Regulation of calcium spiking in mammalian oocytes through a combination of inositol trisphosphate-dependent entry and release

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The basic mechanism responsible for setting up the Ca\(^{2+}\) oscillations that occur during fertilization of mammalian oocytes is still very much of a mystery. These Ca\(^{2+}\) oscillations closely resemble those found in many other cell types and are generated by a process of calcium-induced calcium release (CICR) by channels on internal stores. What remains in contention, however, is the nature of the channels used and how their opening is controlled to produce the periodic bursts of calcium responsible for activating the oocyte.

Both inositol 1,4,5-trisphosphate receptors (InsP\(_3\)Rs) and ryanodine receptors (RYRs) display the process of CICR in mammalian oocytes. There is considerable evidence to indicate that InsP\(_3\)Rs play a primary role in generating calcium signals in oocytes (Miyazaki et al., 1993). While RYRs have also been implicated in wave generation in seaurchin oocytes, evidence concerning their role in mammalian oocytes has been controversial. Some previous evidence seemed to rule out a role for RYRs (Miyazaki et al., 1992; Kline and Kline, 1994) whereas there are other reports suggesting that oocytes possess such receptors (Swann, 1992; Ayabe et al., 1995; Jones et al., 1995; Yue et al., 1995; Tesarik, 1996). On the basis of the recent positive evidence, Tesarik (1995) has proposed an elaborate two-pool model to explain how InsP\(_3\)Rs and RYRs may interact to generate calcium spikes in mammalian oocytes. While there are aspects of this model that are appealing, Tesarik (1996) may have placed too much reliance on the evidence implicating a functional role for the RYRs.

A curious feature of most of the studies implicating RYRs in oocytes is that while there is some response to ryanodine (both stimulatory and inhibitory) there is an apparent lack of any sensitivity of these receptors to caffeine, which is extremely potent in stimulating Ca\(^{2+}\) release from all the known RYR isoforms including RYR3 (Takeshima et al., 1992). Indeed, there are reports indicating that mammalian oocytes are insensitive to caffeine (Miyazaki et al., 1992; Swann, 1992; Kline and Kline, 1994). This apparent lack of caffeine sensitivity must mean either that these oocyte RYRs are different, which in itself would be interesting, or that they are quantitatively insignificant. The latter possibility seems the most likely. There are examples of cells which have identifiable RYRs which are functionally incapable of generating global calcium signals when treated with the classical calcium-mobilizing agent caffeine (Giannini et al., 1995; Bennett et al., 1996). Despite having a very few RYRs, such cells may be capable of responding to ryanodine because this agent has an unusual property of locking RYRs in an open configuration, which

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For the full reference list, please refer to the original article.
might enable them to give a brief calcium signal by by-passing the normal calcium-dependent negative feedback loop. By inserting a permanent leak into the intracellular stores, a small number of RYRs will also prevent the stores from reloading, thus explaining the inhibitory effect of ryanodine on calcium spiking. Therefore, evidence based on the use of ryanodine is difficult to interpret and need not necessarily mean that the RYRs are functionally important with regard to generating calcium spikes in mouse oocytes.

On the basis of these reservations about the contribution of RYRs to calcium signalling in oocytes, there must be some doubt concerning the validity of this two-pool model as applied by Tesarik (1996). However, there are aspects of his two-pool model which are applicable if one assumes the existence of a single InsP$_3$-sensitive pool which can be separated into two functional entities – one responsible for entry and the other giving the regenerative spike. Igusa and Miyazaki (1983) were the first to emphasize the importance of coupling entry and release to explain calcium spiking in mammalian oocytes. Indeed, this Igusa and Miyazaki (1983) model has been adapted as a general model which places considerable emphasis on the role of InsP$_3$ and how it might contribute to the onset and continuation of calcium oscillations (Berridge and Galione, 1988; Berridge, 1991, 1992, 1994; Berridge and Dupont, 1994). The original two-pool model was formulated at a time before we knew that InsP$_3$-sensitive receptors could display CICR which is why we proposed the existence of an InsP$_3$-insensitive store deeper within the cell that displayed the CICR property necessary to generate the spike. Since we now know that the regenerative release responsible for spiking can be achieved by either InsP$_3$-R or RYRs, there is no need for two pools and indeed there is evidence that spiking in oocytes, as in other cells, can be achieved using just the InsP$_3$-sensitive pool (Miyazaki et al., 1992; Fissore and Robl, 1994; Kline and Kline, 1994). What is important, however, is that this InsP$_3$-sensitive pool may have two distinct functions (Berridge, 1992; Friesen et al., 1995).

The basic idea is that the InsP$_3$-sensitive store near the membrane regulates the influx of calcium which is then passed into the deeper stores where it is taken up and then periodically released to give the characteristic repetitive spikes (Cheek et al., 1993; Friesen et al., 1995). A major unsolved question concerns how this oscillatory mechanism is activated at fertilization. Miyazaki et al. (1993) have reviewed the considerable body of evidence favouring a central role for InsP$_3$ during the fertilization of mammalian oocytes. In keeping with the idea that the mechanism resembles that in other cells, a reasonable working hypothesis is that oscillations result from a sperm-induced localized elevation of InsP$_3$ (Cheek et al., 1993).

Just how the spermatozoa trigger an increase in InsP$_3$ is unknown. Mouse oocytes have at least three PLC isoforms (PLC$\beta_1$, PLC$\beta_3$ and PLC$\gamma_1$), any one of which is a candidate to generate InsP$_3$ (Dupont et al., 1996). It has been suggested that oscillin, the newly-defined sperm factor, might act either by enhancing the sensitivity of the intracellular receptors or by stimulating the generation of InsP$_3$ (Parrington et al., 1996). Since InsP$_3$ is generated at the cell membrane, there might be a concentration gradient of InsP$_3$ which may help to accentuate the two discrete functions of the InsP$_3$-sensitive pool (Berridge, 1992). High levels of InsP$_3$ near the cell surface will empty stores near the membrane thereby switching on capacitative calcium entry. In mouse oocytes, a Mn$^{2+}$ quench technique has revealed an increase in capacitative entry which becomes particularly evident as the stores empty during the first spike and then remains elevated during the subsequent period of repetitive spiking (McGuinness et al., in press). What is unusual is that this capacitative entry component is relatively small and this could account for the long periods between spikes which is so characteristic of the oscillations of mammalian oocytes. The low frequency of spiking is not due to some inherent inertia of the release channels because spiking can be accelerated simply by increasing the concentration of external calcium, thus emphasizing the importance of calcium influx as an important determinant of oscillator frequency. As originally envisaged by Igusa and Miyazaki (1983), the enhanced entry of external calcium is a key element of the spiking mechanism.

The other major component of the model concerns the events leading up to the periodic release of calcium from the internal stores. An important property of the InsP$_3$Rs is that they are under dual agonist control because release depends upon both InsP$_3$ and Ca$^{2+}$. An elevation of InsP$_3$ serves to increase their sensitivity to calcium and this effectively converts the cytoplasm into an excitable medium capable of generating calcium waves. Evidence that the cytoplasm of fertilized oocytes becomes such an excitable medium has been confirmed by injecting small test pulses of calcium (Igusa and Miyazaki, 1983; Fissore and Robl, 1994). The specific proposal, therefore, is that the onset of a spike may depend upon a combination of factors including an elevation of InsP$_3$, an influx of external calcium and then the loading of the stores with calcium which may further enhance the sensitivity of the InsP$_3$Rs.

An important component of this hypothesis, therefore, is that the InsP$_3$-sensitive stores have two separate functions. Those near the surface control entry whereas the stores found deeper within the cell are responsible for the global calcium spikes as they become primed by the calcium entering from outside. The distinction between these two functions might be sharpened if there is a gradient of InsP$_3$ spreading in from the cell surface. The existence of such a gradient may explain why it is difficult to precisely duplicate the normal spiking pattern by artificially flooding the cell with calcium (Galione et al., 1994). Similarly, intracytoplasmic sperm injection (ICSI) may not be able to duplicate this non-uniform InsP$_3$ gradient, thus accounting for the different spiking pattern observed with this artificial fertilization procedure.

References


How and why spermatozoa cause calcium oscillations in mammalian oocytes

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A prolonged series of transients, or oscillations, in the intracytoplasmic free Ca\(^{2+}\) ion concentration (Ca\(^{2+}\)i) have been observed at fertilization in all types of mammalian oocytes studied to date (Miyazaki et al. 1993a; Swann and Ozil, 1994). More recently Ca\(^{2+}\)i oscillations have been detected after intracytoplasmic sperm injection (ICSI) in human oocytes (Tesseirik et al., 1994). Whilst other articles in this debate discuss Ca\(^{2+}\) stores and Ca\(^{2+}\)i release channels, we would like to concentrate our attention on two aspects of Ca\(^{2+}\)i oscillations that are exemplified by the story of fertilization. The two outstanding questions for fertilization concern how the spermatozoa initiate the Ca\(^{2+}\)i oscillations, and why mammalian fertilization involves a repetitive Ca\(^{2+}\)i signal.

The first question concerns the signal transduction system that the spermatozoa use to trigger these Ca\(^{2+}\)i oscillations in the oocyte. One hypothesis that has been outlined in many textbooks, is that the spermatozoa interact with surface receptors which then couple to intracellular proteins that lead to the generation of the Ca\(^{2+}\)i mobilizing second messenger inositol 1,4,5-trisphosphate (InsP\(_3\)). The ryanodine receptor is also the Ca\(^{2+}\)i oscillations, and why mammalian fertilization involves a repetitive Ca\(^{2+}\)i signal.

The alternative view of fertilization is that the interaction between receptors on the spermatozoon and oocyte is simply the prelude to fusion and that it is after sperm–oocyte fusion that activation is achieved. Since gamete fusion appears to occur before Ca\(^{2+}\)i release it is possible that the spermatozoa contain a soluble factor that diffuses into the oocyte and causes Ca\(^{2+}\)i release after gamete fusion (Swann, 1993; Whitaker and Swann, 1993). Direct evidence for this hypothesis is the finding that injecting cytosolic extracts from mammalian spermatozoa causes Ca\(^{2+}\)i oscillations and oocyte activation in mouse, hamster and human oocytes (Stice and Robl, 1990; Swann, 1990, 1992, 1994; Homa and Swann, 1994; Dozortsev et al., 1995a). The factor has been shown to be protein-based and only effective when injected into the oocyte cytoplasm.

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