmic reticulum Ca^{2+} pump; FCC, fertilization channels activated by Ca^{2+}.*

a syn-capping process (Singer, 1992 for review). This association leads to T_H cell activation which involves events similar to the egg activation processes. Two soluble tyrosine kinases, $p59^{fyn}$ (Cooke *et al.*, 1991) and $p56^{lck}$ (Voronova and Sefton, 1986) non-covalently linked respectively to CD3 and CD4 may be responsible for this activation. Tyrosine kinase seems to be involved in various intracellular communications also involving integrins (Hynes, 1992 for review; Singer, 1992) and fertilization may perhaps prove to fit a more general scheme of cell activation. Recent results favor such an assumption. A rat sperm protein (DE) involved in sperm-egg fusion has been immunolocalized on the plasma membrane of eggs preincubated with DE (Rochwerger *et al.*, 1992). A patchy labeling probably resulting from DE-induced capping was observed. Capping is a common mechanism during interaction with integrins (Singer, 1992). There is also evidence of competitive inhibition of hamster sperm-egg binding by the peptide RGD (Arg-Gly-Asp), an integrin consensus binding sequence (Bronson and Fusi, 1990). Genes coding for the egg-binding membrane protein PH30 of guinea pig sperm (Primakoff *et al.*, 1987) have been sequenced. Deduced amino-acid sequences show that this protein contains a putative RGD domain for integrin binding (Blobel *et al.*, 1992). PH30 also contains a potential fusion peptide and DE is specifically involved in sperm-egg fusion (Rochwerger *et al.*, 1992).

A model for the events occurring during the latent period

The model proposed below (Fig. 4) is based on the existence of two steps required for sperm-egg fusion (s.l.). In sea urchin eggs, the latent period clearly comprises two steps reflected by the absolute and relative latent period (Shen and Steinhardt, 1984) and the existence of two voltage barriers which the sperm has to overcome to penetrate into the egg (McCulloh and Chambers, 1992). In rat oocytes, DE inhibits sperm penetration into, but not sperm attachment to, the egg (Rochwerger *et al.*, 1992), thus reflecting two steps during sperm interaction with the egg membrane.

It is proposed (Fig. 4A) that a plasma membrane voltage-sensitive egg protein (EVS) is recognized by a sperm «voltage-sensitive» protein (SVS) for specific egg membrane potential values. EVS is a conserved voltage sensor and SVS is responsible for the species specificity of the interaction. This step controls sperm-egg attachment and accounts for the early block to polyspermy.

As a voltage-sensor, EVS, akin to feet structures in skeletal muscle, is connected mechanically to the ryanodine receptor (RR) of cisternae (Cist) of cortical endoplasmic reticulum (CER). SVS binding to EVS is transduced to RR, which becomes sensitized (*, Fig. 4) and the threshold of luminal [Ca^{2+}] necessary to trigger the spontaneous Ca^{2+} release is lowered.

This interaction allows the sperm and egg membranes to come into contact with each other and electrical continuity to be established through gap-junction-like pores (GLP) (Fig. 4B). A non-specific current flows into the egg through these pores and generates a step current and a step depolarization taken as the beginning of the latent period. Subsequently, the contact between the membrane will increase as reported by the increase in phase 1 current.

More intimate contact between the membranes triggered by SVS/EVS interaction allows another step to proceed: a sperm protein which possesses both a fusion domain and an integrin recognition domain (EFP) is recognized by an egg plasma membrane receptor for sperm (PMSR) which is both an integrin and possesses a cytoplasmic tyrosine kinase domain (TyrK) (or a domain linked to soluble tyrosine kinase activation). Binding and subsequent recog-

nition of EFP by PMSR activates tyrosine phosphorylation of specific targets e.g. phospholipase C (PLC). Ins(1,4,5)P3 is produced locally and triggers a localized Ca^{2+} release (Fig. 4C).

Ca^{2+} released locally and Ca^{2+} originating from the non-specific phase 1 current is rapidly taken up by the CER pump (SRP).

Meanwhile, as a consequence of Ca^{2+} influx and cortical Ca^{2+} release, submembrane [Ca^{2+}] increases and Ca^{2+}-sensitive fertilization channels (FCC) open. This marks the onset of the fertilization current. The egg plasma membrane starts to depolarize (Fig. 4D left).

At this stage, there is a second step controlled by the egg potential. The sperm fusion protein will trigger fusion only if the egg is depolarized further than a threshold (reflected by the transition from type I to type II and III responses in sea urchin). Luminal [Ca^{2+}] has then reached a threshold and a burst of Ca^{2+} is liberated (Fig. 4D right). This helps the fusion between egg and sperm membrane to proceed and acts as the trigger of the Ca^{2+} wave.

In the eggs where Ca^{2+} oscillations occurs, it is proposed that cisternae sensitized at the sperm attachment site remain in this state. They subsequently act as a cortical pacemaker which initiates successive waves.

Propagation of the calcium wave

Mechanisms of propagation

Ca^{2+} waves and Ca^{2+} oscillations are regenerative mechanisms resulting from positive feedback. Two basic mechanisms can account for wave propagation (Fig. 5) (for review Tsien and Tsien, 1990; Cheek, 1991; Tsunoda, 1991): a) Ins(1,4,5)P3 liberates Ca^{2+}, which in turn activates Ins(1,4,5)P3 production through PLC activation according to the Ins(1,4,5)P3-induced Ca^{2+} release model (IICR); b) Ca^{2+} itself activates its own liberation as proposed by Ca^{2+}-induced Ca^{2+} release models (CICR). Cross-talk between Ins(1,4,5)P3-sensitive and insensitive Ca^{2+} stores with mutual facilitation and desensitization of Ins(1,4,5)P3 receptor (IR) and ryanodine receptor (RR) have led to several more elaborate models (e.g. Dupont *et al.*, 1991).

Mechanisms of Ca^{2+} wave propagation have been investigated in detail in sea urchin, *Xenopus* and mammalian eggs.

Ca^{2+} wave propagation in sea urchin eggs

Large amounts of Ins(1,4,5)P3 are produced during the Ca^{2+} wave (Ciapa and Whitaker, 1986) and phosphoinositide turnover is massively enhanced, probably in response to [Ca^{2+}] increase (Ciapa *et al.*, 1992). However Ins(1,4,5)P3 injections induce a Ca^{2+} transient very dissimilar to the sperm-induced one (Whalley *et al.*, 1992). Moreover heparin, a competitive inhibitor of Ins(1,4,5)P3 binding to its receptor (Worley *et al.*, 1987; Ghosh *et al.*, 1988; Ferris *et al.*, 1989) does not inhibit sperm induced Ca^{2+} transient (Rakow and Shen, 1990) and reduces its magnitude only slightly (Buck *et al.*, 1992; Whalley *et al.*, 1992). These results suggest that Ins(1,4,5)P3-insensitive Ca^{2+} stores are mainly involved in Ca^{2+} wave production. Ryanodine and caffeine (0.001 and 0.1 M respectively), which are CICR agonists (Schmid *et al.*, 1990), liberate Ca^{2+} in *in vitro* preparations of sea urchin eggs (Fujiwara *et al.*, 1990; Galione *et al.*, 1991; Sardet *et al.*, 1992). Their action is inhibited by CICR inhibitors ruthenium red and procaine. Incubations of sea urchin eggs with high ryanodine or caffeine concentrations (0.001 and 0.1 M respectively for half maximal effect) cause egg cortical reaction (Fujiwara *et al.*, 1990; Buck *et al.*, 1992; Sardet *et al.*, 1992). Ryanodine concentrations used in these studies are three orders of magnitude higher than concentrations active on Ca^{2+} fluxes (Meissner, 1986; Lattanzio *et al.*, 1987). Thus either ryanodine affinity of the egg ryanodine receptor (RR) is very low, or ryanodine effects in sea urchin eggs are not specific. However, RR have been immunolocalized in sea urchin eggs (McPherson *et al.*, 1992), and ryanodine injection (150-400 μM in cytoplasm) triggers fertilization envelope elevation (Galione *et al.*, 1991; Sardet *et al.*, 1992a). Caffeine injections also cause cortical reactions (Galione *et al.*, 1991) though large doses are required (1.6mM in cytoplasm) reflecting results obtained in egg homogenates (Fujiwara *et al.*, 1990; Galione *et al.*, 1991). Ins(1,4,5)P3-insensitive Ca^{2+} stores potentially responsible for CICR are thus present in sea urchin eggs.

But does CICR occur in eggs? Small ryanodine injections (60 μM final concentration) do trigger small slow Ca^{2+} transients (Buck *et al.*, 1992). Above a threshold large and brief Ca^{2+} transients similar to those induced by Ins(1,4,5)P3 are triggered (Galione *et al.*, 1991; Buck *et al.*, 1992). Heparin does not have any effect on these transients proving that ryanodine-induced Ca^{2+} transients do not result from IICR but probably from CICR (Buck *et al.*, 1992). Another argument for CICR lies in the synergistic action of caffeine and

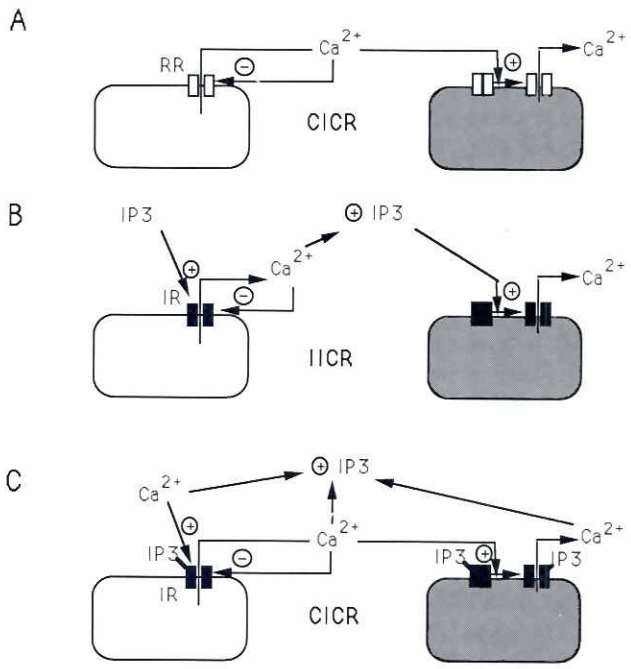

Fig. 5. Mechanisms responsible for Ca^{2+} wave propagation and Ca^{2+} oscillations. *It is assumed that Ca^{2+} stores behave as sums of discrete compartments which are «full» before release (in black) and become «empty» after.* **(A)** *Is the basic CICR mechanism where Ca^{2+} is released from Ins(1,4,5)P3-insensitive Ca^{2+} stores by the ryanodine receptor (RR) and activates its own release by the next store.* **(B)** *Is the basic IICR mechanism where Ca^{2+} released by Ins(1,4,5)P3 activates Ins(1,4,5)P3 synthesis, which in turn releases Ca^{2+} from the next store.* **(C)** *Represents an alternative mechanism based on CICR. IR activation by Ins(1,4,5)P3 is a prerequisite for Ca^{2+} release controlled by Ca^{2+} acting alternatively as an activator and an inhibitor.*

ryanodine *in vitro* (Galione *et al.*, 1991) and *in vivo* (Buck *et al.*, 1992), which has also been reported in other CICR mechanisms (Schmid *et al.*, 1990). cADPR is revealed to activate CICR *in vitro* (Galione *et al.*, 1991) and Ca^{2+} release and cortical reaction *in vivo* (*in vivo* experiments with unpurified fractions, Clapper *et al.*, 1987) at very low concentrations. The CICR pathway thus exists and is functional in sea urchin eggs.

CICR participation in the sperm-induced Ca^{2+} wave has been tested by double egg activation by sperm and GTPγS (Rakow and Shen, 1990). GTPγS photoreleased by UV flash from caged GTPγS causes Ins(1,4,5)P3 production and subsequently Ca^{2+} transients. A second identical UV flash releases only a small amount of Ca^{2+} because Ins(1,4,5)P3 sensitive stores are either desensitized or depleted. In contrast, insemination after a UV pulse does release Ca^{2+}. The latter result together with the heparin insensitivity of the sperm-induced Ca^{2+} wave shows that propagation of the Ca^{2+} wave in the sea urchin egg is mediated by CICR. Ins(1,4,5)P3 sensitive Ca^{2+} stores also exist but are probably desensitized or depleted by sperm-induced Ca^{2+} wave since UV flashes of eggs loaded with caged GTPγS do not cause a significant Ca^{2+} release after sperm action. A recovery period of at least 15 min is necessary to observe Ca^{2+} release by a UV flash after insemination. This would indicate that Ins(1,4,5)P3-sensitive stores are depleted rather than desensitized during fertilization and that they communicate with Ins(1,4,5)P3-insensitive stores during the Ca^{2+} wave as proposed by some models (e.g. Dupont *et al.*, 1991). It is also interesting to note that the sperm-induced transient observed when the egg is inseminated 8 min after a ryanodine injection (40-60 μM which does not induce the cortical reaction) is similar to an Ins(1,4,5)P3-induced transient (without a plateau but with a latent period) (Buck *et al.*, 1992). In contrast the sperm-induced transient after Ins(1,4,5)P3 action is not affected (Rakow and Shen, 1990). This shows that the state of Ins(1,4,5)P3-insensitive stores but not of Ins(1,4,5)P3-sensitive stores have an influence on fertilization Ca^{2+} wave and argues for the depletion of the former by the latter.

In conclusion, CICR appears to be the major mechanism for Ca^{2+} wave propagation in sea urchin eggs. Ca^{2+} released from Ins(1,4,5)P3-insensitive stores would directly release Ca^{2+} through RR and Ins(1,4,5)P3-sensitive stores would provide Ca^{2+} through a yet to be defined pathway. cADPR would be an excellent tool to study the intimate mechanism of CICR in sea urchin especially to define refractory states necessary to explain the progression of the wave (see example of such a mechanism below).

Ca^{2+} wave propagation in Xenopus and hamster eggs

Ins(1,4,5)P3 injections in Xenopus eggs trigger, above a threshold, regenerative Ca^{2+} release by endoplasmic reticulum (for review see Nuccitelli *et al.*, 1989; Busa, 1990). Ins(1,4,5)P3 can cause various Ca^{2+} transient patterns and release mechanisms may be more complex than originally thought (Berridge, 1988; Ferguson *et al.*, 1991; Parker and Ivorra, 1991) with other phosphoinositides involved in its modulation. It is important to note that IR but no RR have been purified and immunodetected in *Xenopus* eggs (Parys *et al.*, 1992) and that eggs pre-injected with antibodies to PtdIns(4,5)P2 are not activated by sperm (Larabell and Nuccitelli, 1992). An IICR mechanism for Ca^{2+} wave propagation therefore seems probable.

Existence of Ins(1,4,5)P3-insensitive Ca^{2+} stores similar to the one reported in sea urchin eggs is very improbable since RR appear to be absent and neither caffeine (Berridge, 1991; Lechleiter *et al.*, 1991; Parker and Ivorra, 1991; DeLisle and Welsh, 1992) nor ryanodine injected (Lechleiter and Clapham, 1992; only small doses (1 μM final concentration) were tested) or externally applied (1 nM to 1 mM in bath) (DeLisle and Welsh, 1992) induce Ca^{2+} release. However, caffeine inhibits Ins(1,4,5)P3-induced Ca^{2+}-activated Cl^- currents (Berridge, 1991; Parker and Ivorra, 1991b) and Ins(1,4,5)P3-induced Ca^{2+} release (Lechleiter *et al.*, 1991; Parker and Ivorra, 1991b). Caffeine does not mediate these inhibitions by inhibiting cAMP degradation (Butcher and Sutherland, 1962; Berridge, 1991; Parker and Ivorra, 1991b), but its mechanism remains unknown. It may be indirect since caffeine does inhibit only the second part of the response to Ins(1,4,5)P3 injections, which is constituted of Ca^{2+} oscillations to Ins(1,4,5)P3 (Berridge, 1991). This could be interpreted by the presence of two Ca^{2+} stores, although this seems unlikely since RR have not been detected. It can also be argued that caffeine does not inhibit IICR but modulates Ca^{2+} release by IR. Pre-injection of antibodies against PtdIns(4,5)P2 occasionally inhibits Ins(1,4,5)P3-induced Ca^{2+} waves and reduces the amount of Ca^{2+} released in eggs which undergo Ca^{2+} waves, but does not alter wave velocity (Larabell and Nuccitelli, 1992). Heparin injections also abolish propagation of Ca^{2+} waves (DeLisle and Welsh, 1992). Thus, both a working Ins(1,4,5)P3 production system and a potential responsiveness for Ins(1,4,5)P3 are necessary for Ca^{2+} release during wave propagation in *Xenopus* eggs. Ins(1,4,5)P3 is therefore the agent of Ca^{2+} release in this system. It has also been noticed that Ca^{2+} sensitive dye fura-2 would be responsible due to its Ca^{2+} buffering capacity for reducing wave velocity to half of their physiological value (Busa and Nuccitelli, 1985; Larabell and Nuccitelli, 1992). This effect is obvious when velocities are compared between different experiments where various dye concentrations are used (e.g. compare Crossley *et al.*, 1988 with Whalley *et al.*, 1992) and between the speed of Ca^{2+}-induced fertilization current (approximately 5 μm/sec) (McCulloh and Chambers, 1991), which is twice as fast as the Ca^{2+} wave recorded with Ca^{2+}-sensitive dyes at similar temperature (Jaffe, L.F., 1991). This can be interpreted by assigning wave propagation to Ca^{2+} diffusion.

Thanks to its large size, the *Xenopus* egg provides a perfect model for studying Ca^{2+} wave propagation with confocal microscopy. When acetylcholine (Ach) is applied externally to *Xenopus* eggs preinjected with mRNA coding for Ach muscarinic receptor, a remarkable Ca^{2+} pattern (monitored with fluo-3) develops (Lechleiter *et al.*, 1991; DeLisle and Welsh, 1992). Pulsating foci produce circular propagating Ca^{2+} waves, which eventually become spiral waves. When two Ca^{2+} wave fronts collide they annihilate each other. Both GTPγS release and Ins(1,4,5)P3 release from caged compounds by UV flash generate similar patterns indicating that previous results originated from Ins(1,4,5)P3 production by Ach (Lechleiter and Clapham, 1992). Such patterns which self-organize from a homogeneous medium have been described by the Belousov-Zahbotinsky chemical reaction (Zaikin and Zahbotinski, 1970), are characteristic of physical excitable media and can be modelled (Markus and Hess, 1990). This model, like other models proposed for Ca^{2+} oscillations, assumes that Ca^{2+} stores behave as an ensemble of distinct compartments (such behavior is likely to exist in *Xenopus* eggs (Parker and Ivorra, 1990a). Ca^{2+} waves propagate because after Ca^{2+} release from a compartment, the latter becomes refractory and a diffusible activator whose production is activated by Ca^{2+} releases Ca^{2+} from the next nearest store. The mean diffusion coefficient for the putative activator in the *Xenopus* egg calculated according to this model is closer to the diffusion

coefficient in cytoplasm estimated for Ca^{2+} than for Ins(1,4,5)P3 (Lechleiter *et al.*, 1991; Lechleiter and Clapham, 1992). However various values have been estimated for these diffusion coefficients (compare with Jaffe, 1991). Moreover, injections of nonhydrolyzable Ins(1,4,5)P3 analogue inositol 1,4,5-trisphosphothioate (IP3S3) cause spiral Ca^{2+} waves (DeLisle and Welsh, 1992; Lechleiter and Clapham, 1992). Since it can be assumed that IP3S3 level remains constant, Ins(1,4,5)P3 oscillations associated with IICR do not appear to be essential to spiral Ca^{2+} wave propagation and IICR would be unlikely to take part to this mechanism. Ca^{2+} injections do trigger waves only if they are preceded by IP3S3 injections (DeLisle and Welsh, 1992). Localized Ins(1,4,5)P3 photorelease by confocal UV scans creates pulsating foci. This effect is enhanced by an IP3S3 preinjection (Lechleiter and Clapham, 1992). These results can be interpreted as showing that a basal Ins(1,4,5)P3 level is required for initiation and propagation of spiral waves by Ca^{2+}. In eggs pre-injected with Ins(1,4,5)P3, further Ins(1,4,5)P3 injections inhibit the oscillating Ca^{2+}-activated Cl- current monitored (Berridge, 1988). With the «confocal dissection» approach it is shown that a large Ins(1,4,5)P3 photorelease corresponding to a large Ca^{2+} release abolishes propagating Ca^{2+} waves. Both Ca^{2+} activation (for $[Ca^{2+}]<250$ nM) and inhibition (for $[Ca^{2+}]>500$ nM) of Ins(1,4,5)P3 Ca^{2+} release have been demonstrated in other systems (Bezprozvanny *et al.*, 1991; Finch *et al.*, 1991). Results reported above have been interpreted by a model derived from CICR (Lechleiter and Clapham, 1992) (Fig. 5C). According to this model, IR need to be in a preactivated state, already bound to Ins(1,4,5)P3 which require a threshold Ins(1,4,5)P3 level in the egg. Ca^{2+} release by IR is controlled by Ca^{2+} itself, activated at the front of the wave and inhibited behind it. In comparison to other models proposed (Berridge and Irvine, 1989; Petersen and Wakui, 1990; Tsien and Tsien, 1990; Tsunoda, 1990), this model is particularly interesting because it is based on unique type of Ca^{2+} store and Ca^{2+} channel which seems to be the case in *Xenopus* eggs. However, sperm-induced Ca^{2+} waves do not seem to display any spiral Ca^{2+} oscillations (Busa and Nuccitelli, 1985; Kubota *et al.*, 1987). A confocal study would be necessary to confirm this point and to ascertain whether spiral waves can be induced by sperm.

In contrast, detectable Ca^{2+} oscillations resulting from successive propagation of Ca^{2+} waves (Miyasaki *et al.*, 1986) occur during hamster egg activation. These eggs thus present two phenomena: wave propagation and Ca^{2+} oscillations. This situation is similar to the spiral waves in *Xenopus* eggs where foci activity oscillates and generates Ca^{2+} waves. Mammalian eggs are a better model for understanding oscillation mechanisms which can be obviously manipulated, though confocal studies to dissect the wave mechanism would be difficult to achieve due to the small size of the eggs. A high molecular weight (>100 kDa) sperm factor (Swann, 1990), Ins(1,4,5)P3 (Miyasaki, 1988; Peres, 1990; Peres *et al.*, 1991; Carrol and Swann, 1992), thimerosal (Swann, 1992) Ca^{2+} (Igusa and Miyazaki, 1983) and ryanodine (Swann, 1992) elicit Ca^{2+} oscillations in hamster and mouse eggs, suggesting that both Ins(1,4,5)P3-sensitive and Ins(1,4,5)P3-insensitive Ca^{2+} stores are present. But, as in *Xenopus* eggs, IR only have been immunodetected (Miyazaki *et al.*, 1992). To dissect the mechanism responsible for Ca^{2+} oscillations, various IICR and CICR activators have been injected during oscillation sequences. A major result comes from injections of monoclonal antibodies directed against IR (Miyazaki *et al.*, 1992). Sperm-induced Ca^{2+} oscillations are then reduced to one or two and are characterized by a slower propagation and release a lower level of Ca^{2+} proportional to the amount of antibodies injected. Heparin also abolishes spontaneous Ca^{2+} oscillations in mouse eggs (Carroll and Swann, 1992). Together, these results strongly suggest that Ins(1,4,5)P3 is responsible for Ca^{2+} release but is not necessarily responsible for a feed-back mechanism (if we assume that Ca^{2+} stores behave as discrete compartments). However, ryanodine abolishes Ca^{2+} oscillations induced either by sperm factor, Ins(1,4,5)P3 or thimerosal (Swann, 1992) and could act by Ca^{2+} inhibition of IR. Ca^{2+} injection triggers single or multiple transients (Igusa and Miyasaki, 1983; Swann, 1991; Miyazaki *et al.*, 1992). Pre-injections with antibodies against IR abolish propagation of single transients and increase the threshold of Ca^{2+} injection to induce regenerative responses (Miyasaki *et al.*, 1992), and multiple Ca^{2+} injections do not promote further Ca^{2+} release but instead progressively inhibit it (Igusa and Miyasaki, 1983; Swann, 1991). After photorelease of caged Ins(1,4,5)P3 by a UV pulse, successive UV pulses liberate smaller amount of Ca^{2+} (Peres *et al.*, 1991), similar to results obtained in *Xenopus* eggs. Thus, Ca^{2+} inhibits Ca^{2+} release by IR and the CICR mechanism proposed for *Xenopus* egg explains results obtained in hamster and mouse eggs. It must be noted that anti-IR antibodies inhibit both oscillations and wave propagation and that a common mechanism is likely to be responsible for both phenomena. However, relationships between Ca^{2+} injections and Ins(1,4,5)P3 level should be investigated using IP3S3 to determine whether Ca^{2+} also activates IR. By using antibodies to PtsIns(4,5,)P2, the requirement of Ins(1,4,5)P3 production for oscillations and wave would be determined. Moreover, effects of the inhibition of Ins(1,4,5)P3 production on the frequency and duration of the oscillations should allow us to posit the existence of an IICR mechanism distinct from the CICR mechanism proposed for *Xenopus* eggs.

Refilling the Ca^{2+} stores

In sea urchin eggs the plateau of fertilization-like Ca^{2+} transients is heavily dependent on external Ca^{2+} (Crossley *et al.*, 1991; Whalley *et al.*, 1992). This suggests the participation of external Ca^{2+} uptake to sustain the Ca^{2+} wave. A large amount of calcium that could potentially increase cytoplasmic $[Ca^{2+}]$ up to 150 μM is released from the internal stores during fertilization of sea urchin eggs (Mohri and Hamaguchi, 1991) and the egg undergoes a net loss of calcium as large as 20% of the total calcium within 40 min after fertilization (Arzania and Chambers, 1976) and estimated to be 7 to 15% (depending on the species) within 4 min (Gillot *et al.*, 1991). A large part of this loss can be accounted for by cortical granule exocytosis ($[Ca^{2+}]$ has been estimated to be higher than 1 mM in cortical granules) (Gillot *et al.*, 1991). However, the fertilization Ca^{2+} wave originates mainly if not only from endoplasmic reticulum-derived stores (Gillot *et al.*, 1990 for review; Terasaki and Sardet, 1991) and mitochondria are likely to be a sink for Ca^{2+} (Eisen and Rey, 1985; Girard *et al.*, 1991). If so, Ca^{2+} stores from the endoplasmic reticulum are depleted by the massive Ca^{2+} release during the Ca^{2+} wave and need to be replenished after and possibly even during the wave. Can this be explained in terms of known Ca^{2+} transport mechanisms? (for review see Carafoli, 1987). The plasma membrane calcium pump is outwardly directed and causes loss of calcium. The Ca^{2+}/Na^{+} exchanger with its high dissociation constant for Ca^{2+} is involved mainly in Ca^{2+} homeostasis control and even during depolarization expels calcium from the cell. The calcium pump of the reticulum may also be inefficient alone. There may therefore be another pathway.

A special pathway for Ca^{2+} influx dependent on both Ins(1,4,5)P3 and Ins(1,3,4,5)P4 (produced by the Ins(1,4,5)P3-3, kinase) has been a matter of controversy (reviews by Irvine *et al.*, 1988; Swann and Whitaker, 1990). Seminal studies indicated that Ins(1,3,4,5)P4 activates sea urchin eggs by a pathway dependent on external Ca^{2+} when coinjected with an intracellular Ca^{2+} mobilizer (Irvine and Moor, 1986). This result led to intense studies in other cell types (reviewed in Irvine, 1990; Meldolesi *et al.*, 1991) and recent results have shown that there are at least two distinct paths for Ca^{2+} influx after Ca^{2+} release from intracellular stores, a plasma membrane Ins(1,3,4,5)P4-activated Ca^{2+} channel (Luckhoff and Clapham, 1992) and an Ins(1,3,4,5)P4-independent Ca^{2+} influx activated by depletion of intracellular Ca^{2+} stores (Hoth and Penner, 1992). However, in sea urchin eggs, the results reported above have not been observed in subsequent studies (Irvine and Moor, 1987; Crossley *et al.*, 1998) and moreover Ins(1,4,5)P3-induced Ca^{2+} waves are independent of external Ca^{2+} (Crossley *et al.*, 1988; Whalley *et al.*, 1992). Although these results would seem to demonstrate an absence of Ins(1,3,4,5)P4-dependent Ca^{2+} influx, Ins(1,3,4,5)P4 is most probably produced by the increase in phosphoinositide turnover during the wave and may have a role which has been overlooked.

In *Xenopus* eggs dialogue between Ins(1,4,5)P3 and Ins(1,3,4,5)P4 has also been examined. Ins(1,3,4,5)P4 injections also trigger Ca^{2+} transients. These transients differ from Ins(1,4,5)P3-induced transients by the lag period which precedes them and by their monophasic aspect (Ferguson *et al.*, 1991; Parker and Ivorra, 1991a). It has been shown that the lag phase is not the result of Ins(1,3,4,5)P4 conversion into Ins(1,4,5)P3 during the first minutes after injection (Ferguson *et al.*, 1991) and that it does not act as a competitive inhibitor of 5-monoesterase, which degrades Ins(1,4,5)P3 (Parker and Ivorra, 1991a). However, the Ins(1,3,4,5)P4-induced Ca^{2+} transient is independent of extracellular Ca^{2+}. Synergistic action of Ins(1,3,4,5)P4 on Ca^{2+} release by Ins(1,4,5)P3 has been observed when low doses of Ins(1,3,4,5)P4 are injected before Ins(1,4,5)P3 photorelease (Parker and Ivorra, 1991a). Thus in *Xenopus* eggs Ins(1,3,4,5)P4 is likely to play a role during CICR but the mechanism is unclear and may not involve a Ca^{2+} influx.

Biological significance of fertilization waves

It can be proposed teleologically that such a precisely controlled phenomenon may not be simply the consequence of an out-of-equilibrium physical system (Lechleiter *et al.*, 1991). Does this spatial and temporal phenomenon have any consequences which might differ from a uniform increase of the $[Ca^{2+}]c$? Considering the large size of eggs in some species, it can be argued that the simple diffusion of calcium from outside (which is claimed to happen in the protostome *Urechis* (Gould and Stephano, 1987, 1989 for review)) would not allow such a rapid cytoplasmic $[Ca^{2+}]$ increase. In addition to allowing rapid Ca^{2+} release, some observations give clues about eventual roles for the wave. First of all, some other cellular events occur as wave. The fertilization current travels as a wave along the egg surface in sea urchin and *Xenopus* eggs (McCulloh and Chambers, 1991). During the cortical reaction in sea urchin (Kline and Nuccitelli, 1985; Gillot *et al.*, 1991), *Xenopus* (Grey *et al.*, 1974) and Medaka fish (Gilkey *et al.*, 1978), but not hamster (Stewart-Savage and Bavister, 1991), the exocytosis of the cortical granules takes place as a wave. Similarly, a contraction wave crosses the egg in the same direction as the calcium wave in ascidians (Speksnijder *et al.*, 1989; Brownlee and Dale, 1990), *Xenopus* (Kline and Nuccitelli, 1985) and probably sea urchin (Stricker *et al.*, 1992). As already mentioned, the cortical endoplasmic reticulum of the sea urchin egg undergoes a wave of temporary disorganization (Terasaki and Jaffe, 1991). The pH increase has also been recorded as a wave in the *Xenopus* egg (Grandin and Charbonneau, 1992). This last result has been recently obtained with pH-sensitive electrodes positioned at different distances from an artificial activation site. The whole wave lasts 80±20 sec.

These observations nonetheless push the question one step further. The calcium wave pattern can be related to the subsequent events activated by high $[Ca^{2+}]$. But why should subsequent events occur as waves?.

The ascidian egg is, as far as we know, the only example of a real implication of the wave in the future development of the egg. Indeed, the contraction wave directed by the calcium wave controls the segregation of a specific area of the cytoplasm rich in mitochondria, the myoplasm. The myoplasm is confined in the dorsal pole of the vegetal hemisphere where the contraction ends (Speksnijder *et al.*, 1989). Thus the calcium wave in the ascidian egg arguably directs the future dorsoventral polarity of the embryo. At present, the wave pattern has not been claimed to have other biological significance. It can thus be considered as an organizer that coordinates kinetics of chemical reactions involved in subsequent fertilization-associated events.

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Summary

In deuterostome species, after fertilization, the egg undergoes a transient increase in free cytoplasmic calcium. This increase begins at the sperm entry point and propagates across the egg towards the opposite pole. This phenomenon is referred to as the calcium wave.

Conflicting theories have arisen about the mechanism of wave initiation by sperm. Some results favor Ca^{2+} channel transfer from the sperm to the egg, others favor signal transduction via a sperm receptor linked to G-protein and second messenger production pathway. Other results have raised the possibility of the existence of a diffusible sperm factor delivered into the egg during gamete fusion. These models are reviewed and criticized. The existence and nature of a sperm receptor and the potential involvement of tyrosine kinase is discussed.

The propagation of the wave relies on a positive feedback mechanism. Recent evidence favors a role for calcium itself in Calcium-Induced Calcium Release processes which also involve Inositol(1,4,5)-trisphosphate. The actual activation of inositol phosphate turnover and the resulting increase in Ins(1,3,4,5)P4 concentration may be involved in refilling internal calcium stores during and after the calcium wave.

Ultimately the biological significance of the wave pattern, expressed in many early fertilization events, is questioned.

KEY WORDS: *fertilization, Ca^{2+} wave, Ca^{2+} oscillations, signal transduction*

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