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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 Ca²⁺ ion concentration (Ca²⁺) have been observed at fertilization in all types of mammalian oocytes studied to date (Miyazaki et al., 1993a; Swann and Ozil, 1994). More recently Ca²⁺ oscillations have been detected after intracytoplasmic sperm injection (ICSI) in human oocytes (Tesarik et al., 1994). Whilst other articles in this debate discuss Ca²⁺ stores and Ca²⁺ release channels, we would like to concentrate our attention on two aspects of Ca²⁺ oscillations that are exemplified by the story of fertilization. The two outstanding questions for fertilization concern how the spermatozoa initiate the Ca²⁺ oscillations, and why mammalian fertilization involves a repetitive Ca²⁺ signal.

The first question concerns the signal transduction system that the spermatozoa use to trigger these Ca²⁺ oscillations. 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 Ca²⁺ mobilizing second messenger inositol 1,4,5-trisphosphate (InsP₃) (Jaffe, 1990; Foltz and Shilling, 1991; Miyazaki et al., 1993a). This idea lacks direct support because the molecules that have been identified on the surface of mammalian spermatozoa that bind them to oocytes fail to cause Ca²⁺ release or oocyte activation (Myles, 1993). Furthermore, experiments in hamster and mouse oocytes show that Ca²⁺ oscillations triggered by receptor activation, or even by direct InsP₃ injection, have different dynamics to those triggered by the spermatozoa at fertilization (Miyazaki et al., 1990; Swann, 1991, 1992, 1994; Galione et al., 1994). The most specific inhibition experiment that has been used to support the receptor–InsP₃ hypothesis involves injecting a monoclonal antibody raised against the InsP₃ receptor. Injecting this monoclonal antibody blocks all forms of Ca²⁺ oscillations and activation in mouse and hamster oocytes (Miyazaki et al., 1992b, 1993a). The InsP₃ receptor is also the Ca²⁺ release channel and the antibody acts on the channel portion of the molecule and not on the InsP₃ binding site (Nakade et al., 1991). Injection of heparin, which directly competes with InsP₃ at its binding site, does not block Ca²⁺ oscillations at fertilization in hamster oocytes (Miyazaki et al., 1993b). These results, therefore, show that the InsP₃ receptor/channel is involved in Ca²⁺ release at fertilization, but they do not prove that InsP₃ is the molecule that the spermatozoa use to activate this channel. As we shall explain, the character of the block to Ca²⁺ release caused by the InsP₃ receptor antibody is consistent with the alternative explanation of signalling at fertilization.

The alternative view of fertilization is that the interaction between receptors on the spermatozoon and oocyte are simply the prelude to fusion and that it is after sperm–oocyte fusion that activation is achieved. Since gamete fusion appears to occur before Ca²⁺ release it is possible that the spermatozoa contain a soluble factor that diffuses into the oocyte and causes Ca²⁺ 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 Ca²⁺ oscillations and oocyte activation in mouse, hamster and human oocytes (Stice and Robl, 1990; Swann, 1990; Swann, 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.
The cover of Molecular Human Reproduction displays a ribbon diagram of the peptide backbone of the α-subunit (grey) and β-subunit (purple). The amino acid side chains are shown in stick representation. The carbohydrate moiety is shown by space-filling CPK representation. The CPK colours red, blue and green refer to the atoms oxygen, nitrogen and carbon. For further details see Contents page.
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(Swann, 1990; Stice and Robl, 1990; Dozortsev et al., 1995a).

Since it is inside the spermatozoon, the soluble sperm factor hypothesis also offers a simple explanation for the finding that injecting whole spermatozooza causes activation and oscillations, and that injection of permeabilized spermatozooza does not activate human oocytes (Tesarik et al., 1994; Dozortsev et al., 1995a). Injecting the sperm factor also appears sufficient to trigger other events in activation such as second polar body emission, pronuclear formation, exocytosis (Stice and Robl, 1990; Swann, 1990), and can lead to embryonic development up to at least the blastocyst stage in the mouse (Y.Lawrence and K.R.S.Cuthbertson, unpublished data).

Sperm factor activity has now been correlated with a novel protein called oscillin (Parrington et al., 1996). Oscillin is a high molecular weight protein made of 33 kDa subunits. It appears to be expressed to far greater levels in spermatozooza than any other tissue (Parrington et al., 1996). Oscillin is concentrated in the intracellular part of the equatorial segment in mammalian spermatozooza (Parrington et al., 1996). The equatorial segment of the spermatozooa is the region that first fuses with the oocyte membrane in mammals (Yanagimachi, 1994), so oscillin is ideally located for its prospective role in causing oscillations which appear to start within minutes after gamete fusion in mammals. We do not yet know how oscillin causes Ca\(^{2+}\) oscillation in oocytes. Its mechanism of action is liable to involve components common to most cells since sperm extracts can also trigger Ca\(^{2+}\) oscillation in neurons and liver cells (Currie et al., 1992; Berrie et al., 1996). A novel mechanism of action is suggested by the fact that oscillin has significant sequence homology with a bacterial form of glucosamine phosphate deaminase (Parrington et al., 1996). Whatever the exact sequence of events that leads oscillin to trigger Ca\(^{2+}\) oscillations it seems reasonable to speculate that it eventually involves Ca\(^{2+}\) release via the channels in the endoplasmic reticulum. Since mammalian oocytes contain both InsP\(_3\) and ryanodine-sensitive Ca\(^{2+}\) release channels (Miyazaki et al., 1992b; Ayabe et al., 1995), oscillin may mediate its effects through either of these channels (Swann, 1993, 1996).

The block of fertilization by InsP\(_3\) initiates Ca\(^{2+}\) release by the InsP\(_3\) receptor antibody on or after the fertilization of the oocytes (Swann and Ozil, 1995a). In addition, these studies showed that the frequency and pattern of the Ca\(^{2+}\) release was correlated to the rate of pronuclear formation as well as the rate of development of pre- and post-implantation stages (Ozil, 1990; Vitullio and Ozil, 1992). Only these early events are seen if all but the initial Ca\(^{2+}\) transient is prevented at fertilization (Kline and Kline, 1992). These data clearly show that a single physiological Ca\(^{2+}\) release transient is not enough to cause entry into the first embryonic cell cycle. It is possible that oscillations in Ca\(^{2+}\) might be a way for the spermatozooa to guarantee oocyte activation to the pronuclear stage. This view is supported by studies on electrical pulse activation of mouse and rabbit oocytes which demonstrated that the maximal efficiency in activating eggs, i.e. 100% activation of oocytes of any postovulatory age, was only achieved with repetitive, not singular, pulses of Ca\(^{2+}\) (Vitullio and Ozil, 1992; Ozil and Swann, 1995). In addition, these studies showed that the frequency and pattern of the Ca\(^{2+}\) transients affected both the rate of pronuclear formation as well as the rate of development of pre- and post-implantation stages (Ozil, 1990; Vitullio and Ozil, 1992; Ozil and Swann, 1995). What is remarkable about these findings is that the initial stimulus which represents only a fraction of the life span of the embryo has such profound effects days after its occurrence.

From an evolutionary perspective, it seems unlikely that the continued reproduction of a species would rely on an inefficient signalling mechanism. Although we suggest that the mammalian spermatozooa trigger repetitive Ca\(^{2+}\) increases in order to ensure oocyte activation, we are still left with the question as to why it is necessary for the oocyte to be given a repetitive stimulus. Eggs or oocytes of phylogenetically lower organisms, such as those of the sea urchin and frog, show only a single Ca\(^{2+}\) transient at fertilization (Whitaker and Swann, 1993). The explanation for these differences may be in the length of the cell cycle which is considerably longer in mammals than in the sea urchin and frog. By the time a *Xenopus* embryo is made of hundreds of cells, a mouse embryo is only mid-way through the second cell cycle. The very longevity of the cell cycle in mammals may necessitate persistent Ca\(^{2+}\) oscillations...
in order to maintain the decrease in the activity of maturation promoting factor (MPF) which otherwise holds the oocyte at metaphase II (Collas et al., 1993). A sustained rise in Ca$^{2+}$ may not be an option for the oocyte since this would be expected to drain the cell’s ATP and result in cell death. Thus one likelihood is that mammals have evolved an oscillatory Ca$^{2+}$ signalling mechanism to ensure release from meiotic arrest into what is a lengthy embryonic first cell cycle. In this respect it is worth noting that the inability of the spermatozoon to generate a sufficient number of Ca$^{2+}$ transients at fertilization may be a possible cause of failed fertilization in some oocytes in either conventional in-vitro fertilization (IVF) or in ICSI treatments (Dozortsev et al., 1995b; Flaherty et al., 1995). It remains to be investigated if this is related to the concentration of oscillin in the spermatozoon.

References


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