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CALCIUM SIGNALLING AT FERTILIZATION

KARL SWANN*, ALEX MCDUGALL[†] AND MICHAEL WHITAKER[†]

*MRC Experimental Embryology and Teratology Unit, St George's Hospital Medical School, Cranmer Terrace, London, SW17 0RE. [†]Department of Physiology, University College London, Gower Street, London, WC1E 6BT (address correspondence to M. Whitaker).

It is generally agreed that fertilization in deuterostomes is accompanied by a large intracellular calcium wave that triggers the onset of development, but we still do not know exactly how the calcium wave is generated. The question has two parts: how does interaction of sperm and egg initiate the calcium wave, and how does the calcium wave spread across the cell? Two provisional answers are available to the first part of the question, one involving receptor-G-protein interactions of the sort that mediate transmembrane signal transduction in somatic cells, the other injection of an activating messenger when sperm and egg fuse. Both these ideas are being actively pursued; the dialectic is productive, albeit no synthesis is in sight. We discuss their strengths and weaknesses. The second part of the question can now be much more precisely formulated: thanks to the recent flush of interest in calcium waves in somatic cells, new ideas and new experimental tools are available. The work on somatic cells repays a debt to eggs, where the basic properties of calcium waves were first set out, ten years before they turned up in somatic cells.

INTRODUCTION

In deuterostomes, the fertilizing sperm triggers a calcium increase within the egg that travels as a wave from the point of sperm-egg interaction to its antipode (Jaffe, 1980; 1983). The calcium wave activates the egg and is necessary and sufficient as a signal to trigger the onset of development (Steinhardt *et al.*, 1974; Lorca *et al.*, 1991; see Whitaker & Steinhardt, 1982, and Whitaker & Patel, 1990, for reviews). But we should not forget the sperm. The incorporation of the sperm nucleus into the egg cytoplasm is an essential element of syngamy and maintenance of diploidy. What we shall discuss here is the link between egg activation and sperm egg fusion. The link is a strong one: except in very special circumstances (Longo, 1978; Chambers, 1989) an egg is never activated after insemination without at the same time incorporating a sperm. How is the link forged?

Looked at from the sperm's point of view, there are two obvious strategies for gaining entry. The well-bred sperm might knock on the door and wait for the egg to respond. A less delicate strategy is to barge in without bothering to ring. The first of these strategies finds a physical realization in the idea of G-protein-mediated transmembrane signalling. On this hypothesis (Jaffe *et al.*, 1988), the sperm interacts with a G-protein-linked membrane receptor to generate an intracellular message that triggers the calcium wave, fusion of the sperm and egg plasma membranes, and sperm incorporation. It waits for

an answer. The less genteel strategy corresponds to an older idea from rougher times (Jaffe, 1983), where, without waiting for a by-your-leave, the sperm fuses with the egg, so injecting an activating messenger that sets off the calcium wave. These two usefully-contrasting ideas form the basis for a discussion of the cellular messengers and mechanisms that generate the fertilization calcium wave. We shall discuss only data from frog, mouse, hamster and sea urchin eggs.

KNOCK AND THE DOOR WILL BE OPENED

The idea that a G-protein signal transduction system might operate at fertilization had its beginnings in the discovery of a polyphosphoinositide (PPI) messenger system in eggs (Turner *et al.*, 1984; Whitaker & Irvine, 1984) and grew alongside the discoveries that GTP binding proteins linked receptor proteins to generation of the PPI messengers inositol trisphosphate (InsP₃) and diacylglycerol (DAG).

PPI messengers at fertilization

There is a marked increase in the turnover of the PPI lipids at fertilization in sea urchin eggs (Turner *et al.*, 1984; Ciapa *et al.*, 1992), the hallmark of activation of the PPI signalling system. Both InsP₃ and DAG are produced during the fertilization calcium transient or after activation of eggs with calcium ionophore (Ciapa & Whitaker, 1986; Ciapa *et al.*, 1992) and sea urchin eggs have a calcium-dependent phosphoinositidase C that may be responsible for this (Whitaker & Aitchison, 1985). Micro-injecting InsP₃ triggers the calcium wave and activates sea urchin (Whitaker & Irvine, 1984), frog (Busa *et al.*, 1985) mouse (Kurasawa *et al.*, 1989; Kline & Kline, 1992; Swann, 1992) and hamster eggs (Miyazaki, 1988; Cran *et al.*, 1988). In sea urchins, the calcium wave is independent of external calcium (Crossley *et al.*, 1988), implying release of calcium from an internal store. Indeed, sea urchin homogenates have a non-mitochondrial InsP₃-sensitive calcium sequestration system (Clapper *et al.*, 1987) that has its counterpart in the endoplasmic reticulum of the intact egg (Henson *et al.*, 1989, 1990; Terasaki *et al.*, 1991; Terasaki & Sardet, 1991). A similar set of results is obtained from frog egg homogenates (Galione *et al.*, 1992).

Sea urchin eggs, then, have a functioning PPI messenger system at fertilization. Some or all of the InsP₃ and DAG produced at fertilization is a consequence of the calcium increase, not a cause. Data from frog and hamster eggs suggests that they are analogous, but is not extensive: no measurements of PPI turnover have been made. How comfortably can we sustain the view that InsP₃ triggers or facilitates the calcium wave at fertilization? With ease in hamster and frog eggs, where blocking the InsP₃ receptor [InsP₃R] (Miyazaki *et al.*, 1992; Galione *et al.*, 1992) prevents the intracellular calcium wave. Less happily in sea urchin eggs, where the InsP₃R antagonist, heparin, fails to block the calcium wave (Rakow & Shen, 1990; Crossley *et al.*, 1991; Galione *et al.*, 1993; Lee *et al.*, 1993). We shall return to this; differences between eggs that have calcium waves with apparently identical properties (Jaffe, 1983) is one of the themes of this survey.

The sperm, the receptor and G proteins

The receptor proteins that mediate sperm-egg binding in sea urchin and guinea pig have been characterized (Foltz & Lennarz, 1990, 1992; Blobel *et al.*, 1992); for guinea pig, primary sequence data are available and the receptor shows homology to integrin receptors. Is there a link with signal transduction? Integrin receptor occupancy has been shown to lead to the generation of calcium signals (Jaconi *et al.*, 1991), perhaps by stimulating tyrosine kinase activity (Kornberg *et al.*, 1991), and tyrosine kinase activity can trigger PPI turnover *via* phospholipase C γ (Rhee, 1991). Note, though, that integrin receptors do not interact, as far as is known, with the multimeric G proteins. The sequence data for the sea urchin sperm-receptor protein shows no obvious homology with any signal-transducing receptor (Foltz *et al.*, 1993), but activation of a tyrosine kinase precedes the fertilization calcium transient (Ciapa *et al.*, 1991), a finding that at least raises the possibility of tyrosine-kinase-linked signal transduction. The idea of a G-protein-linked signal transduction system at fertilization does not gain much support from what is known about the receptor side of things. Its strengths lie in another quarter.

Trimeric GTP binding proteins are activated by guanosine 5'-triphosphothioate (GTP γ S) and so are sea urchin (Turner *et al.*, 1986), hamster (Miyazaki, 1988) and frog eggs (Kline *et al.*, 1991). Proteins ribosylated by cholera and pertussis toxins (toxins with G-protein targets) can be identified in sea urchin egg plasma membrane (Turner *et al.*, 1987). Cholera toxin activates sea urchin eggs (Turner *et al.*, 1987) but pertussis toxin does not inhibit fertilization in sea urchin (Turner *et al.*, 1987), frog (Kline *et al.*, 1991), starfish (Shilling *et al.*, 1989) or hamster (Miyazaki, 1988). The initial successes with GTP γ S encouraged the elegant approach of injecting brain mRNA-derived cDNA, and cDNA coding for the G-protein-linked 5-HT receptor, into oocytes (Kline *et al.*, 1988; Shilling *et al.*, 1990). When the oocytes matured, they had incorporated the receptors themselves into the egg membrane. Adding 5-HT triggered a fertilization-like calcium transient and egg activation. In mammalian eggs, there is no need for experiments with receptor cDNA: there are G-protein-linked 5-HT receptors in the hamster (Miyazaki *et al.*, 1990) and G-protein-linked acetylcholine receptors in the mouse (Swann, 1992) that generate calcium oscillations. This latter fact did not discourage Williams *et al.* (1992) from introducing mRNA coding for the ACh receptor into mouse oocytes to show that ACh application would activate eggs. These and the GTP γ S experiments amply demonstrate that eggs have a G-protein-linked PPI messenger system, a pre-requisite if the G-protein mechanism is to operate at fertilization. The G proteins are sufficient to evoke a fertilization-like calcium transient, but are they necessary?

Proving that G-proteins are necessary at fertilization has been more difficult. One tool used has been GDP β S, an inhibitory guanine nucleotide analogue. GDP β S was originally reported to block elevation of the fertilization envelope in sea urchin egg after insemination (Turner *et al.*, 1986), a result taken to imply that G proteins were necessary for the fertilization response. Subsequently it was shown that GDP β S blocked only envelope elevation, not the fertilization calcium transient (Crossley *et al.*, 1991); the latter outcome turns the meaning of the experiment on its head, indicating that G

proteins do not mediate the fertilization response. Recall that the fertilization calcium transient in sea urchin eggs is not blocked by the InsP_3R antagonist heparin; the $\text{GTP}\gamma\text{S}$ -induced calcium transient *is* (Crossley *et al.*, 1991). The simplest interpretation of these data is that while $\text{GTP}\gamma\text{S}$ triggers a calcium transient by generating InsP_3 , the sperm does not. The G-protein idea also predicts that production of InsP_3 and DAG is the result of receptor occupancy. However, if sea urchin eggs are inseminated after micro-injecting the calcium chelator BAPTA, no DAG production is detectable (Crossley *et al.*, 1991), apparently indicating that the production of the PPI messengers is the result, rather than the cause of the calcium increase. All the experiments designed to test whether G proteins are essential for signal transduction at fertilization in sea urchin eggs have so far come up negative. In the hamster egg, things are a little better.

In hamster eggs, micro-injecting $\text{GDP}\beta\text{S}$ has been shown to block the calcium response at fertilization, without affecting InsP_3 -induced calcium release from the egg's internal store (Miyazaki, 1988). Thus, there is evidence that, in hamster eggs, G proteins may be necessary for fertilization signal transduction. This is the only piece of evidence in any egg that points to an essential role for G proteins at fertilization. Even for hamster eggs, this pure-seeming proof is not unalloyed, as the following experiments demonstrate. Treating hamster eggs with phorbol ester mimics the natural down regulation of the receptor-G-protein response (in this it differs from the fertilization response, which does not down regulate: Swann *et al.*, 1989; Miyazaki *et al.*, 1990) and inhibits the series of calcium transients that usually follow injection of $\text{GTP}\gamma\text{S}$ (Swann *et al.*, 1989); the fertilization calcium transients are unaffected. The sperm is clearly not behaving like $\text{GTP}\gamma\text{S}$. If these latter experiments are accepted as evidence against the G-protein idea, then the inhibition of fertilization by $\text{GDP}\beta\text{S}$ has to be explained away. In frog oocytes, $\text{GDP}\beta\text{S}$ blocks all but the first of a series of calcium transients that can be generated by InsP_3 (Sealfon *et al.*, 1990), perhaps because G proteins are involved in the translocation of calcium from one internal store to another (Ghosh *et al.*, 1989). This is one way to explain inhibition of fertilization by $\text{GDP}\beta\text{S}$, but it is not a very compelling argument. We are left with a clear contradiction between the two sets of experiments in hamster eggs.

To sum up for G proteins: there is abundant evidence for a G-protein-linked PPI messenger system in eggs that, when stimulated, will bring about egg activation; it is much less certain that the mechanism necessarily operates at fertilization. The worst case is the sea urchin egg, where neither $\text{GDP}\beta\text{S}$ nor heparin blocks the fertilization calcium wave. The best case is the hamster egg where blocking either G proteins or the InsP_3R can prevent the fertilization calcium transient. But even in hamster, the fact that the response to $\text{GTP}\gamma\text{S}$ can be down regulated without affecting the fertilization response should make us cautious about swallowing the bait whole.

Opening the door

How do we explain sperm-egg fusion and sperm incorporation if the sperm first interacts with a G-protein-linked transmembrane receptor? Sperm-egg fusion has not received much attention in the context of the G-protein model. Our simple analogy

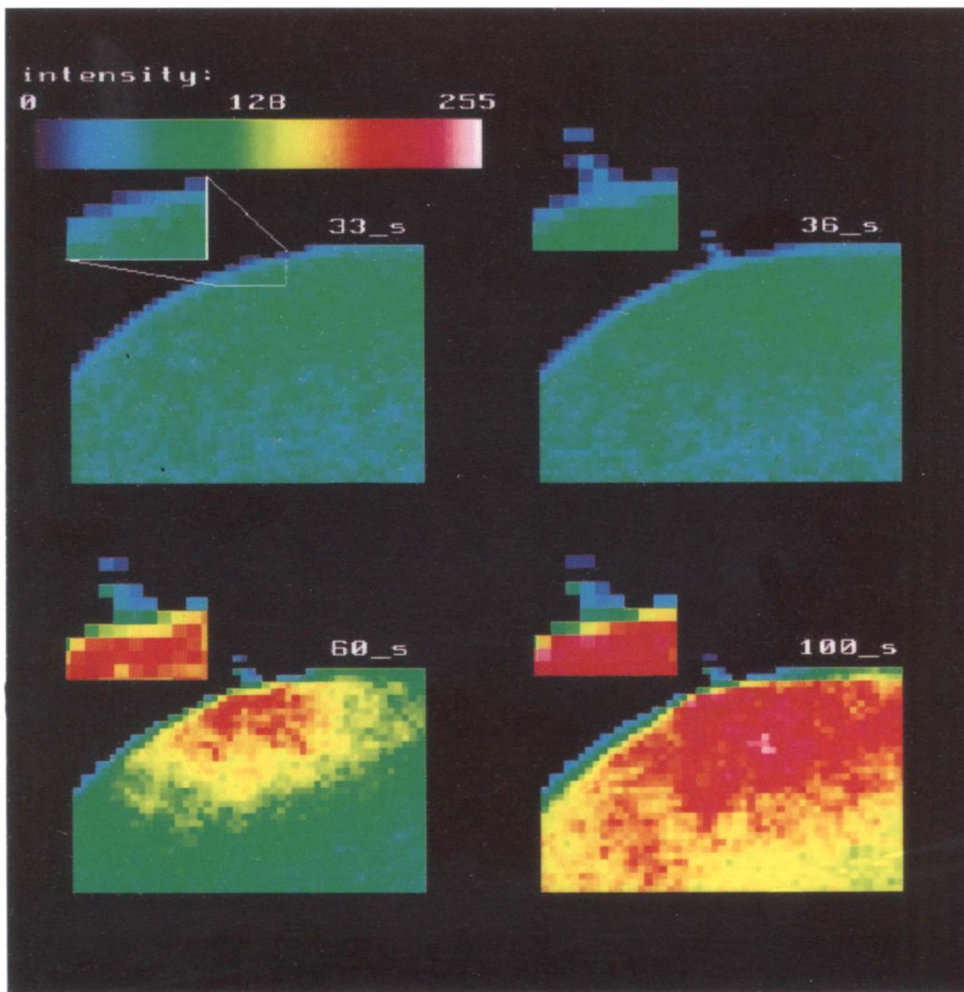


Figure 1. Sperm-egg fusion in the sea urchin at fertilization. Confocal image of changes in the cytoplasmic calcium concentration during fertilization in sea urchin eggs in equatorial section using the fluorescent indicator dye calcium green. Cold tones represent low intensities and warm tones high intensities. In the 3-s interval between the first and second images, dye has begun to diffuse from the egg into the sperm as cytoplasmic continuity is established. The third and fourth images show the initiation of the calcium wave. Pixels are 1 μm square.

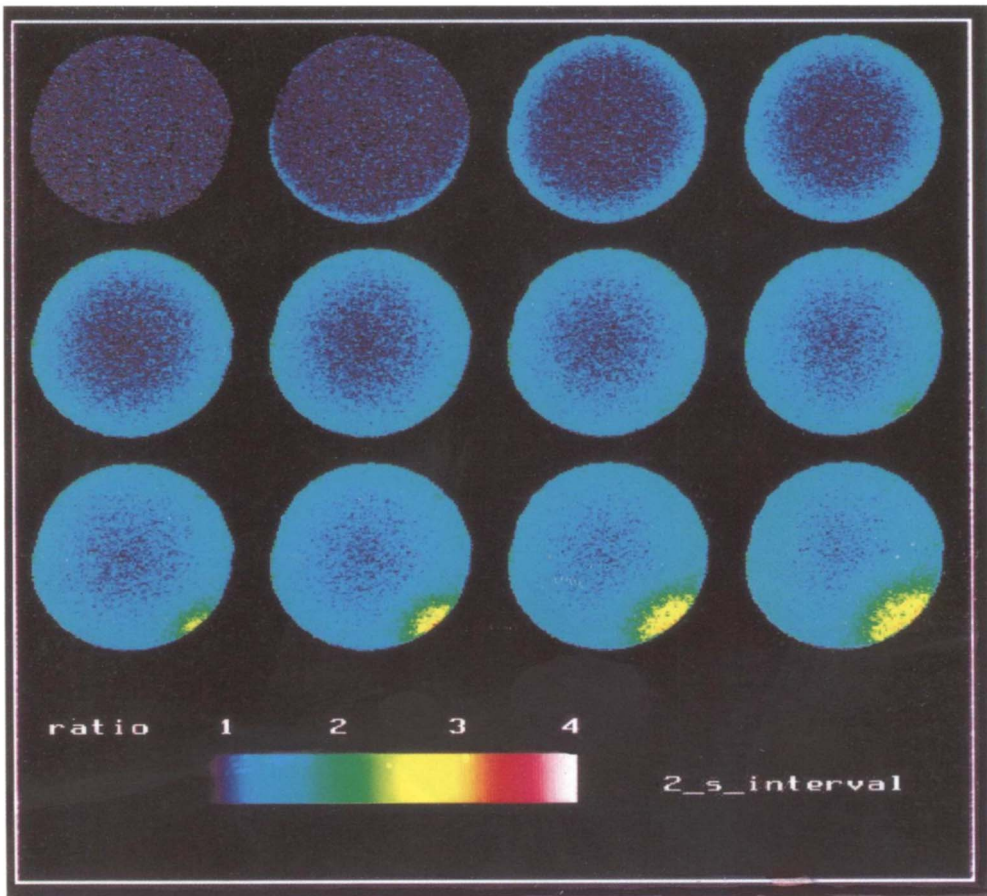


Figure 3. The latency of the fertilization response in the egg of the sea urchin, *Lytechinus pictus* (16°C). Confocal ratio image of Ca_i in the sea urchin egg at fertilization. Cold tones represent low Ca_i and warm tones high Ca_i . The first detectable response at fertilization is seen at the egg periphery. The response is rapid and occurs within tens of milliseconds: the smile visible in the second image is an artefact due to influx beginning half-way through the confocal scan cycle. Each scan lasted 1 s; there is a 1-s interval between scans. The influx occurs through voltage-gated calcium channels: it is sensitive to removal of external calcium and to calcium-channel blockers. It is due to the calcium action potential triggered by the sperm-induced depolarizing current that marks the beginning of the latent period (Whitaker *et al.*, 1989). The calcium wave begins in the eighth image, 12 s after the initial sperm-egg interaction. This 12 s is the latent period. Ratio images were obtained by dividing each image, pixel by pixel, by a control image obtained at the beginning of the experiment, to give a signal independent of dye concentration.

suggests that sperm-egg fusion is triggered when the egg answers the sperm by raising its intracellular calcium concentration. Figures 1 and 2 show the way in which a calcium indicator dye signal changes at fertilization in the region of sperm-egg fusion. Diffusion of the dye into the sperm from the egg cytoplasm *precedes* the calcium increase in the egg. A further increase in the dye signal may reflect a calcium increase in the sperm; this occurs *after* the calcium increase in the egg. This single observation is not consistent with the view that sperm-egg fusion is triggered by the egg's calcium transient. Nor does it support the view that calcium from the sperm sets off the egg's calcium wave (Jaffe, 1983, 1990), an idea known as the calcium bomb hypothesis. Also ranged against the idea of a calcium bomb are the observations that sperm incorporation is *facilitated* in sea urchin eggs when changes in calcium within the egg are prevented by injection of calcium chelators (Swann *et al.*, 1992) or calcium channel blockers (McCulloh *et al.*, 1989, 1990).

Despite the apparent shortcomings of the calcium bomb idea, sperm-egg fusion itself is more easily situated in the older (Loeb, 1913) model of fertilization: it must precede egg activation, since activation is said to be due to the diffusion of an activating messenger from sperm to egg through the cytoplasmic channel formed by the fusion of sperm and egg. Fusion must be a natural consequence of the binding of the sperm to its receptor, the receptor now being seen as analogous to cell adhesion receptors like the integrins, not to signalling receptors like those for 5-HT. The idea lacks strong support,

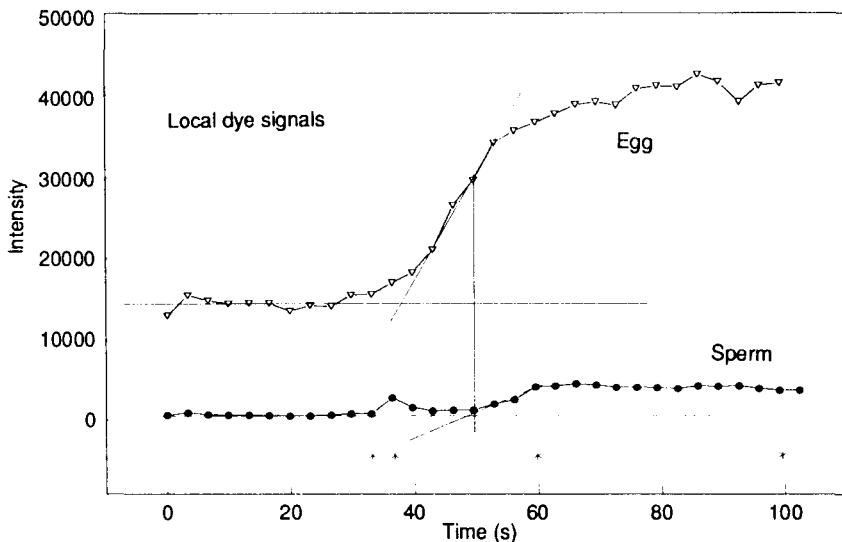


Figure 2. Sperm-egg fusion in the egg of the sea urchin, *Lytechinus pictus* (16°C) at fertilization. The intensity signal from the eight pixels corresponding to the dye signal (Figure 1) from the sperm is compared with the signal from eight pixels just beneath the plasma membrane of the egg at the sperm entry site. The asterisks indicate the four images shown in Figure 1. The intensity signal measures both dye concentration and calcium concentration, though not independently. The comparison shows that dye transfer begins and ends before calcium starts to increase in the egg. There is a second increase in signal in the sperm that may correspond to a calcium increase in the sperm cytoplasm triggered by the increase in the egg. The latency of the fertilization response was artificially increased in this experiment by co-injecting heparin (300 $\mu\text{g ml}^{-1}$) with the calcium green (10 μM). Elapsed time from the beginning of data acquisition, some 2 min after insemination at very low sperm density ($<10^9 \text{ ml}^{-1}$), is shown. For methods see Galione *et al.* (1992).

but it is not far-fetched: the guinea pig sperm-receptor protein contains a viral fusion peptide motif (Blobel *et al.*, 1992) and bindin, the sperm acrosomal protein that binds to the sea urchin egg receptor, is fusogenic (Glabe, 1985a,b; Glabe *et al.*, 1991; Lopez *et al.*, 1993). The difficulties with the G-protein idea, particularly as applied to sea urchin eggs, have stimulated a re-investigation of the older model, with interesting and useful results.

BREAKING AND ENTERING

If indeed the sperm puts its shoulder to the door at fertilization without waiting for an answer from the egg, then sperm-egg fusion must precede the calcium wave. There is considerable latitude here, because the time between sperm-egg interaction and the onset of the calcium transient can be as long as seven minutes in mouse eggs (Jaffe *et al.*, 1983), though in hamster eggs it is only a few seconds (Miyazaki & Igusa, 1981). In mammals the length of this latent period is a property of the sperm: mouse sperm give rise to a seven minute latency when fertilizing hamster eggs (Igusa *et al.*, 1983). The latency of the fertilization response has received most attention in sea urchin eggs, in part because it attracted early interest (Allen & Griffen, 1958), but largely because the initial event at fertilization that marks the beginning of the latent period is easily recorded with precision in sea urchin eggs.

The first detectable event at fertilization in sea urchin eggs is an inward current with a very rapid onset (Dale *et al.*, 1978). It precedes the fertilization calcium wave by 15-20 s and marks the moment of productive sperm-egg interaction, corresponding to the time at which fertilization can no longer be prevented by addition of spermicides (Whitaker *et al.*, 1989), and to the point at which the electrical block to polyspermy (Jaffe, 1976) operates (Shen & Steinhardt, 1984). There is a gap between sperm-egg interaction and egg activation. Allen & Griffin (1958) first identified it as the 10-15 s period between the time that sperm cease to be susceptible to a spermicide like KCl and the time at which egg activation (FE elevation) occurs. They called it the latent period. The time that elapses between the current step and the calcium transient (Figure 3) is identical to the latent period defined by Allen & Griffin (1958) and by other criteria (Whitaker *et al.*, 1989; Swann *et al.*, 1992). In an interesting series of technically-demanding experiments, Chambers and his colleagues estimated the time that elapsed between the current step and sperm-egg fusion. The initial approach was to fix single voltage-clamped sea urchin eggs chemically at various times after the current step was recorded. Once the eggs were fixed, fusion was assayed by monitoring dye diffusion from egg to sperm (Hinckley *et al.*, 1986), or, even more remarkable, by taking serial thin sections of a single egg for analysis in the electron microscope (Longo *et al.*, 1990). Cytoplasmic continuity (dye diffusion) is detected 4-8 s after the current step; fusion, as defined by continuity of gamete plasma membranes observed in the electron microscope, occurs 5 s or more after the step. That is, fusion clearly occurs before the onset of the calcium wave.

A later, more precise, approach dispensed with chemical fixation entirely and monitored fusion by measuring the electrical capacitance change that occurs in the egg

membrane when cytoplasmic continuity is established between the gametes (McCulloh & Chambers, 1992). The increase in membrane capacitance that signals fusion occurs *at the same time* as the current step, with a very rapid onset. Sperm-egg fusion, then, is fertilization, in the sense that it occurs at precisely the time that spermicides cease to be effective and at which the electrical polyspermy block operates.

ONCE INSIDE

Cytoplasmic messengers

A sperm using the muscular method to gain entry by fusion with an egg must then activate it. There is no consensus about what the activating agent may be, except that it must be present in the sperm as it fuses with the egg (Jaffe, 1983, 1990; Iwasa *et al.*, 1990; Whalley *et al.*, 1992). Calcium (Schackmann, 1989), InsP_3 (Iwasa *et al.*, 1990) and cGMP (Kopf *et al.*, 1979) all increase markedly in sea urchin sperm during the acrosome reaction just prior to fusion. Microinjection of InsP_3 (Whitaker & Irvine, 1984) and cGMP (Whalley *et al.*, 1992) will activate the calcium wave in sea urchin eggs. Injection of calcium will not (Swann & Whitaker, 1986). The most potent activating agent of this sort is cyclic ADP-ribose (cADPr; Clapper *et al.*, 1987; Lee *et al.*, 1989; Galione *et al.*, 1991; Galione, 1992). It is not yet known whether it is present in sperm. cADPr has the advantage in potency: it is not clear if there is enough InsP_3 or cGMP in sperm to trigger the calcium wave (Whitaker & Swann, 1993). Remember also that the InsP_3 antagonist heparin does not block fertilization in the sea urchin (Crossley *et al.*, 1991). For the sea urchin sperm, cADPr looks the most likely, but perhaps only because least is known about it.

Hamster and frog eggs produce perfect regenerative calcium waves in response to calcium microinjection (Igusa & Miyazaki, 1983; Busa & Nuccitelli, 1985; Jaffe, 1990), showing classical calcium-induced calcium release (CICR: Endo, 1977; Fabiato, 1985). In these eggs, fertilization is blocked by InsP_3 antagonists (Miyazaki *et al.*, 1992; Galione *et al.*, 1992). Calcium is elevated in mammalian sperm at fertilization, as is InsP_3 (Florman *et al.*, 1989); a case for either or both as the activating messenger clearly exists in frog or hamster, where, at least for the latter egg, neither cGMP nor cADPr is effective (Swann, unpublished observations). But mammalian eggs offer a new idea: the suggestion that the activating messenger at fertilization is a protein (Swann & Whitaker, 1990; Swann, 1990).

The protein factor is isolated from sperm cytosol and triggers the repetitive and persistent calcium oscillations that are a peculiar feature of fertilization in hamster eggs (Miyazaki *et al.*, 1986, 1993). This is interesting, because none of the other agents that elicit calcium responses in the hamster egg (calcium itself, InsP_3 , $\text{GTP}\gamma\text{S}$) reproduce the longevity of the response seen at fertilization and with the protein factor. The factor is unique, too, among the cytoplasmic messengers in mimicking the sensitization of CICR (Igusa & Miyazaki, 1983) which accompanies fertilization (Swann, 1990; Swann, 1991; Whitaker & Swann, 1993).

Which, if any, of these cytoplasmic messengers is responsible for triggering the

fertilization calcium response is one question that awaits an answer. The other question is how the calcium wave, once begun, spreads across the egg.

Calcium waves

Calcium waves are highly explosive, both in eggs (Jaffe, 1980) and in somatic cells (Berridge & Galione, 1988; Jacob, 1990). The positive feedback that precipitates the explosion appears to be an intrinsic property of the calcium release channels that rapidly dump calcium from its membrane store into the cytoplasm. These channels undergo calcium-induced calcium release (CICR). The classical CICR of muscle (Endo, 1977) is mediated by the so-called ryanodine receptor (RYR) (Lai *et al.*, 1988; Takeshima *et al.*, 1989). A novel form of CICR (Finch *et al.*, 1991; Bezprozvanny *et al.*, 1991; Missiaen *et al.*, 1991) has been demonstrated with the InsP_3 receptor, where calcium increases the affinity of the receptor for InsP_3 . The InsP_3R is very closely related structurally to the RYR (Ross *et al.*, 1989; Furuichi *et al.*, 1989; Mignery *et al.*, 1989; Südhof *et al.*, 1991). There is a functional parallel, too, with the suggestion that the RYR is the target of the calcium mobilizing agent cADPr (Galione *et al.*, 1991; Galione, 1992). Either of the two calcium release channels might sustain the calcium wave at fertilization. Which is it?

There are two approaches to the question, one descriptive, the other functional. The descriptive approach has demonstrated the presence of InsP_3R in hamster eggs (Miyazaki *et al.*, 1992) and *Xenopus* oocytes (Parys *et al.*, 1992) and of RYR in sea urchin (McPherson *et al.*, 1992). The functional approach uses agonists and antagonists of the two channels and is perhaps more informative. The results are summarized in Table 1. To précis them further: InsP_3R are functionally active in frog, mouse, hamster and sea urchin eggs. RYR

Table 1. *The InsP_3 receptor and the ryanodine receptor.*

	Frog	Mouse	Hamster	Sea urchin
<i>Agonists that cause Ca^{2+} release</i>				
InsP_3	+ ¹	+ ²	+ ³	+ ⁴
cADPr	- ⁵	- ⁶	- ⁷	+ ⁸
ryanodine	- ⁵	+ ²	?	+ ⁹
caffeine	- ⁵	- ²	- ¹⁰	+ ¹¹
<i>Antagonists that block CICR/fertilization</i>				
heparin	+ ⁵	?	?	- ⁵
InsP_3 sponge / InsP_3R Ab	+ ¹²	?	+ ¹³	- ¹⁴
ryanodine	?	+ ²	?	?
ruthenium red	-	?	?	- ⁵
heparin + ruthenium red	?	?	?	+ ⁵
<i>Conclusion</i>				
RYR	-	+/-	?	+
InsP_3R	+	+	+	+

+, efficacy; -, lack of efficacy; ?, not tested. ¹Busa *et al.* (1985); ²Kurasawa *et al.* (1989); ³Miyazaki (1988); ⁴Whitaker & Irvine (1984); ⁵Galione *et al.* (1993); ⁶Kline *et al.* (1992); ⁷Swann (unpublished observation); ⁸Clapper *et al.* (1987); Lee *et al.* (1989); ⁹Galione *et al.* (1991); Sardet *et al.* (1992); ¹⁰Miyazaki (1990); ¹¹Galione *et al.* (1991); Buck *et al.* (1992); ¹²Nuccitelli *et al.* (1992); ¹³Miyazaki *et al.* (1992); ¹⁴K. Mikoshiba (personal communication).

are active in sea urchin and mouse eggs and, possibly, in hamster, though the RYR in mammalian eggs does not respond to either caffeine or cADPr.

The frog egg is insensitive to RYR agonists and antagonists. The prediction that CICR in the frog egg is mediated by InsP_3R is borne out by the observation that the InsP_3 antagonist, heparin, blocks CICR in frog eggs (Galione *et al.*, 1993). When heparin is micro-injected into frog eggs, it blocks not only CICR but the fertilization calcium wave, too. It now seems fairly certain that the fertilization calcium wave in the frog is based on InsP_3R -CICR, as are the small calcium waves that can be induced artificially in immature frog oocytes (Lechleiter & Clapham, 1992). The sea urchin, on the other hand, has both functional InsP_3R and RYR (Fujiwara *et al.*, 1990). Agonists of either receptor trigger a regenerative calcium response and antagonists to either channel block CICR only when present together (Galione *et al.*, 1992; Lee *et al.*, 1993). The fertilization calcium wave is effectively blocked only when antagonists of both channel types are present. It now seems that the fertilization calcium wave in the sea urchin relies on both RYR-CICR and InsP_3R -CICR. Mammalian eggs resemble the frog egg in having a CICR and fertilization wave that is blocked by an InsP_3 antagonist (Miyazaki *et al.*, 1992, 1993). We could take it that the hamster fertilization wave is generated solely by InsP_3R -CICR, were it not that ryanodine, the idiosyncratic RYR agonist, causes regenerative calcium oscillations in mouse eggs and inhibits the mouse egg's periodic calcium oscillations when they are generated by other means (Swann, 1992). It is possible that while RYR-CICR cannot alone sustain a calcium wave in mammalian eggs, it nonetheless contributes to the calcium wave, enhancing InsP_3R -CICR.

Putting two and two together

One interesting outcome of these recent experiments is the realization that an apparently identical phenomenon, the fertilization calcium wave (Jaffe, 1983), may show a multiplicity of mechanisms in different eggs. In the sea urchin, two CICR channels, InsP_3R and RYR, contribute to the wave and generate a richness of choice: any or all of calcium, InsP_3 , cADPr and cGMP may co-operate to initiate the calcium wave. In the frog egg, the sole participation of the InsP_3R narrows our choice: calcium or InsP_3 . The mammalian egg lies somewhere in between. Each egg may be different.

Or we can point to similarities. It used to be said that CICR existed in frog (Busa *et al.*, 1985) and hamster (Igusa & Miyazaki, 1983), but not in sea urchin eggs (Swann & Whitaker, 1986). Now CICR is demonstrable in all as the mediator of the fertilization calcium wave. Another possible unity of mechanism is suggested by the observation that the activating factor from hamster sperm cytoplasm triggers calcium release in both frog and sea urchin homogenates (Galione and Swann, unpublished observations). There is convergence as well as divergence here.

There are still two models of fertilization, one involving G proteins and InsP_3 , the other fusion and a cytoplasmic messenger. They offer the possibility of a clear choice, but we are still waiting for the information that will give us a final answer.

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