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THE ROLE OF Ca^{2+} IN SIGNAL TRANSDUCTION FOLLOWING FERTILIZATION IN *FUCUS SERRATUS*

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Summary

The marine brown alga *Fucus serratus* represents one of the few multicellular plant species in which the process of fertilization can be studied relatively easily. Fertilization marks the onset of a cascade of events associated with egg activation. Fertilization in *Fucus serratus* bears several superficial similarities to fertilization in several animal systems. The essential features of *Fucus serratus* egg activation are compared with those of protostome and deuterostome animal systems. Ca^{2+} is required for egg activation in *Fucus serratus* and cytosolic [Ca^{2+}] changes can be observed in fertilizing eggs. However, these are small and variable in comparison with those occurring in deuterostomes, and fertilization can proceed normally in the absence of any global cytosolic Ca^{2+} transients. A model for egg activation in *Fucus serratus* is presented, invoking a role for both Ca^{2+} influx and localized propagation of the sperm signal around the plasma membrane by an as yet unidentified mechanism. Polarity in *Fucus serratus* is acquired a considerable time after fertilization and the role of cytosolic Ca^{2+} gradients in the acquisition and expression of polarity is discussed. The problem of the signals associated with the onset of the cell cycle in the fertilized *Fucus serratus* egg is also addressed.

Introduction

Fertilization of a large non-motile egg by a smaller motile sperm is a common reproductive strategy amongst multicellular animals and plants. Egg activation represents the sequence of events leading to the initiation of development of the egg. Activation events have been extensively characterized in sea urchin and other deuterostomes (e.g. Whitaker and Swann, 1993; Berger, 1992) but remain more obscure in most protostomes and plants. The marine brown alga *Fucus serratus* continues to provide the bulk of our knowledge of the processes associated with egg activation in a multicellular plant. The release of eggs and sperm into the surrounding sea water from separate male and female plants makes this one of the few easily accessible plant fertilization systems. This review will summarize our present knowledge of the events associated with and following fertilization, leading to the development of the *Fucus serratus* zygote and embryo, emphasizing similarities and differences with the signal-transduction mechanisms associated with activation of various animal eggs. Particular attention will be paid to the role of Ca^{2+} in signal transduction during egg activation and to zygote polarization.

Key words: cytoplasmic Ca^{2+}, fertilization, *Fucus serratus*, polarization, egg activation.

The unfertilized egg

Many animal eggs possess animal–vegetal polarity marked by cytological and molecular features (e.g. Giudice, 1986; Gurdon, 1992). For example, ascidian eggs possess animal–vegetal polarity (Jeffery, 1984), and sperm entry, which establishes the pattern of ooplasmic segregation, occurs in the animal hemisphere (Speksnijder *et al.* 1989*a*). In amphibians and *Drosophila melanogaster*, maternal mRNAs and their products form gradients that establish the general organisation of the larval body (Dawid, 1992; St Johnston and Nusslein-Volhard, 1992). In contrast, the unfertilized *Fucus serratus* egg does not show any sign of polarity. The egg membrane is uniformly covered with an amorphous gelatinous layer (Levring, 1952). The pronucleus remains at the centre of the egg throughout fertilization (Brawley *et al.* 1976*b*; Swope and Kropf, 1993). The cytoskeleton is evenly distributed around the cortex (Kropf *et al.* 1990; Kropf, 1992) and organelles and numerous cytoplasmic inclusions are distributed evenly in the egg (Brawley *et al.* 1976*a*). Maternal mRNA stored as ribonucleoproteins (Hetherington *et al.* 1990) is present, but information is lacking concerning its distribution.

The electrical properties of the *Fucus serratus* egg show similarities with those of several animal eggs. The *Fucus serratus* egg plasma membrane contains voltage-gated channels and can elicit action-potential-like voltage responses when depolarized in current-clamp mode (Taylor and Brownlee, 1993). Voltage-clamp analysis of the currents underlying this response reveals the presence of an inward current which activates rapidly and inactivates slowly on depolarization of the plasma membrane from resting levels of −55mV to potentials more positive than −30mV. Further depolarization elicits a more slowly activating outward current. Inhibitor and ion-substitution experiments show that Ca^{2+} could carry the inward current and that K^+ carries the outward current (Taylor and Brownlee, 1993). Similar Ca^{2+}-dependent action potentials are found in deuterostomes such as sea urchins (e.g. David *et al.* 1988; Lynn *et al.* 1988) and protostomes such as *Urechis caupo* (Jaffe *et al.* 1979). Their relative roles during the fertilization potential and in egg activation will be discussed below.

Responses to fertilization

Early events

In the sea urchin egg, fusion with a sperm is followed, after a short ‘latent period’ (Allen and Griffin, 1958) during which the sperm signal is transduced to the egg, by the series of events depicted in Fig. 1. The corresponding events characterized in the fertilizing *Fucus serratus* egg are juxtaposed. As with the sea urchin, a fertilization potential marks the onset of egg activation in *Fucus serratus*. It takes place as a depolarization from −50mV to membrane potentials more positive than −20mV (Brawley, 1991; Taylor and Brownlee, 1993), followed by a repolarization lasting approximately 5min.

Fig. 1. Comparison of the time courses for the main events associated with and following fertilization in sea urchin and in *Fucus serratus* eggs.

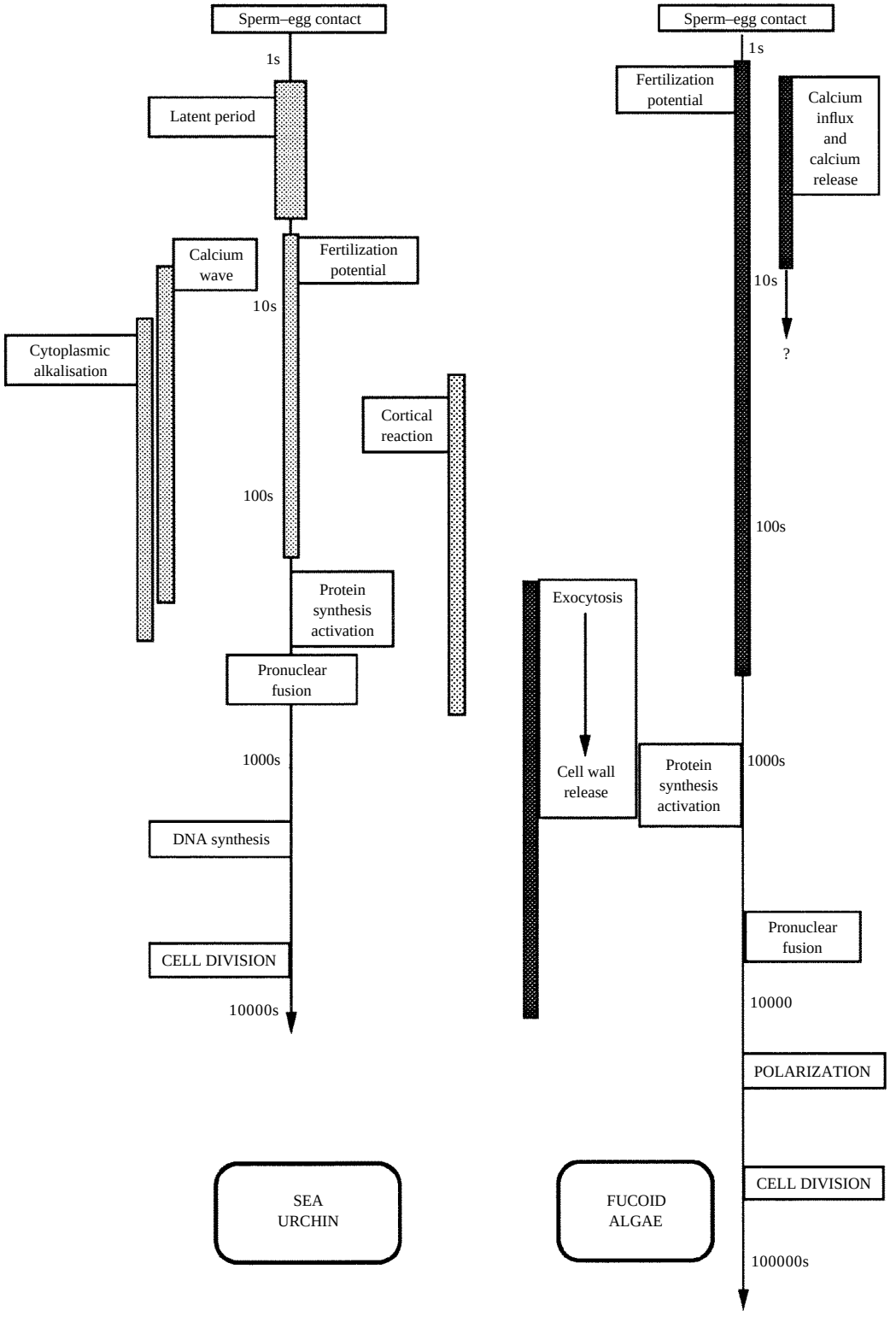
Sperm–egg contact
1s
Latent period
Calcium wave
Fertilization potential
10s
Cytoplasmic alkalisation
Cortical reaction
100s
Protein synthesis activation
Pronuclear fusion
1000s
DNA synthesis
CELL DIVISION
10000s
SEA URCHIN
Sperm–egg contact
1s
Fertilization potential
Calcium influx and calcium release
10s
?
100s
Exocytosis
Cell wall release
Protein synthesis activation
1000s
Pronuclear fusion
10000
POLARIZATION
CELL DIVISION
100000s
FUCOID ALGAE

Fig. 1

In all deuterostome eggs studied, a major early activation event is a rise in the concentration of free cytoplasmic Ca^{2+}, $[Ca^{2+}]_i$ (for reviews, see Whitaker and Swann, 1993; Jaffe, 1991; Berger, 1992). This propagates from the site of sperm entry through the egg cytosol as a concentration wave. As we will discuss below, though elevation of $[Ca^{2+}]_i$ is involved in fertilization in protostomes and in fucoid algae, fundamental differences exist in the mechanisms underlying this elevation.

Polyspermy blocks

Mechanisms providing a barrier to polyspermy have evolved in many cases where fertilization is external and multiple sperm–egg encounters are likely (Ginsburg, 1988). Though exceptions exist (e.g. the ctenophore *Beroe ovata*, Carre *et al.* 1990), the fertilization potential provides an initial transient block to polyspermy and a more permanent block is established later by modification of the egg surface. In the sea urchin (and also in fish and amphibians), a wave of exocytosis sets a hard fertilization envelope (Moser, 1939). In ascidians, modification of the cortical cytoskeleton during the contraction wave can prevent further sperm entry (Sawada and Osanai, 1981). The case with protostomes, such as the marine worm *Urechis caupo*, is less clear (Gould and Stephano, 1989), though a wave of fertilization-induced exocytosis is absent in most species studied. When internal fertilization is the rule, a barrier against polyspermy is lacking, as in many birds, or refined, as in mammals where exocytosis liberates an enzyme that modifies the sperm receptor ZP3 of the zona pellucida (Bleil and Wasserman, 1980). In the *Fucus serratus* egg, the cortical area is rich in vesicles full of electron-clear material. After fertilization, the vesicles fuse with each other (Brawley *et al.* 1976*a*) and with the plasma membrane (Peng and Jaffe, 1976), releasing polysaccharides on the outside of the plasma membrane into the cell wall (for a review, see Evans *et al.* 1982). The onset of cell wall release probably occurs within seconds of fertilization and can be detected minutes later (Brawley and Bell, 1987; Evans *et al.* 1982; Roberts and Brownlee, 1993). The cell wall is released uniformly and simultaneously on the egg surface (Roberts and Brownlee, 1993; Peng and Jaffe, 1976) and constitutes a mechanical barrier against polyspermy.

The cell wall and Fucus serratus *zygote development*

The cell wall provides an effective protection against mechanical stresses and sudden variations of the external osmotic environment (Brawley and Johnston, 1991). Moreover, the cell wall is essential for growth and development. In parallel with an increase in K^+ conductance (Taylor and Brownlee, 1993), internal osmotic pressure increases rapidly after fertilization (Allen *et al.* 1972). This provides the turgor that is necessary for rhizoid germination (Torrey and Galun, 1970). Unpolarized *Fucus serratus* embryos cannot establish an axis without a cell wall (Kropf *et al.* 1988). During axis fixation, membrane and cell wall are connected through a vitronectin-like protein at the site of rhizoid germination (Wagner *et al.* 1992). The first mitosis starts 12–18h after fertilization, after the cell wall has played its major part in the establishment of the polarization template (axis fixation) and made possible the increase of turgor crucial for germination and, more generally, for growth (Torrey and Galun, 1970). Cell wall release following fertilization

in *Fucus serratus* thus appears to have a much broader and fundamental developmental significance than the cortical reaction in animal eggs.

Metabolic and molecular changes and Fucus serratus *egg activation*

Fertilization in *Fucus serratus* is also accompanied by a series of biochemical events. Metabolic oxygen consumption increases, doubling within minutes of fertilization (Whitaker, 1931; Levring, 1952) and remaining constant throughout the first cell cycle. However, this is distinct from the sharp transient in oxygen consumption observed in sea urchin eggs which is related to ovoperoxidase activity during fertilization membrane hardening (Shapiro, 1991; Heinecke and Shapiro, 1992).

In animal eggs, stored maternal mRNA in the form of messenger ribonucleoprotein (mRNP) is used to support zygote development up to the mid-blastula stage (Davidson, 1986). Although maternal mRNAs represent a potential pool for synthesis of major proteins (e.g. actin) in *Fucus serratus* (Masters *et al.* 1992; Kropf *et al.* 1989), *de novo* mRNA synthesis starts about 1h after fertilization (Koehler and Linsken, 1967; Peterson and Torrey, 1968). Transcription of essential mRNAs for future zygote development is complete within 4h, after which actinomycin D does not prevent polarization or the first cell division (Quatrano, 1968; Kropf *et al.* 1989). RNA translation starts after 1 h (Koehler and Linskens, 1967; Peterson and Torrey, 1968) and is required until rhizoid germination (Kropf *et al.* 1989; Quatrano, 1968).

In summary, fertilization mechanisms in *Fucus serratus* eggs bear some significant similarities, at least superficially, with their animal counterparts. These include the generation of a fertilization potential, the presence of two successive blocks to polyspermy, the signalling by sperm of the onset of metabolic activity and the presence of stored maternal mRNA. An important singularity of *Fucus serratus* fertilization is the late appearance of zygote polarity and first cleavage. The unfertilized egg is not polarized and fertilization itself does not reorganize the cytoplasm as is often the case in animals. Polarization relies entirely on environmental cues and fertilization provides the machinery to transduce the signals. For example, zygotes acquire sensitivity to the main polarizing vector (blue light) only 4–6h after fertilization (Whitaker, 1936; Bentrup, 1963). Cell division is completed approximately 20h after the onset of fertilization of fucoid eggs (Allen and Kropf, 1992). This is relatively slow. By comparison, the first cell cycle is completed 85–95min after fertilization of sea urchin eggs (Epel, 1990). In the next sections we will discuss the signal transduction mechanism underlying these responses in *Fucus serratus.*

Signal transduction and egg activation

Receptors and early signals

A gene coding for a sperm receptor in the plasma membrane of the sea urchin egg has recently been cloned (Foltz *et al.* 1993). The deduced amino acid sequence does not possess any similarities with other receptors involved in signal transduction. This and other arguments have weakened the case for the involvement of a receptor/G-protein in transduction of the sperm signal (Whitaker and Swann, 1993; Berger, 1992). As an

alternative, evidence has accumulated suggesting that an activating sperm factor is injected during gamete fusion. Injections of sperm extracts from *Urechis caupo* (Gould and Stephano, 1989), sea urchin and mammals (Whitaker and Crossley, 1990) can cross-activate eggs of various species. This is the only treatment which mimics closely the various events involved in egg activation (e.g. Swann *et al.* 1992). An additional hypothesis involves multiple receptors for the sperm similar to the recognition between helper T cells and antigen-presenting B cells (a case of self/non-self recognition, Abraham *et al.* 1992). Sperm receptors could be linked to tyrosine kinase activity, which has been characterized in sea urchins (Ciapa and Epel, 1991; Peaucellier *et al.* 1993).

In *Urechis caupo*, a potential egg activator is an acrosomal protein (Gould and Stephano, 1991). A hexapeptide isolated from the protein proves to have similar biological activity to the native protein and to trigger meiotic resumption in oyster oocytes (Gould and Stephano, 1991). This implies the existence of a sperm receptor which could be a sodium channel (Gould-Somero, 1981).

In *Fucus serratus*, glycoproteins which may be involved in sperm–egg recognition have been characterized immunologically on the surface of both sperm and eggs (Jones *et al.* 1990; Stafford *et al.* 1992, 1993). Surface glycoproteins are distributed in discrete domains on the egg surface. Their possible roles in signal transduction during *Fucus serratus* egg activation remain to be characterized.

Egg activation and cytoplasmic Ca^{2+}

Two general models of egg activation have been proposed on the basis of studies with deuterosomes and protosomes (Jaffe, 1983).

Deuterostomes

The deuterostome model of egg activation is characterized by a wave or waves of elevated $[Ca^{2+}]_i$ traversing the egg. These originate from the point of sperm entry and involve the liberation of Ca^{2+} from internal stores. This is thought to signal, at least in part, the cascade of events responsible for initiating zygote development. $[Ca^{2+}]_i$ has been reported to rise to levels of up to $10\,\mu mol\,l^{-1}$ from resting levels of $100–200 nmol\,l^{-1}$ (e.g. Brownlee and Dale, 1990; Mohri and Hamaguchi, 1991). Ca^{2+} wave propagation has been investigated in detail in sea urchin, *Xenopus laevis* and mouse eggs and two basic mechanisms have been proposed: (a) inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3]-induced Ca^{2+} release (IICR), which relies on liberation of Ca^{2+} from Ins(1,4,5)P_3-sensitive stores, which then stimulates further Ins(1,4,5)P_3 production (by Ca^{2+} activation of phospholipase C) and (b) Ca^{2+}-induced Ca^{2+} release (CICR), where elevated $[Ca^{2+}]_i$ stimulates release from Ins(1,4,5)P_3-insensitive stores. These mechanisms are thought not to be mutually exclusive and more complex models involving cross-talk between them have been suggested (e.g. Ciapa *et al.* 1993; Dupont *et al.* 1991).

The first detectable event during deuterostome egg activation is an increase in membrane conductance and depolarization due to the activation of sperm-gated cation channels at the point of sperm entry (McCulloh and Chambers, 1991; Dale *et al.* 1978). This is associated with a transient influx of Ca^{2+} into the egg (McDougall *et al.* 1993; Shen and Buck, 1993) and marks the onset of the latent period, which is followed by an

action potential and the fertilization potential (Allen and Griffin, 1958; Whitaker *et al.* 1989; McCulloh and Chambers, 1991; McDougall *et al.* 1993). The fertilization potential results from Ca^{2+} activation of cation channels in the sea urchin as Ca^{2+} levels increase during the Ca^{2+} wave (Chambers and deArmendi, 1979; David *et al.* 1988). In *Xenopus laevis*, the fertilization potential involves Ca^{2+} activation of plasma membrane Cl^- channels (Ferguson *et al.* 1990).

The sea urchin Ca^{2+} wave is thought to be propagated predominantly by CICR. Potent CICR agonists, ryanodine and caffeine, stimulate egg activation (Galione *et al.* 1991; Sardet *et al.* 1992) and the ryanodine binding sites have been localized in the cortical region (McPherson *et al.* 1992). Although Ins(1,4,5)P_3 turnover is dramatically enhanced during sea urchin egg activation (Turner *et al.* 1984), heparin [a potent competitive inhibitor of Ins(1,4,5)P_3 binding to its receptor] does not inhibit the sperm-induced Ca^{2+} transient (Rakow and Shen, 1990), suggesting that there is no requirement for Ins(1,4,5)P_3 during wave propagation. However, in *Xenopus laevis* and hamster eggs, heparin does abolish the Ca^{2+} wave (DeLisle and Welsh, 1992) and antibodies to phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2], the precursor of Ins(1,4,5)P_3, inhibit sperm activation of the egg (Miyasaki *et al.* 1992; Larabell and Nuccitelli, 1992). Furthermore, only the Ins(1,4,5)P_3 receptor has been purified from *Xenopus laeivs* eggs and neither ryanodine nor caffeine is able to induce Ca^{2+} release (Parys *et al.* 1992). A mechanism of wave propagation based on IICR is likely to operate in these eggs. The Ca^{2+} wave and other aspects of deuterostome egg activation have been thoroughly discussed recently (Berger, 1992; Whitaker and Swann, 1993).

Protostomes

A unifying feature of deuterostome egg activation is its independence of the presence of external Ca^{2+} (Schmidt *et al.* 1982). In contrast, a requirement for external Ca^{2+} is well documented during protostome egg activation, namely in *Urechis caupo, Spisula solidissima* and *Barnea candida*. Egg activation can be prevented in Ca^{2+}-depleted sea water despite sperm penetration (Allen, 1953). As with the sea urchin, the fertilization potential in *Urechis caupo* is preceded by an action potential (Jaffe *et al.* 1979). The initial depolarization phase results from an increased conductance due to gating of Na^+ channels at the point of sperm fusion (Jaffe *et al.* 1979; Gould-Somero, 1981). This is amplified by activation of voltage-gated Ca^{2+} channels, which allow a considerable Ca^{2+} influx into the egg during the first few seconds of the fertilization potential (Jaffe *et al.* 1979; Johnston and Paul, 1977). The prolonged phase of the fertilization potential is due largely to Na^+ influx (Jaffe *et al.* 1979). Protostome eggs can also be activated by depolarization of the plasma membrane with high external $[K^+]$, reflecting the opening of voltage-gated Ca^{2+} channels.

Few direct measurements of $[Ca^{2+}]_i$ during protostome egg activation have been made. Brassard *et al.* (1988) reported increased fluorescence of the Ca^{2+} indicator Quin-2 during artificial activation of *Barnea candida* eggs. A more recent report (Freeman and Ridgeway, 1993) demonstrated increased luminescence of an endogenous aequorin-like Ca^{2+}-activated photoprotein in the cytoplasm of the eggs of the hydrozoans *Phialidium gregarium* and *Mitrocomella polydiademata* during fertilization. The magnitude of the

Ca^{2+} elevation underlying this is unknown. Improved spatiotemporal measurements are necessary to determine the presence or absence of a Ca^{2+} wave. Several Ca^{2+}-dependent events occur as a wave during deuterostome egg activation, e.g. cortical granule exocytosis or cortical reaction (Moser, 1939) and contraction (Brownlee and Dale, 1990), indicating indirectly the presence of the Ca^{2+} wave. However, no such 'fertilization waves' have been reported during protostome egg activation. Cortical granules are present in *Urechis caupo* and surface coat elevation occurs as a slow irregular release taking approximately 20min (Tyler, 1932).

A range of protostome eggs can be activated by A23187 in Ca^{2+}-free sea water (Paul, 1975), demonstrating at least the presence of internal Ca^{2+} stores. Furthermore, PtdIns(4,5)P_2 turnover is increased in *Spisula solidissima* eggs at fertilization, and the injection of Ins(1,4,5)P_3 causes germinal vesicle breakdown and egg activation (Bloom *et al.* 1988).

Fucus serratus

Fucus serratus egg activation is also dependent on the presence of external Ca^{2+}. Eggs will not exhibit fertilization potentials in low (<1.0mmol l^{-1}) Ca^{2+} concentrations. Tracer experiments using $^{45}Ca^{2+}$ reveal a sperm-induced Ca^{2+} influx lasting up to 5min and representing a three- to fourfold increase above pre-fertilization levels (Roberts and Brownlee, 1993). Our unpublished observations demonstrate increased Ca^{2+} influx by monitoring the Mn^{2+} quench of Fura-2 fluorescence (O'Sage *et al.* 1989) in Fura-2–dextran-loaded *Fucus serratus* eggs coincident with the onset of the fertilization potential. The *Fucus serratus* fertilization potential is characterized by a rapid initial depolarization phase, followed by a slower depolarization to values more positive than −20mV and a slow repolarization to pre-fertilization potentials (Brawley, 1991; Taylor and Brownlee, 1993). There is no evidence for an initial action potential. Removal of external Na^+ significantly reduces the initial depolarization during the fertilization potential (Brawley, 1991), while removing external Ca^{2+} immediately after the onset of the fertilization potential diminishes the magnitude of the subsequent depolarization and inhibits egg activation (S. K. Roberts and C. Brownlee, unpublished observations). This contrasts with *Urechis caupo*, where removal of Ca^{2+} did not affect the prolonged depolarization phase of the fertilization potential (Jaffe *et al.* 1979). The kinetics and activation threshold of the Ca^{2+} channels in the *Fucus serratus* plasma membrane are consistent with their having a role in carrying Ca^{2+} into the egg during the fertilization potential (Taylor and Brownlee, 1993; see above). Though increased Ca^{2+} influx occurs during *Fucus serratus* egg activation, photometric measurements of Ca^{2+} in Fura-2–dextran-loaded eggs have so far failed to show any explosive increases in $[Ca^{2+}]_i$ (Roberts and Brownlee, 1993). In a proportion of the eggs studied (approximately 30%), relatively small whole-egg $[Ca^{2+}]_i$ elevations to around 300nmol l^{-1} (from 100–200nmol l^{-1} resting $[Ca^{2+}]_i$) were observed, coincident with the fertilization potential, while in others no elevations were detected (Roberts and Brownlee, 1993). Parallel studies using confocal laser scanning microscopy never showed a fertilization-associated Ca^{2+} wave but rather small increases in $[Ca^{2+}]_i$, possibly originating from the cortical region, consistent in magnitude with those measured with Fura-2–dextran.

Injection of appropriate amounts of Ca^{2+} buffers (BAPTA and Br_2BAPTA) completely inhibit any fertilization-associated whole-egg $[Ca^{2+}]_i$ elevation while allowing normal egg activation and subsequent zygote development (Roberts and Brownlee, 1993). However, injection of higher concentrations of Ca^{2+} buffer (>1mmol l^{-1} $[Br_2BAPTA]_i$ and >3–4mmol l^{-1} $[BAPTA]_i$) did inhibit egg activation, suggesting a role for elevated $[Ca^{2+}]_i$, at least in the early stages of egg activation. Furthermore, Br_2BAPTA is a more effective buffer than BAPTA (K_d values of 3.6 μmol l^{-1} and 0.7 μmol l^{-1} respectively; Speksnijder *et al.* 1989*b*) in preventing egg activation, including cell wall exocytosis. Assuming that a Ca^{2+} buffer will be most efficient at buffering $[Ca^{2+}]_i$ close to its K_d value, a $[Ca^{2+}]_i$ elevation to micromolar levels would appear to be necessary for *Fucus serratus* egg activation. The failure to detect such an elevation using photometry can be explained if the elevation is localized and so averaged out in whole-egg $[Ca^{2+}]_i$ measurements. These results are consistent with the occurrence of a localized $[Ca^{2+}]_i$ elevation just beneath the plasma membrane which mediates fertilization-associated cell wall secretion.

Three possible models for the role of Ca^{2+} in the propagation of the sperm signal to the rest of the egg in *Fucus serratus* are presented in Fig. 2. Signal transduction *via* a Ca^{2+} wave passing through the egg causing significant elevation of $[Ca^{2+}]_i$, either through CICR or through a more complex Ca^{2+} release pathway (Fig. 2A) can be largely discounted since large elevations in $[Ca^{2+}]_i$ have not been observed during *Fucus serratus* egg activation. Furthermore, buffering global Ca^{2+} transients does not necessarily prevent egg activation. Egg activation resulting from simple Ca^{2+} influx through voltage-regulated channels (the protostome model) (Fig. 2B) could explain the small global $[Ca^{2+}]$ elevations, if these occurred locally, and would also explain the lack of effects of BAPTA buffers if the influx were sufficient to overcome locally the capacity of the buffer. In this case, the initial depolarization induced by the sperm would propagate rapidly around the egg during the initial depolarization phase of the fertilization potential. However, this model does not explain the inability to activate *Fucus serratus* eggs by simple depolarization. Therefore, an additional signal is required which either prevents spontaneous inactivation of the Ca^{2+} channels during fertilization or augments the Ca^{2+} influx. This additional signal would need to propagate around the egg, at least beneath the plasma-membrane. Possibilities include a plasma-membrane-located biochemical cascade, such as a protein kinase cascade, or a highly localized CICR or IICR mechanism (Fig. 2C) which could act together with Ca^{2+} influx to stimulate exocytosis. This would depend on the localization of releasable Ca^{2+} stores close to the plasma membrane. Indeed, smooth endoplasmic reticulum does occur in close proximity to the plasma membrane (Brawley *et al.* 1976*b*), though it is not clear whether its distribution is restricted to this region.

Polarization

Fucus serratus egg polarity is eventually determined by the environment, though it is not known whether the fertilizing sperm sets an initial axis. It has been proposed that the site of sperm entry determines an initial labile axis subsequently over-ridden by

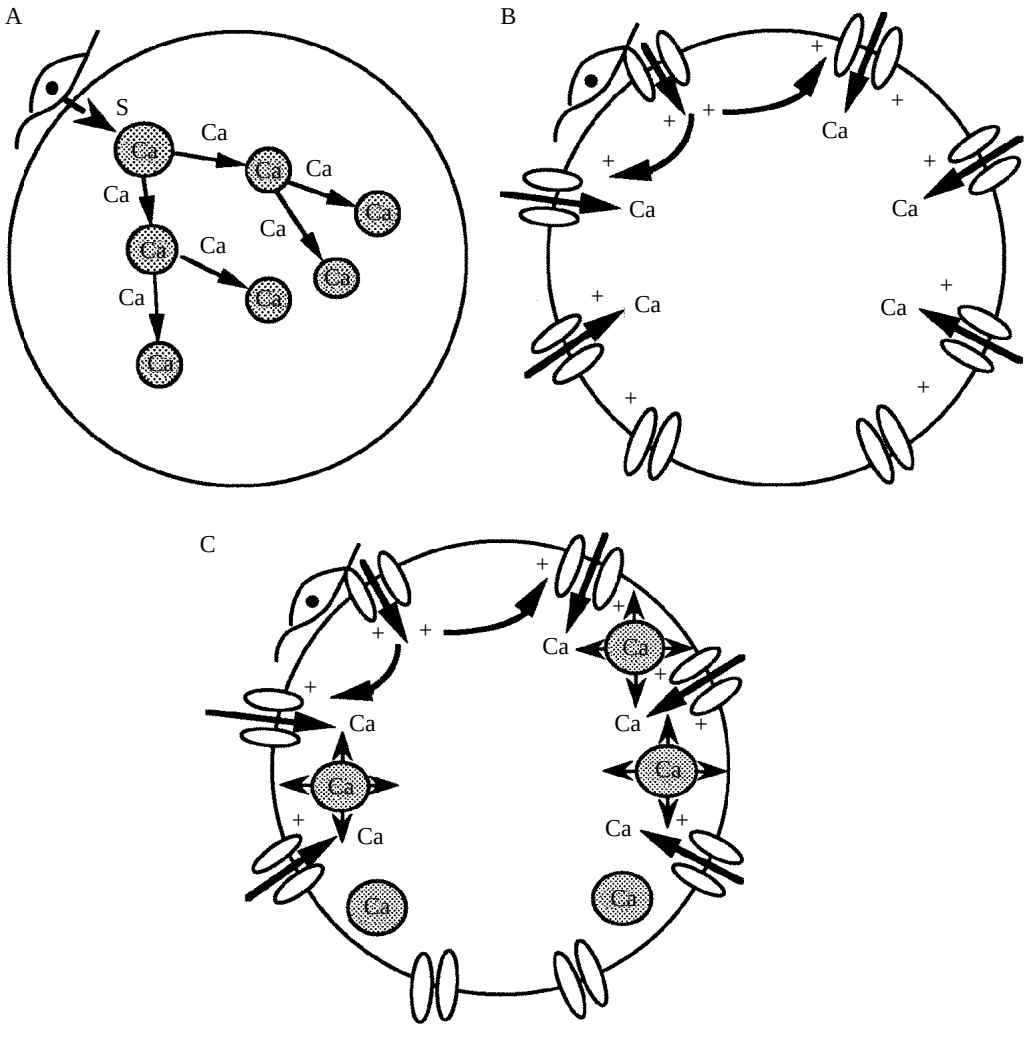

Fig. 2. Hypothetical models to explain the transduction of the sperm signal (S) throughout the egg during fertilization in *Fucus serratus.* (A) Propagation of a Ca^{2+} wave *via* intracellular release throughout the egg. (B) Propagation *via* depolarization and Ca^{2+} influx. (C) Propagation *via* influx and Ca^{2+} release from intracellular stores located just beneath the plasma membrane. See text for discussion.

environmental gradients (Knapp, 1931). Indeed this site is marked transiently by the male pronucleus surrounded by a high density of mitochondria (Brawley *et al.* 1976*a*). According to the speed of male pronuclear migration ($0.2 mm min^{-1}$) (Swope and Kropf, 1993), this asymmetry would last for the first 4h in the zygote. However, strong arguments for the existence of an internal axis remain elusive and experimental difficulties arise because many vectors polarize the zygote and the site of sperm entry is difficult to observe. However, zygotes subjected to polarized light germinate two rhizoids 130–160˚ from each other (Jaffe, 1958), i.e. they establish two axes. It is difficult to

Fig. 3. Ratio confocal images of cytosolic [Ca^{2+}] in germinating *Fucus serratus* zygotes. Zygotes were loaded with a mixture of the Ca^{2+} indicator Calcium Green and SNARF, both conjugated to 50000 M_r dextran. The Ca^{2+}-dependent signal of Calcium Green was divided by the Ca^{2+}-insensitive SNARF fluorescence at the SNARF pH isosbestic emission wavelength (600nm). (For details see Berger and Brownlee, 1993.) (A) [Ca^{2+}] elevation at the growing rhizoid apex (red represents high [Ca^{2+}], blue represents low [Ca^{2+}]). (B) The same zygote after microinjection with Br_2BAPTA, showing the absence of the [Ca^{2+}] elevation at the rhizoid apex. (C) Control zygote, microinjected with FITC–dextran (Ca^{2+}-insensitive) and SNARF. FITC–dextran fluorescence, monitored at the same wavelengths as Calcium Green in A and B was divided by the SNARF fluorescence. This ratio image shows no gradients.

interpret this in terms of a single preformed axis which would be reorientated in two nearly opposite directions at once (Weisenseel *et al.* 1979). The cell biology of polarization in fucoid algae has recently been reviewed in detail (Kropf, 1992). An as yet unresolved argument concerns the role of Ca^{2+} in polar axis fixation and rhizoid germination. Hurst and Kropf (1991) showed that K^+ was the only external ion essential for polar axis fixation. In contrast, Speksnijder *et al.* (1989*b*) showed that Ca^{2+} buffer injections could prevent zygote development by facilitating Ca^{2+} diffusion and abolishing localized Ca^{2+} gradients, presumably including the high [Ca^{2+}] at the growing rhizoid apex. Our own measurements of cytosolic [Ca^{2+}] in germinating *Fucus serratus* zygotes do indeed show that injection of Br_2BAPTA can abolish the locally elevated [Ca^{2+}] at the growing rhizoid apex (Fig. 3). The experiments of Speksnijder *et al.* (1989*b*), while demonstrating an essential role for a [Ca^{2+}] gradient during rhizoid germination, did not directly test whether buffer-treated zygotes had fixed polar axes. Monitoring the pattern of $[Ca^{2+}]_i$ in polarizing zygotes by a ratiometric method using confocal microspcopy has also shown that an early $[Ca^{2+}]_i$ gradient marks the future site of rhizoid germination (Berger and Brownlee, 1993). This $[Ca^{2+}]_i$ gradient is amplified during germination and maintained at the tip of growing rhizoids (Fig. 3). It has been proposed that the gradient originates from spatial regulation of membrane Ca^{2+} conductances (Taylor *et al.* 1992). To investigate this hypothesis, a laser microsurgery method has been designed to remove small regions of the zygote cell wall (Taylor and Brownlee, 1993). This allows access to an undisturbed membrane area for a patch-clamp electrode. Future experiments using this technique will be aimed at characterizing further the role of plasma membrane ion channels in the polarization of *Fucus serratus* zygotes.

Cell cycle activation

Fucus serratus eggs are probably arrested in phase G1 of the cell cycle, following the meiotic and mitotic divisions of the primary oocyte (McCully, 1968; Callow *et al.* 1985). In sea urchins, a Ca^{2+} transient is thought to trigger cell cycle re-initiation following fertilization by targetting cell cycle control proteins (Whitaker and Patel, 1990). In *Fucus serratus*, the absence of a large global fertilization-associated Ca^{2+} transient leaves open the question of what triggers the cell cycle. A Ca^{2+}-independent process may be responsible, for example MAP kinases (mitogen-activated protein kinases) which play a role in signal transduction in animal cells. These are activated by epidermal growth factor

(EGF) and nerve growth factor (NGF) and are coupled by receptors belonging to the tyrosine kinase family. Homologues in budding yeast are part of the pathway inducing a differentiation programme involved in mating (see Sprague, 1992, for a recent review). A Ca^{2+}-dependent protein kinase C is thought to regulate egg activation events in *Xenopus laevis* (Bement, 1992) and hamster (Gallicano *et al.* 1993). In sea urchin, an increase in cytosolic pH plays a role (in conjunction with $[Ca^{2+}]_i$) in the activation of protein synthesis (Winkler *et al*. 1980). In *Xenopus laevis,* a fertilization-induced cytosolic alkalization closely follows M-phase promoting factor (MPF) inactivation (Charbonneau and Grandin, 1992). In protostome eggs, fertilization-induced changes in cytosolic pH are inferred from acid release by the egg following activation (Paul, 1975). An amiloride-sensitive Na^+/H^+ exchanger activity is present in *Fucus serratus* zygotes (Gibbon and Kropf, 1993) and intracellular pH has been measured with pH-sensitive microelectrodes. After fertilization, a very slow and small cytosolic acidification (from 7.5–7.35 at 3h) has been detected (Gibbon and Kropf, 1993). So far there is no evidence for any early pH transients following fertilization.

The absence of any large global Ca^{2+} transient that may be related to reinitiation of the cell cycle may simply reflect the likelihood that the cell cycle may not be activated immediately following fertilization. In *Fucus serratus*, cell division is not observed until approximately 16h post-fertilization. A long-delayed first cell division may reflect the requirement to register and respond to the prevailing light direction in order to maximize the probability of survival. Data on the precise timing of phases of the cell cycle in *Fucus serratus* are urgently required before progress can be made towards understanding the signal transduction processes involved.

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