Human Reproduction Update, Vol.8, No.4 pp. 313–321, 2002

Signalling mechanisms of mammalian oocyte activation

Carmen J.Williams

Center for Research on Reproduction and Women's Health and Department of Obstetrics and Gynecology, University of Pennsylvania, 1313 BRB II/III, 421 Curie Blvd, PA 19104–6142, USA. E-mail: cjwill@mail.med.upenn.edu

Intensive study over the past 10 years has resulted in major advances in our understanding of the signalling mechanisms required for the transformation of the oocyte into an embryo capable of developing into a new individual. This review focuses on the signal transduction events that occur subsequent to the initial sperm–oocyte plasma membrane interaction, with particular emphasis on the mechanism(s) by which sperm initiate these signalling pathways. Downstream signalling/effector molecules that respond to sperm-induced calcium oscillations and appear to have important roles in oocyte activation are then discussed. Finally, the mechanisms of physiological oocyte activation are compared with events of oocyte activation that occur after ICSI.

Key words: fertilization/ICSI/meiosis/oocyte/oocyte activation

TABLE OF CONTENTS

Introduction

Fertilization is a multistep process involving interactions of the sperm with the outer vestments of the oocyte, namely the cumulus cell layer and zona pellucida (ZP), penetration of the sperm through these structures, and eventually direct interaction of the sperm and oocyte plasma membranes. After this plasma membrane interaction occurs, a signal transduction cascade is initiated that results in the conversion of the oocyte to a diploid embryo capable of forming a new organism; this process is known as 'oocyte activation'. The signal transduction pathways that are utilized during oocyte activation have been studied for many years. Although calcium has long been identified as a universally important second messenger during oocyte activation, the signalling mechanisms by which the fertilizing sperm induces shifts in intracellular calcium concentration within the oocyte are only now beginning to be understood. In this review, the literature regarding signal transduction mechanisms that are known to be important for mammalian oocyte activation will be outlined, and some recent findings that shed light on the mechanisms by which sperm trigger these signalling pathways will be highlighted. Next, some of the known signalling effector molecules that are important for the successful transformation of the oocyte to a cleaving embryo will be detailed. Lastly, differences between the physiological process of oocyte activation and oocyte activation that occurs in response to ICSI will be described along with the potential impact of these differences on development.

Oocyte activation: overview

Oocyte activation includes a large number of well defined morphological and biochemical endpoints, some of which occur within seconds or minutes of sperm–oocyte plasma membrane interaction, and some that occur over the course of several hours (Yanagimachi, 1994; Schultz and Kopf, 1995). One of the earliest events of oocyte activation is an increase in the level of intracellular calcium. In mammals, levels of intracellular calcium oscillate repetitively for a period of up to several hours after sperm–oocyte fusion (Cuthbertson *et al.*, 1981; Miyazaki and Igusa, 1981; Miyazaki *et al.*, 1993). Elevations in intracellular calcium cause the exocytosis of cortical granules that are located in the cortical region of the oocyte below the plasma membrane (Abbott and Ducibella, 2001). These granules contain enzymes that are released into the perivitelline space, resulting in modifications of the ZP that prevent additional sperm from binding to and penetrating the ZP (Bleil and Wassarman, 1981; Endo *et al.*, 1987b). These alterations in the ZP constitute an important mechanism by which some mammals, including humans, prevent polyspermy. There is also evidence for a block to polyspermy at the level of the plasma membrane that is important in certain mammals, e.g. rabbits (Yanagimachi, 1994), but the mechanisms by which this membrane block occurs are unknown, and may or may not involve cortical granules.

Many changes in the sperm head components occur upon exposure to the oocyte cytoplasm. During spermiogenesis within

the testis, the sperm chromatin is packaged into a highly condensed structure, a process that requires the replacement of histone proteins by protamines (Yanagimachi, 1994). In addition, the sperm nucleus becomes surrounded by a structure known as the perinuclear theca (PT) that is comprised of at least six distinct proteins (Oko *et al.*, 2001). Shortly after sperm–oocyte fusion, the sperm nuclear envelope breaks down, and the PT is removed from around the chromatin, a process in which oocyte microvilli appear to actively participate (Sutovsky *et al.*, 1996, 1997). The chromatin subsequently undergoes a process of decondensation that begins in the posterior region of the sperm head and progresses to the anterior portion (Terada *et al.*, 2000). Sperm chromatin decondensation requires reduction of disulphide bonds, which is accomplished by reduced glutathione present in the oocyte cytoplasm (Perreault *et al.*, 1988). During decondensation, the protamines that package the chromatin are replaced by oocyte-derived histones (Perreault, 1990).

The sperm tail also enters the oocyte at fertilization. Sperm mitochondria dissociate from the midpiece of the tail but remain aggregated together in the oocyte cytoplasm (Sutovsky *et al.*, 1996). Mitochondrial proteins have been marked during spermatogenesis with ubiquitin tags that result in proteasome-mediated destruction of these organelles (Sutovsky *et al.*, 1999, 2000). As a result, sperm mitochondria do not persist beyond the preimplantation embryo stages, and only maternal mitochondria are inherited (Hutchison *et al.*, 1974; Giles *et al.*, 1980; Cummins *et al.*, 1997). In most mammals, the sperm centriole is responsible for organization within the oocyte cytoplasm of the sperm aster, a microtubular structure that is required for the generation of the microtubular network important for pronuclear migration (Schatten, 1994; Simerly *et al.*, 1995; Sutovsky *et al.*, 1996). One exception to this paternal inheritance of the centriole is in mice, in which maternal cytoplasmic microtubule organizing centres nucleate the microtubules utilized for pronuclear migration (Schatten, 1994). Most of the remaining structural elements of the sperm tail rapidly disappear after incorporation into the oocyte (Sutovsky *et al.*, 1996).

A critical component of oocyte activation is the resumption of meiosis II that occurs during the time that the sperm chromatin is decondensing in the oocyte cytoplasm; this resumption marks re-entry of the oocyte into the cell cycle. The cell cycle is controlled by a balance of the activities of kinases and phosphatases that modulate the activity of cellular proteins. Morphological changes observed during meiotic resumption include rotation of the metaphase spindle (in some species), entry into anaphase, and emission of the second polar body. At this point, the DNA originating from the female gamete becomes haploid.

Prior to fertilization, the meiotic cell cycle of the mammalian oocyte is arrested at metaphase II because of the presence of active maturation promoting factor (MPF) (Verlhac *et al.*, 1993). MPF is a cell cycle modulator comprised of two subunits, $p34^{cdc2}$ kinase and cyclin B1, and is responsible for inducing spindle assembly, chromatin condensation and nuclear envelope breakdown (Murray and Hunt, 1993). During metaphase II arrest, MPF remains active due to the presence of 'cytostatic factor' which is comprised at least in part of the proteins Mos (Colledge *et al.*, 1994) and Emi1 (Reimann and Jackson, 2002) which prevent the degradation of cyclin B1. Shortly after sperm–oocyte fusion, elevation of intracellular calcium induces cyclin degradation (Lorca *et al.*, 1993) and Mos undergoes ubiquitin-dependent degradation by the proteasome (Ishida *et al.*, 1993; Nishizawa *et al.*, 1993). The resulting loss of active cytostatic factor and the inactivation of MPF allows entry of the female chromatin into anaphase II (Murray and Hunt, 1993).

Another protein kinase important in regulation of the meiotic cell cycle is mitogen-activated protein (MAP) kinase. MAP kinases are thought to phosphorylate chromosomal proteins important for maintaining chromatin in a condensed state during the transition from meiosis I to meiosis II, and to prevent nuclear envelope formation by phosphorylation of nuclear lamins (Murray and Hunt, 1993). During oocyte activation, MAP kinase activity begins to decrease somewhat later than the decrease in MPF activity, and this decrease is required before pronuclear envelope formation can occur (Moos *et al.*, 1995).

Several hours after sperm–oocyte fusion, the calcium oscillations cease (Jones *et al.*, 1995), the male and female pronuclei form, and DNA synthesis begins. Under the control of a microtubular network, the two pronuclei migrate to the centre of the newly formed 1-cell zygote. After pronuclear apposition in the centre of the zygote, the pronuclear membranes break down and the chromosomes undergo syngamy in preparation for the first cleavage division.

Major changes in the pattern of protein synthesis occur during oocyte activation (Latham *et al.*, 1991). These changes result in part from the recruitment of maternal mRNAs that are present in the cytoplasm of the oocyte but not translated prior to oocyte activation (Cascio and Wassarman, 1982), and in part from post-translational modifications of existing proteins (Van Blerkom, 1981). Finally, transcription and translation of proteins encoded by the newly formed embryonic genome begin in the late 1-cell stage in the mouse, and during later preimplantation embryo development in the human; this process is known as zygotic gene activation.

Although this brief description of oocyte activation includes a number of biochemical and morphological changes that are well defined, it is likely that many other changes are occurring during oocyte activation that are important for embryo development. In the following sections, the best known second messengers and protein effectors responsible for oocyte activation will be described, and areas in which the signalling pathways responsible for oocyte activation events remain unknown will be highlighted.

Second messengers in oocyte activation

Calcium

As mentioned above, calcium is a universally important second messenger at fertilization in all species studied to date (Miyazaki *et al.*, 1993). In mammals, a short latent period of several minutes occurs after sperm–oocyte fusion that is followed by an increase in the level of intracellular calcium. The rise in calcium proceeds in a wavelike fashion from the site of sperm–oocyte fusion across the oocyte (Miyazaki *et al.*, 1986; Deguchi *et al.*, 2000) and rises from a basal level of 50–100 nmol/l to a peak of 600–1000 nmol/l, depending on the species (Miyazaki *et al.*, 1993). This initial calcium response may persist for several minutes, as in the mouse (Kline and Kline, 1992), or may entail several high frequency calcium transients, as in the hamster (Miyazaki *et al.*, 1992).

Subsequent to the initial rise in calcium, repetitive calcium oscillations occur with a periodicity of between 2 and 30 min, and continue for variable periods of time up to several hours (Miyazaki *et al.*, 1993), ending by the time of pronuclear formation (Jones *et al.*, 1995; Deguchi *et al.*, 2000). To maintain the repetitive oscillations, the intracellular calcium stores require replenishment by the uptake of extracellular calcium (Igusa and Miyazaki, 1983; Kline and Kline, 1992).

Although most lower species do not generate multiple calcium transients, these are critical for successful oocyte activation in mammals (Ozil, 1990; Kline and Kline, 1992). Spatial and temporal characteristics of calcium oscillations are widely used to regulate many different cellular responses, including cell proliferation, gene transcription and vesicle secretion (Berridge *et al.*, 1998). A direct relationship between successful oocyte activation and the frequency and amplitude of the calcium oscillations has been documented by experiments in which a specific number of electrical pulses resulting in defined increases in intracellular calcium was administered to mouse oocytes, and the success of subsequent oocyte activation was measured (Vitullo and Ozil, 1992). Oocytes exposed to either abnormally high or low amplitude calcium oscillations failed to form pronuclei. In addition, when the oocytes were exposed to a lower total duration of oscillations, they failed to complete second meiosis. A recent study confirmed that an abnormally high frequency and duration of calcium oscillations induces abnormal patterns of oocyte activation, and can induce cell death in aged oocytes (Gordo *et al.*, 2000). These studies highlight the importance of strict temporal and spatial regulation of calcium oscillations for mammalian oocyte activation.

Inositol 1,4,5-trisphosphate (IP_3)

IP_3 is a second messenger produced by the hydrolysis of phosphatidyl-inositol 4,5-bisphosphate (PIP_2) by phosphoinositide-specific phospholipase C (PLC). Calcium release during mammalian oocyte activation is induced by the action of IP_3 on IP_3 receptors (IP_3R) present in the membrane compartment of cytoplasmic calcium stores (Miyazaki *et al.*, 1992). This process is known as 'IP_3-induced calcium release', and is the calcium release mechanism utilized by many hormones and neurotransmitters to alter cell functions (Berridge, 1993; Schuster *et al.*, 2002). IP_3R is itself a cation channel that is regulated by calcium in addition to IP_3. IP_3 binding to IP_3R triggers the release of calcium through IP_3R by increasing the sensitivity of IP_3R to calcium. Because relatively low calcium levels induce opening of the IP_3R channel, release of calcium from cytoplasmic stores provides a positive feedback mechanism for the generation of further calcium release into the cytoplasm. The positive feedback is reversed above a critical calcium concentration, at which point high calcium levels cause the IP_3R channel to close. This allows a net re-uptake of cytoplasmic calcium by the activity of sarco-endoplasmic reticulum calcium ATPases (Lechleiter *et al.*, 1998; Schuster *et al.*, 2002) and the subsequent return of the cytoplasmic calcium to basal levels. In this way, alternating feedback regulation of calcium release results in the occurrence of repetitive oscillations in intracellular calcium levels (Berridge, 1993; Schuster *et al.*, 2002).

A role for IP_3 during oocyte activation was first suggested by experiments in the sea urchin that demonstrated polyphosphoinositide turnover at fertilization (Turner *et al.*, 1984). An increase in IP_3 content has been measured within seconds of fertilization in *Xenopus laevis* oocytes (Stith *et al.*, 1993), supporting the idea that this second messenger is important for oocyte activation. In addition, microinjection of IP_3 into mammalian oocytes can result in repetitive calcium oscillations and cortical granule exocytosis (Miyazaki *et al.*, 1993). Further support for the critical role of IP_3 during mammalian oocyte activation was provided by a series of elegant experiments by Miyazaki *et al*. in which IP_3R-mediated calcium release function was blocked in hamster oocytes by microinjection into oocytes of an IP_3R antibody prior to insemination. This antibody completely inhibited calcium oscillations in response to the fertilizing sperm, whereas a non-function-blocking antibody had no effect (Miyazaki *et al.*, 1992); similar studies have confirmed this finding in the mouse (Xu *et al.*, 1994).

Of note, microinjection of a single bolus of IP_3 only can induce oocyte activation in mouse oocytes obtained from the oviduct several hours after ovulation, known as 'old oocytes'. 'Young oocytes' obtained immediately after ovulation do not respond to IP_3 microinjection (Xu *et al.*, 1997) unless it is given in a sustained or pulsatile fashion (Swann, 1992; Jones and Nixon, 2000). Similarly, the ability of calcium ionophore to artificially activate oocytes is dependent on the amount of time after ovulation, with more success in activating 'old oocytes'. These experiments suggest that either effectors in addition to IP_3, or an amplification or repetition of the IP_3 signal, are required for successful oocyte activation, and that the ability of increases in intracellular calcium to induce complete oocyte activation increases with time after ovulation. One explanation for this phenomenon is that in the absence of active protein synthesis after oocyte maturation, the levels of important cell cycle regulatory proteins may decrease with time after ovulation. In that case, a lower amplitude/frequency of calcium oscillations induced by IP_3 or calcium ionophore could result in degradation of these proteins, e.g. cyclin B1, to the same extent as that induced by sperm in a 'young oocyte'.

sn-1,2 diacylglycerol (DAG)

In addition to the formation of IP_3, hydrolysis of PIP_2 by PLC results in the formation of another second messenger, DAG. DAG and its mimetics cause the activation of protein kinase C (PKC), a calcium-dependent kinase that has multiple downstream effectors in somatic cells, including cytoskeletal proteins, transcription factors and proteins involved in the process of exocytosis. Although there have been no direct measurements of DAG production in fertilized mammalian oocytes, due to limiting amounts of material, oocytes of lower species have a measurable increase in the amount of DAG present immediately after fertilization (Eckberg and Szuts, 1993; Stith *et al.*, 1997). A role for DAG in mammalian oocyte activation is supported by experiments in which exposure of mouse oocytes to DAG mimetics has been shown to induce calcium oscillations, cortical granule exocytosis and ZP modifications (Cuthbertson and Cobbold, 1985; Endo *et al.*, 1987a,b).

Signalling pathways initiating oocyte activation

There has been intensive debate over the past several years regarding the signalling mechanism(s) by which the fertilizing

sperm initiates the repetitive calcium oscillations required for successful oocyte activation. As outlined above, PLC-mediated generation of IP_3 by hydrolysis of PIP_2 is thought to be critically important for the generation of these calcium oscillations. A role for oocyte PLC was confirmed by experiments demonstrating that an aminosteroid PLC inhibitor prevented sperm-induced calcium oscillations in the mouse oocyte (Dupont *et al.*, 1996). Several different isoforms of PLC have been described that are activated by distinct mechanisms. PLC enzymes are divided into four major types, denoted PLCβ, PLCγ, PLCδ and PLCε (Rhee, 2001). These PLC isoforms can be activated in response to heterotrimeric G proteins, low molecular weight G proteins, receptor or non-receptor tyrosine kinases and lipid-derived second messengers (Rhee, 2001). Echinoderm oocytes appear to be activated via stimulation of PLCγ by a Src-like tyrosine kinase (Carroll *et al.*, 1997; Giusti *et al.*, 2000); the PLC isoform that is utilized during mammalian oocyte activation is as yet unknown.

The hypothesis that a sperm surface molecule might act as a ligand to induce receptor-mediated activation of oocyte PLC has been tested by a number of laboratories. It was shown that stimulation of oocyte PLC by ligand stimulation of specific G protein-coupled receptors introduced into the oocyte resulted in oocyte activation (Kline *et al.*, 1988; Shilling *et al.*, 1990; Williams *et al.*, 1992; Moore *et al.*, 1993). However, blocking antibodies that inhibited this response by interfering with native G protein function in the oocyte had no inhibitory effect on sperm-induced oocyte activation (Williams *et al.*, 1998). Similarly, inhibition of receptor-mediated activation of PLCγ did not prevent sperm-induced oocyte activation in the mouse (Mehlmann *et al.*, 1998). These results suggest that a plasma membrane receptor-mediated mechanism is not responsible for activation of PLC by the sperm.

An alternative hypothesis, that is now generally accepted, is that a factor within the sperm that enters the oocyte at the time of sperm–oocyte fusion is responsible for the generation of IP_3 and calcium oscillations. This 'sperm factor' hypothesis is supported by the fact that sperm extracts of many different species, when microinjected into oocytes, result in calcium oscillations and other events of oocyte activation (Dale *et al.*, 1985; Stice and Robl, 1990; Swann, 1990; Homa and Swann, 1994). Because the sperm factor is heat- and protease-sensitive (Swann, 1990), it is thought to be a protein; however, identification of the sperm factor has not yet been accomplished. To be considered the sperm factor, a protein should have certain characteristics that are based on results of many experiments regarding the signalling mechanisms by which sperm activate oocytes at fertilization. First, the sperm factor should be found in association with isolated sperm heads (Kuretake *et al.*, 1996) and should have a molecular weight of 30–100 kDa (Parrington *et al.*, 1996, 2002; Kimura *et al.*, 1998; Kyozuka *et al.*, 1998). Second, the sperm factor should induce calcium oscillations resembling those seen at fertilization (Swann, 1990), and it should be active when microinjected into oocytes in amounts that approximate those present in a single spermatozoon. In addition, these calcium oscillations should be induced via the IP_3-induced calcium release mechanism (Oda *et al.*, 1999). Lastly, if a candidate sperm factor is truly a physiological activator of the oocyte, it should be possible to prevent sperm-induced oocyte activation by blocking the function of the sperm factor.

Several candidate proteins have been proposed to be the sperm factor. The first protein isolated that was thought to represent the sperm factor, oscillin (Parrington *et al.*, 1996), was later shown to have no ability to induce calcium oscillations (Wolny *et al.*, 1999). Another candidate sperm factor protein was the truncated c-kit receptor (tr-kit) (Sette *et al.*, 1997). Although microinjection of extracts of cells expressing this protein into mouse oocytes was shown to cause many oocyte activation events, most of the endogenous protein is associated with the sperm tail, not the sperm head (Sette *et al.*, 1997), and recombinant tr-kit protein has not been shown to induce repetitive calcium oscillations in oocytes. In addition, the mechanism by which tr-kit induces oocyte activation appears to be different from that utilized by the sperm (Mehlmann *et al.*, 1998; Sette *et al.*, 1998). There has been considerable interest in the possibility that a sperm-associated PLC could be the sperm factor (Jones *et al.*, 1998). However, known isoforms of PLC present in sperm do not co-purify with sperm factor activity (Parrington *et al.*, 2002), and recombinant forms of several PLC isoforms do not induce calcium oscillations when microinjected into mouse oocytes (Jones *et al.*, 2000). These latter results support the possibility that the sperm factor activates an oocyte PLC rather than being itself a PLC.

Although the initial reports described the sperm factor as a soluble sperm component, later reports suggested that the sperm factor is located within the sperm PT (Kimura *et al.*, 1998; Perry *et al.*, 2000). A hypothetical model of the initiation of oocyte activation by a sperm PT protein is shown in Figure 1. Recently, a sperm PT protein known as PT32 was cloned, and is a viable candidate for the mammalian sperm factor (Oko *et al.*, 2001; Wu *et al.*, 2001). Evidence supporting this role for PT32 includes its localization in the post-acrosomal region of the sperm head, which is one of the first regions of the PT to be exposed to the oocyte cytoplasm (Sutovsky *et al.*, 1997). In addition, the biochemical properties of PT32 mimic those previously reported for the sperm factor, including its apparent molecular weight of 32 kDa and the ability to be extracted from sperm by freeze–thaw procedures or reducing agents (Oko *et al.*, 2001). The most exciting finding to date regarding PT32 as a potential sperm factor is that the recombinant protein is capable of inducing oocyte activation in porcine oocytes, as assayed by morphological markers including pronuclear formation, when microinjected into the oocyte in amounts close to those estimated to be present in a single spermatozoon (Sutovsky *et al.*, 2001). However, verification of PT32 as the physiological sperm factor awaits documentation that the recombinant protein induces calcium oscillations when microinjected into oocytes, and that inhibition of its function prevents sperm-induced oocyte activation. In addition, the mechanism by which PT32 might initiate oocyte activation, and the molecule(s) with which it interacts within the oocyte, are as yet unknown.

Evidence is mounting that the sperm factor may activate a PLC in mammalian oocytes via the activation of a Src-family tyrosine kinase. These kinases are important during oocyte activation in echinoderms, sea urchins and frogs (Carroll *et al.*, 1997; Livingston *et al.*, 1998; Glahn *et al.*, 1999; Abassi *et al.*, 2000; Giusti *et al.*, 2000). The candidate mammalian sperm factor PT32 contains consensus amino acid sequences that bind to 'WW domains', protein modules related to Src-homology domains that are frequently found in signalling molecules that interact with

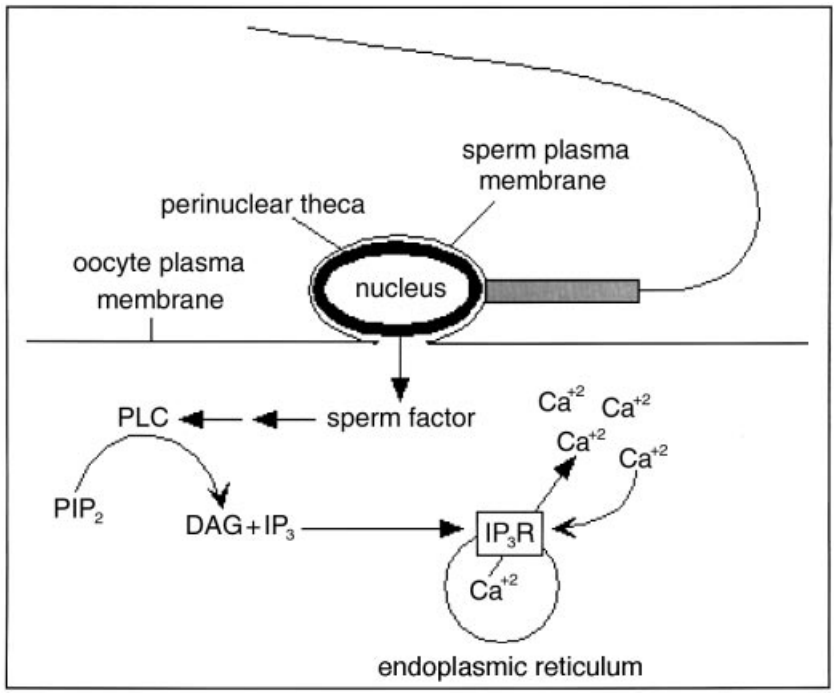

Figure 1. Hypothetical model of oocyte activation by a sperm factor. Sperm–oocyte plasma membrane fusion allows exposure of the sperm perinuclear theca to the oocyte cytoplasm and results in release of the sperm factor. The sperm factor activates a signalling cascade that could involve a Src-family kinase, and results in the activation of an oocyte PLC. The oocyte PLC catalyses the hydrolysis of PIP_2 to form DAG and IP_3. IP_3 acts on IP_3R present in the endoplasmic reticulum to cause the release of calcium. Calcium itself participates in feedback regulation of further calcium release from the endoplasmic reticulum, and together with other modulators of IP_3R function results in oscillations in intracellular calcium levels required for successful oocyte activation.

Src-family kinases (Sudol *et al.*, 1995). At least two different Src-family kinases are present in mammalian oocytes, including c-Fyn (Talmor *et al.*, 1998) and c-Yes (Sutovsky *et al.*, 2001). Because PT32 has homology to WW Domain Binding Protein 2 (Wu *et al.*, 2001), a protein identified as a ligand for the WW domain of Yes-associated protein (Chen and Sudol, 1995), it has been postulated that c-Yes serves as a downstream effector of PT32-induced oocyte activation (Sutovsky *et al.*, 2001). In fact, microinjection of recombinant c-Yes into bovine oocytes can induce some oocyte activation events, supporting the idea that this kinase or its substrates are involved (Sutovsky *et al.*, 2001). However, whether or not c-Yes, c-Fyn or other Src-family tyrosine kinases directly or indirectly activate PLC during mammalian fertilization remains to be determined.

Targets of calcium signalling during oocyte activation

The downstream molecules that are regulated by calcium oscillations during oocyte activation are beginning to be identified but are not yet well defined in mammalian systems. However, extensive studies in *Xenopus laevis* have defined a number of effector proteins, likely also to be involved in mammalian oocyte activation that will be described here. Calmodulin is a calcium-binding protein that regulates the activity of many different downstream effector proteins, including the calmodulin-dependent protein kinases (CaMK) and phosphatases. In *Xenopus* oocytes, calmodulin regulates IP_3-induced calcium release by modulating the activity of CaMKII; CaMKII-mediated phosphorylation of the IP_3R inhibits IP_3-induced calcium release (Matifat *et al.*, 2001). Calmodulin and CaMKII may indirectly modulate IP_3-induced calcium release by activating the enzyme IP_3 3-kinase that converts IP_3 to IP_4 (Hague *et al.*, 1999). In addition, there is evidence that CaMKII is responsible for inducing cyclin B ubiquitination and subsequent degradation by proteasomes, resulting in the inactivation of MPF (Lorca *et al.*, 1993). In the mouse, CaMKII is transiently activated at fertilization (Winston and Maro, 1995; Johnson *et al.*, 1998), and with calmodulin becomes associated with the meiotic spindle within minutes of the elevation in intracellular calcium, suggesting a role in regulating the progression of meiosis. CaMKII activity was shown recently to be required for centrosome duplication that allows the progression from metaphase to anaphase (Matsumoto and Maller, 2002). In addition, CaMKII is likely to be involved in cortical granule exocytosis (Abbott and Ducibella, 2001), and to be responsible for cytoskeletal rearrangements that occur during oocyte activation (Johnson *et al.*, 1998).

Calpain, a calcium-dependent cysteine protease, is activated by calcium-induced autolysis at fertilization (Watanabe *et al.*, 1989; Malcov *et al.*, 1997), and in the rat localizes to the region around the meiotic spindle after calcium-induced oocyte activation (Malcov *et al.*, 1997). Calpain functions to regulate cell cycle proteins (Santella, 1998), to regulate microtubule assembly/disassembly (Billger *et al.*, 1988) and to regulate nuclear proteins (Mellgren, 1991). All of these functions are important during oocyte activation, but as yet calpain has not been shown directly to modify of any of these proteins in the oocyte.

Two other calcium-dependent effector molecules appear to be important for oocyte activation. First, there is evidence that the calcium and phospholipid-dependent protein kinase, PKC, is activated after fertilization (Gallicano *et al.*, 1997). Although the

function(s) of PKC during oocyte activation are not yet well defined, PKC activity may promote cortical granule exocytosis (Endo *et al.*, 1987b; Abbott and Ducibella, 2001), second polar body emission (Gallicano *et al.*, 1997) and remodelling of the oocyte's internal cytoskeleton (Gallicano *et al.*, 1995). One of the difficulties with interpreting studies of PKC function during oocyte activation is that many of these studies utilize pharmacological modulators of PKC, such as phorbol esters, that also may have effects on other signalling pathways (Jones, 1998). A second calcium-dependent effector molecule that may be important during oocyte activation is the calcium/calmodulin-dependent serine/threonine protein phosphatase 2B, or calcineurin. Calcineurin is implicated in the control of transcription in many cell types (Crabtree, 2001), and could function in this role as gene transcription is beginning during zygotic gene activation. In addition, calcineurin has been shown to regulate IP_3R function by altering its phosphorylation status (Cameron *et al.*, 1995) and therefore may have a role in regulating calcium oscillatory behaviour during oocyte activation.

The ways in which calcium oscillations are decoded by the cell to produce distinct cellular responses is currently a subject of intense study, and the process of oocyte activation provides an important model system for this question (Berridge *et al.*, 1998). Studies in other cell types have shown that CaMKII, calpain and calcineurin activity can each be regulated by variations in the frequency of calcium oscillations (De Koninck and Schulman, 1998; Li *et al.*, 1998; Tompa *et al.*, 2001). The relocalization of CaMKII and calpain to the region of the oocyte meiotic spindle demonstrates that spatial regulation of these signalling effector molecules also occurs and is probably critical for the resumption of meiosis. In this regard, scaffolding proteins could tether these effectors to the meiotic spindle, but there is no direct evidence as yet that scaffolding proteins play an important role during oocyte activation.

Oocyte activation after ICSI

The technique of ICSI, first performed in the hamster (Uehara and Yanagimachi, 1976), was developed in the human as a treatment for male factor infertility in cases of fertilization failure (Palermo *et al.*, 1992). ICSI requires that laboratory personnel select a single spermatozoon and microinject it into the cytoplasm of a metaphase II-arrested oocyte. Although this procedure bypasses many of the normal sperm–oocyte interactions, most oocytes used for ICSI subsequently activate and become cleavage stage embryos capable of full term development. This finding suggests that the signalling events important for oocyte activation could be triggered by sperm components in the absence of sperm–ZP and sperm–oocyte plasma membrane interactions, supporting the idea that a sperm factor is responsible for the initiation of oocyte activation. Although ICSI clearly is not a physiological process, it is routinely used in the clinical setting and has been used to generate preimplantation embryos for transfer that have resulted in the births of thousands of children worldwide (Van Steirteghem *et al.*, 1998; Bonduelle *et al.*, 2002; see also http://www.cdc.gov/nccdphp/drh/art.htm). However, concerns have been raised that differences between oocyte activation events induced by a microinjected sperm and those induced by a sperm that interacts with the oocyte plasma membrane may be important later in development.

One difference between ICSI and normal fertilization is in the timing of calcium oscillations. Although the first calcium transient normally begins within a few minutes of sperm–oocyte fusion, calcium oscillations in ICSI begin after a delay of ~30 min to several hours in the mouse and human (Tesarik *et al.*, 1994; Nakano *et al.*, 1997; Yanagida *et al.*, 2001). This finding is consistent with the observation that other events of oocyte activation after ICSI, including polar body emission and pronuclear formation, are highly variable and can differ by several hours between oocytes that underwent ICSI at approximately the same time (Nagy *et al.*, 1994). This delay could be explained by differences in the length of time required for the oocyte to break down the sperm plasma membrane and remove the acrosome and its membrane components, allowing exposure of the sperm factor within the PT to the oocyte cytoplasm. In fact, differences in sperm treatments prior to ICSI that may result in different amounts of damage to the sperm plasma membrane have been shown to affect the timing of onset of calcium oscillations and oocyte activation after ICSI (Kasai *et al.*, 1999; Yanagida *et al.*, 2001). Since the oocyte cortical cytoskeleton participates in active removal of the sperm plasma membrane and PT (Sutovsky *et al.*, 1997), the fact that the microinjected sperm bypasses this interaction may explain the longer period of time before the sperm factor is able to initiate calcium oscillations. As a result, the oocyte may be 'older', as measured by the amount of time from the LH surge, at the time calcium oscillations begin. Physiological consequences of activating older oocytes have been determined in mouse model systems, and include a decrease in reproductive fitness and longevity in the resulting offspring (Tarin *et al.*, 1999, 2002).

Similarly, experiments in primates indicate that there is a delay in sperm chromatin decondensation after ICSI, particularly in the anterior portion of the sperm head (Ramalho-Santos *et al.*, 2000). This finding could be explained by the persistence of the acrosomal and PT structures overlying the anterior sperm head after ICSI that prevents the exposure of this region to decondensing factors in the oocyte cytoplasm (Ramalho-Santos *et al.*, 2000). This difference, combined with the preferential localization of the sex chromosomes in the anterior region of the sperm head (Luetjens *et al.*, 1999; Terada *et al.*, 2000; Sbracia *et al.*, 2002), may explain in part the increase in sex chromosomal abnormalities in children born after ICSI (Bonduelle *et al.*, 1998). Likely as a result of the delay in decondensation, DNA synthesis, which is required prior to syngamy and the first cleavage, is delayed in both the male and female pronuclei after ICSI (Ramalho-Santos *et al.*, 2000).

ICSI places sperm components into the oocyte cytoplasm that do not normally enter the oocyte, including the acrosome and outer plasma membrane of the sperm head. In addition, the cortical granules that normally undergo exocytosis at fertilization may be retained within the oocyte cytoplasm after ICSI. Although they are eventually degraded or lost, it is unknown if retention of these components could have later effects on development. Of particular concern are the digestive enzymes present within the acrosome that, when released into the oocyte cytoplasm, could affect the activities of important cellular proteins. One way to address this problem would be to ensure that the individual sperm

chosen for ICSI has undergone acrosomal exocytosis prior to microinjection. In addition to avoiding the release of digestive enzymes into the oocyte, removal of the acrosome would enhance the exposure of the sperm chromatin and PT, including the sperm factor, to the oocyte cytoplasm.

Conclusions

The signal transduction pathways responsible for mammalian oocyte activation are not completely understood, but recent studies have provided excellent candidates for some of the critically important molecules and signalling pathways that have long eluded identification. Key areas of future investigation will include the determination of additional molecules in the signal transduction pathways of oocyte activation, mechanisms of spatial regulation of these pathways, and the mechanisms by which calcium oscillations are decoded to result in oocyte activation and successful preimplantation embryo development. Elucidation of these signalling pathways will provide a scientific basis for the refinement of procedures used in the assisted reproductive technologies, particularly ICSI, such that these procedures may more faithfully replicate in-vivo oocyte activation and theoretically result in better outcomes for infertile couples.

Acknowledgements

The author thanks Bayard Storey, Stuart Moss and Brian Jones for critical reading of the manuscript, and Peter Sutovsky and Richard Oko for helpful discussions. This work was supported by a Burroughs Wellcome Fund Career Award in the Biomedical Sciences.

References

Abassi, Y.A., Carroll, D.J., Giusti, A.F., Belton, R.J. Jr and Foltz, K.R. (2000) Evidence that Src-type tyrosine kinase activity is necessary for initiation of calcium release at fertilization in sea urchin eggs. *Dev. Biol.*, **218**, 206–219.

Abbott, A.L. and Ducibella, T. (2001) Calcium and the control of mammalian cortical granule exocytosis. *Front. Biosci.*, **6**, D792–D806.

Berridge, M.J. (1993) Inositol trisphosphate and calcium signalling. *Nature*, **361**, 315–325.

Berridge, M.J., Bootman, M.D. and Lipp, P. (1998) Calcium—a life and death signal [news]. *Nature*, **395**, 645–648.

Billger, M., Wallin, M. and Karlsson, J.O. (1988) Proteolysis of tubulin and microtubule-associated proteins 1 and 2 by calpain I and II. Difference in sensitivity of assembled and disassembled microtubules. *Cell Calcium*, **9**, 33–44.

Bleil, J.D. and Wassarman, P.M. (1981) Mammalian sperm–egg interaction: Fertilization of mouse eggs triggers modifications of the major zona pellucida glycoprotein, ZP2. *Dev. Biol.*, **86**, 189–197.

Bonduelle, M., Wilikens, A., Buysse, A., Van Assche, E., Devroey, P., Van Steirteghem, A.C. and Liebaers, I. (1998) A follow-up study of children born after intracytoplasmic sperm injection (ICSI) with epididymal and testicular spermatozoa and after replacement of cryopreserved embryos obtained after ICSI. *Hum. Reprod.*, **13** (Suppl. 1), 196–207.

Bonduelle, M., Liebaers, I., Deketelaere, V., Derde, M.P., Camus, M., Devroey, P. and Van Steirteghem, A. (2002) Neonatal data on a cohort of 2889 infants born after ICSI (1991–1999) and of 2995 infants born after IVF (1983–1999). *Hum. Reprod.*, **17**, 671–694.

Cameron, A.M., Steiner, J.P., Roskams, A.J., Ali, S.M., Ronnett, G.V. and Snyder, S.H. (1995) Calcineurin associated with the inositol 1,4,5-trisphosphate receptor-FKBP12 complex modulates Ca^{2+} flux. *Cell*, **83**, 463–472.

Carroll, D.J., Ramarao, C.S., Mehlmann, L.M., Roche, S., Terasaki, M. and Jaffe, L.A. (1997) Calcium release at fertilization in starfish eggs is mediated by phospholipase Cγ. *J. Cell. Biol.*, **138**, 1303–1311.

Cascio, S.M. and Wassarman, P.M. (1982) Program of early development in the mammal: post-transcriptional control of a class of proteins synthesized by mouse oocytes and early embryos. *Dev. Biol.*, **89**, 397–408.

Chen, H.I. and Sudol, M. (1995) The WW domain of Yes-associated protein binds a proline-rich ligand that differs from the consensus established for Src homology 3-binding modules. *Proc. Natl Acad. Sci. USA*, **92**, 7819–7823.

Colledge, W.H., Carlton, M.B., Udy, G.B. and Evans, M.J. (1994) Disruption of c-mos causes parthenogenetic development of unfertilized mouse eggs [see comments]. *Nature*, **370**, 65–68.

Crabtree, G.R. (2001) Calcium, calcineurin and the control of transcription. *J. Biol. Chem.*, **276**, 2313–2316.

Cummins, J.M., Wakayama, T. and Yanagimachi, R. (1997) Fate of microinjected sperm components in the mouse oocyte and embryo. *Zygote*, **5**, 301–308.

Cuthbertson, K.S.R. and Cobbold, P.H. (1985) Phorbol ester and sperm activate mouse oocytes by inducing sustained oscillations in cell Ca^{2+}. *Nature*, **316**, 541–542.

Cuthbertson, K.S., Whittingham, D.G. and Cobbold, P.H. (1981) Free Ca^{2+} increases in exponential phases during mouse oocyte activation. *Nature*, **294**, 754–757.

Dale, B., DeFelice, L.J. and Ehrenstein, G. (1985) Injection of a soluble sperm fraction into sea urchin eggs triggers the cortical reaction. *Experientia*, **41**, 1068–1070.

De Koninck, P. and Schulman, H. (1998) Sensitivity of CaM kinase II to the frequency of Ca^{2+} oscillations. *Science*, **279**, 227–230.

Deguchi, R., Shirakawa, H., Oda, S., Mohri, T. and Miyazaki, S. (2000) Spatiotemporal analysis of Ca(2+) waves in relation to the sperm entry site and animal–vegetal axis during Ca(2+) oscillations in fertilized mouse eggs. *Dev. Biol.*, **218**, 299–313.

Dupont, G., McGuinness, O.M., Johnson, M.H., Berridge, M.J. and Borgese, F. (1996) Phospholipase C in mouse oocytes: Characterization of β and gamma isoforms and their possible involvement in sperm-induced Ca^{2+} spiking. *Biochem. J.*, **316**, 583–591.

Eckberg, W.R. and Szuts, E.Z. (1993) Diacylglycerol content of *Chaetopterus* oocytes during maturation and fertilization. *Dev. Biol.*, **159**, 732–735.

Endo, Y., Mattei, P., Kopf, G.S. and Schultz, R.M. (1987a) Effects of a phorbol ester on mouse eggs: dissociation of sperm receptor activity from acrosome reaction-inducing activity of the mouse zona pellucida protein, ZP3. *Dev. Biol.*, **123**, 574–577.

Endo, Y., Schultz, R.M. and Kopf, G.S. (1987b) Effects of phorbol esters and a diacylglycerol on mouse eggs: Inhibition of fertilization and modification of the zona pellucida. *Dev. Biol.*, **119**, 199–209.

Gallicano, G.I., McGaughey, R.W. and Capco, D.G. (1995) Protein kinase M, the cytosolic counterpart of protein kinase C, remodels the internal cytoskeleton of the mammalian egg during activation. *Dev. Biol.*, **167**, 482–501.

Gallicano, G.I., McGaughey, R.W. and Capco, D.G. (1997) Activation of protein kinase C after fertilization is required for remodeling the mouse egg into the zygote. *Mol. Reprod. Dev.*, **46**, 587–601.

Giles, R.E., Blanc, H., Cann, H.M. and Wallace, D.C. (1980) Maternal inheritance of human mitochondrial DNA. *Proc. Natl Acad. Sci. USA*, **77**, 6715–6719.

Giusti, A.F., Xu, W., Hinkle, B., Terasaki, M. and Jaffe, L.A. (2000) Evidence that fertilization activates starfish eggs by sequential activation of a Src-like kinase and phospholipase cgamma. *J. Biol. Chem.*, **275**, 16788–16794.

Glahn, D., Mark, S.D., Behr, R.K. and Nuccitelli, R. (1999) Tyrosine kinase inhibitors block sperm-induced egg activation in *Xenopus laevis*. *Dev. Biol.*, **205**, 171–180.

Gordo, A.C., Wu, H., He, C.L. and Fissore, R.A. (2000) Injection of sperm cytosolic factor into mouse metaphase II oocytes induces different developmental fates according to the frequency of [Ca(2+)](i) oscillations and oocyte age. *Biol. Reprod.*, **62**, 1370–1379.

Hague, F., Matifat, F., Brule, G. and Collin, T. (1999) The inositol (1,4,5)-trisphosphate 3-kinase of *Xenopus* oocyte is activated by CaMKII and involved in the regulation of InsP3-mediated Ca^{2+} release. *FEBS Lett.*, **449**, 70–74.

Homa, S.T. and Swann, K. (1994) A cytosolic sperm factor triggers calcium oscillations and membrane hyperpolarizations in human oocytes. *Hum. Reprod.*, **9**, 2356–2361.

Hutchison, C.A. 3rd, Newbold, J.E., Potter, S.S. and Edgell, M.H. (1974) Maternal inheritance of mammalian mitochondrial DNA. *Nature*, **251**, 536–538.

Igusa, Y. and Miyazaki, S. (1983) Effects of altered extracellular and

intracellular calcium concentration on hyperpolarizing responses of the hamster egg. *J. Physiol.*, **340**, 611–632.
Ishida, N., Tanaka, K., Tamura, T., Nishizawa, M., Okazaki, K., Sagata, N. and Ichihara, A. (1993) Mos is degraded by the 26S proteasome in a ubiquitin-dependent fashion. *FEBS Lett.*, **324**, 345–348.
Johnson, J., Bierle, B.M., Gallicano, G.I. and Capco, D.G. (1998) Calcium/calmodulin-dependent protein kinase II and calmodulin: regulators of the meiotic spindle in mouse eggs. *Dev. Biol.*, **204**, 464–477.
Jones, K.T. (1998) Protein kinase C action at fertilization: overstated or undervalued? *Rev. Reprod.*, **3**, 7–12.
Jones, K.T. and Nixon, V.L. (2000) Sperm-induced Ca(2+) oscillations in mouse oocytes and eggs can be mimicked by photolysis of caged inositol 1,4,5-trisphosphate: evidence to support a continuous low level production of inositol 1,4,5-trisphosphate during mammalian fertilization. *Dev. Biol.*, **225**, 1–12.
Jones, K.T., Carroll, J., Merriman, J.A., Whittingham, D.G. and Kono, T. (1995) Repetitive sperm-induced Ca^{2+} transients in mouse oocytes are cell cycle dependent. *Development*, **121**, 3259–3266.
Jones, K.T., Cruttwell, C., Parrington, J. and Swann, K. (1998) A mammalian sperm cytosolic phospholipase C activity generates inositol trisphosphate and causes Ca^{2+} release in sea urchin egg homogenates. *FEBS Lett.*, **437**, 297–300.
Jones, K.T., Matsuda, M., Parrington, J., Katan, M. and Swann, K. (2000) Different Ca^{2+}-releasing abilities of sperm extracts compared with tissue extracts and phospholipase C isoforms in sea urchin egg homogenate and mouse eggs. *Biochem. J.*, **346** (Pt 3), 743–749.
Kasai, T., Hoshi, K. and Yanagimachi, R. (1999) Effect of sperm immobilisation and demembranation on the oocyte activation rate in the mouse. *Zygote*, **7**, 187–193.
Kimura, Y., Yanagimachi, R., Kuretake, S., Bortkiewicz, H., Perry, A.C.F. and Yanagimachi, H. (1998) Analysis of mouse oocyte activation suggests the involvement of sperm perinuclear material. *Biol. Reprod.*, **58**, 1407–1415.
Kline, D. and Kline, J.T. (1992) Repetitive calcium transients and the role of calcium in exocytosis and cell cycle activation in the mouse egg. *Dev. Biol.*, **149**, 80–89.
Kline, D., Simoncini, L., Mandel, G., Maue, R.A., Kado, R.T. and Jaffe, L.A. (1988) Fertilization events induced by neurotransmitters after injection of mRNA in *Xenopus* eggs. *Science*, **241**, 464–467.
Kuretake, S., Kimura, Y., Hoshi, K. and Yanagimachi, R. (1996) Fertilization and development of mouse oocytes injected with isolated sperm heads. *Biol. Reprod.*, **55**, 789–795.
Kyozuka, K., Deguchi, R., Mohri, T. and Miyazaki, S. (1998) Injection of sperm extract mimics spatiotemporal dynamics of Ca^{2+} responses and progression of meiosis at fertilization of ascidian oocytes. *Development*, **125**, 4099–4105.
Latham, K.E., Garrels, J.I., Chang, C. and Solter, D. (1991) Quantitative analysis of protein synthesis in mouse embryos. I. Extensive reprogramming at the one- and two-cell stages. *Development*, **112**, 921–932.
Lechleiter, J.D., John, L.M. and Camacho, P. (1998) Ca^{2+} wave dispersion and spiral wave entrainment in *Xenopus laevis* oocytes overexpressing Ca^{2+} ATPases. *Biophys Chem.*, **72**, 123–129.
Li, W., Llopis, J., Whitney, M., Zlokarnik, G. and Tsien, R.Y. (1998) Cell-permeant caged InsP3 ester shows that Ca^{2+} spike frequency can optimize gene expression [see comments]. *Nature*, **392**, 936–941.
Livingston, B.T., VanWinkle, C.E. and Kinsey, W.H. (1998) Protein tyrosine kinase activity following fertilization is required to complete gastrulation, but not for initial differentiation of endoderm and mesoderm in the sea urchin embryo. *Dev. Biol.*, **193**, 90–99.
Lorca, T., Cruzalegui, F.H., Fesquet, D., Cavadore, J.C., Mery, J., Means, A. and Doree, M. (1993) Calmodulin-dependent protein kinase II mediates inactivation of MPF and CSF upon fertilization of *Xenopus* eggs. *Nature*, **366**, 270–273.
Luetjens, C.M., Payne, C. and Schatten, G. (1999) Non-random chromosome positioning in human sperm and sex chromosome anomalies following intracytoplasmic sperm injection. *Lancet*, **353**, 1240.
Malcov, M., Ben-Yosef, D., Glaser, T. and Shalgi, R. (1997) Changes in calpain during meiosis in the rat egg. *Mol. Reprod. Dev.*, **48**, 119–126.
Matifat, F., Hague, F., Brule, G. and Collin, T. (2001) Regulation of InsP3-mediated Ca^{2+} release by CaMKII in *Xenopus* oocytes. *Pflugers Arch.*, **441**, 796–801.
Matsumoto, Y. and Maller, J.L. (2002) Calcium, calmodulin and CaMKII requirement for initiation of centrosome duplication in *Xenopus* egg extracts. *Science*, **295**, 499–502.
Mehlmann, L.M., Carpenter, G., Rhee, S.G. and Jaffe, L.A. (1998) SH2 domain-mediated activation of phospholipase Cγ is not required to initiate Ca^{2+} release at fertilization of mouse eggs. *Dev. Biol.*, **203**, 221–232.
Mellgren, R.L. (1991) Proteolysis of nuclear proteins by mu-calpain and m-calpain. *J. Biol. Chem.*, **266**, 13920–13924.
Miyazaki, S. and Igusa, Y. (1981) Fertilization potential in golden hamster eggs consists of recurring hyperpolarizations. *Nature*, **290**, 702–704.
Miyazaki, S., Hashimoto, N., Yoshimoto, Y., Kishimoto, T., Igusa, Y. and Hiramoto, Y. (1986) Temporal and spatial dynamics of the periodic increase in intracellular free calcium at fertilization of golden hamster eggs. *Dev. Biol.*, **118**, 259–267.
Miyazaki, S.-I., Yuzaki, M., Nakada, K., Shirakawa, H., Nakanishi, S., Nakade, S. and Mikoshiba, K. (1992) Block of Ca^{2+} wave and Ca^{2+} oscillation by antibody to the inositol 1,4,5-trisphosphate receptor in fertilized hamster eggs. *Science*, **257**, 251–255.
Miyazaki, S.-I., Shirakawa, H., Nakada, K. and Honda, Y. (1993) Essential role of the inositol 1,4,5-trisphosphate receptor/Ca^{2+} release channel in Ca^{2+} waves and Ca^{2+} oscillations at fertilization of mammalian eggs. *Dev. Biol.*, **158**, 62–78.
Moore, G.D., Kopf, G.S. and Schultz, R.M. (1993) Complete mouse egg activation in the absence of sperm by stimulation of an exogenous G protein-coupled receptor. *Dev. Biol.*, **159**, 669–678.
Moos, J., Visconti, P.E., Moore, G.D., Schultz, R.M. and Kopf, G.S. (1995) Potential role of mitogen-activated protein kinase in pronuclear envelope assembly and disassembly following fertilization of mouse eggs. *Biol. Reprod.*, **53**, 692–699.
Murray, A. and Hunt, T. (1993) *The Cell Cycle.* Oxford University Press, Oxford, UK, p. 251.
Nagy, Z.P., Liu, J., Joris, H., Devroey, P. and Van Steirteghem, A. (1994) Time-course of oocyte activation, pronucleus formation and cleavage in human oocytes fertilized by intracytoplasmic sperm injection. *Hum. Reprod.*, **9**, 1743–1748.
Nakano, Y., Shirakawa, H., Mitsuhashi, N., Kuwabara, Y. and Miyazaki, S. (1997) Spatiotemporal dynamics of intracellular calcium in the mouse egg injected with a spermatozoon. *Mol. Hum. Reprod.*, **3**, 1087–1093.
Nishizawa, M., Furuno, N., Okazaki, K., Tanaka, H., Ogawa, Y. and Sagata, N. (1993) Degradation of Mos by the N-terminal proline (Pro2)-dependent ubiquitin pathway on fertilization of *Xenopus* eggs: possible significance of natural selection for Pro2 in Mos. *EMBO J.*, **12**, 4021–4027.
Oda, S., Deguchi, R., Mohri, T., Shikano, T., Nakanishi, S. and Miyazaki, S. (1999) Spatiotemporal dynamics of the [Ca2+]i rise induced by microinjection of sperm extract into mouse eggs: preferential induction of a Ca2+ wave from the cortex mediated by the inositol 1,4,5-trisphosphate receptor. *Dev. Biol.*, **209**, 172–185.
Oko, R., Aul, R.B., Wu, A. and Sutovsky, P. (2001) The sperm head cytoskeleton. In Robaire, B., Chemes, H. and Morales, C.R. (eds) *Andrology in the 21st Century.* Medimond Publishing Co., Englewood, NJ, USA, pp. 37–51.
Ozil, J.P. (1990) The parthenogenetic development of rabbit oocytes after repetitive pulsatile electrical stimulation. *Development*, **109**, 117–127.
Palermo, G., Joris, H., Devroey, P. and Van Steirteghem, A.C. (1992) Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet*, **340**, 17–18.
Parrington, J., Swann, K., Shevchenko, V.I., Sesay, A.K. and Lai, F.A. (1996) Calcium oscillations in mammalian eggs triggered by a soluble sperm protein. *Nature*, **379**, 364–368.
Parrington, J., Jones, M.L., Tunwell, R., Devader, C., Katan, M. and Swann, K. (2002) Phospholipase C isoforms in mammalian spermatozoa: potential components of the sperm factor that causes Ca^{2+} release in eggs. *Reproduction*, **123**, 31–39.
Perreault, S.D. (1990) Regulation of sperm nuclear reactivation during fertilization. In Bavister, B.D., Cummins, J. and Roldan, E.R.S. (eds) *Fertilization in Mammals.* Serono Symposia, USA, Norwell, MA, USA, pp. 285–296.
Perreault, S.D., Barbee, R.R. and Slott, V.L. (1988) Importance of glutathione in the acquisition and maintenance of sperm nuclear decondensing activity in maturing hamster oocytes. *Dev. Biol.*, **125**, 181–186.
Perry, A.C., Wakayama, T., Cooke, I.M. and Yanagimachi, R. (2000) Mammalian oocyte activation by the synergistic action of discrete sperm head components: induction of calcium transients and involvement of proteolysis. *Dev. Biol.*, **217**, 386–393.
Ramalho-Santos, J., Sutovsky, P., Simerly, C., Oko, R., Wessel, G.M., Hewitson, L. and Schatten, G. (2000) ICSI choreography: fate of sperm structures after monospermic rhesus ICSI and first cell cycle implications. *Hum. Reprod.*, **15**, 2610–2620.

Reimann, J.D. and Jackson, P.K. (2002) Emi1 is required for cytostatic factor arrest in vertebrate eggs. *Nature*, **416**, 850–854.

Rhee, S.G. (2001) Regulation of phosphoinositide-specific phospholipase C. *Ann. Rev. Biochem.*, **70**, 281–312.

Santella, L. (1998) The role of calcium in the cell cycle: facts and hypotheses. *Biochem. Biophys Res. Commun.*, **244**, 317–324.

Sbracia, M., Baldi, M., Cao, D., Sandrelli, A., Chiandetti, A., Poverini, R. and Aragona, C. (2002) Preferential location of sex chromosomes, their aneuploidy in human sperm, and their role in determining sex chromosome aneuploidy in embryos after ICSI. *Hum. Reprod.*, **17**, 320–324.

Schatten, G. (1994) The centrosome and its mode of inheritance: the reduction of the centrosome during gametogenesis and its restoration during fertilization. *Dev. Biol.*, **165**, 299–335.

Schultz, R.M. and Kopf, G.S. (1995) Molecular basis of mammalian egg activation. In Pederson, R.A. and Schatten, G. (eds) *Current Topics in Developmental Biology*, Vol. 30. Academic Press, Inc., San Diego, USA, pp. 21–62.

Schuster, S., Marhl, M. and Hofer, T. (2002) Modelling of simple and complex calcium oscillations. *Eur. J. Biochem.*, **269**, 1333–1355.

Sette, C., Bevilacqua, A., Bianchini, A., Mangia, F., Geremia, R. and Rossi, P. (1997) Parthenogenetic activation of mouse eggs by microinjection of a truncated c-kit tyrosine kinase present in spermatozoa. *Development*, **124**, 2267–2274.

Sette, C., Bevilacqua, A., Geremia, R. and Rossi, P. (1998) Involvement of phospholipase Cγl in mouse egg activation induced by a truncated form of the C-kit tyrosine kinase present in spermatozoa. *J. Cell. Biol.*, **142**, 1063–1074.

Shilling, F., Mandel, G. and Jaffe, L.A. (1990) Activation by serotonin of starfish eggs expressing the rat serotonin 1c receptor. *Cell. Regul.*, **1**, 465–469.

Simerly, C., Wu, G.J., Zoran, S., Ord, T., Rawlins, R., Jones, J., Navara, C., Gerrity, M., Rinehart, J. and Binor, Z. (1995) The paternal inheritance of the centrosome, the cell's microtubule-organizing center, in humans, and the implications for infertility. *Nature Med.*, **1**, 47–52.

Stice, S.L. and Robl, J.M. (1990) Activation of mammalian oocytes by a factor obtained from rabbit sperm. *Mol. Reprod. Dev.*, **25**, 272–280.

Stith, B.J., Goalstone, M., Silva, S. and Jaynes, C. (1993) Inositol 1,4,5-trisphosphate mass changes from fertilization through first cleavage in *Xenopus laevis*. *Mol. Biol. Cell*, **4**, 435–443.

Stith, B.J., Woronoff, K., Espinoza, R. and Smart, T. (1997) *sn*-1,2-diacylglycerol and choline increase after fertilization in *Xenopus laevis*. *Mol. Biol. Cell*, **8**, 755–765.

Sudol, M., Chen, H.I., Bougeret, C., Einbond, A. and Bork, P. (1995) Characterization of a novel protein-binding module—the WW domain. *FEBS Lett.*, **369**, 67–71.

Sutovsky, P., Navara, C.S. and Schatten, G. (1996) Fate of the sperm mitochondria, and the incorporation, conversion, and disassembly of the sperm tail structures during bovine fertilization. *Biol. Reprod.*, **55**, 1195–1205.

Sutovsky, P., Oko, R., Hewitson, L. and Schatten, G. (1997) The removal of the sperm perinuclear theca and its association with the bovine oocyte surface during fertilization. *Dev. Biol.*, **188**, 75–84.

Sutovsky, P., Moreno, R.D., Ramalho-Santos, J., Dominko, T., Simerly, C. and Schatten, G. (1999) Ubiquitin tag for sperm mitochondria. *Nature*, **402**, 371–372.

Sutovsky, P., Moreno, R.D., Ramalho-Santos, J., Dominko, T., Simerly, C. and Schatten, G. (2000) Ubiquitinated sperm mitochondria, selective proteolysis, and the regulation of mitochondrial inheritance in mammalian embryos. *Biol. Reprod.*, **63**, 582–590.

Sutovsky, P., Wu, A., Takahashi, D., Benmusa, A., Schatten, G., Dominko, T. and Oko, R. (2001) Oocyte activation by mammalian sperm perinuclear theca proteins PT 32 and tyrosine kinase c-Yes. *Mol. Biol. Cell*, **12** (Suppl.), 114a.

Swann, K. (1990) A cytosolic sperm factor stimulates repetitive calcium increases and mimics fertilization in hamster eggs. *Development*, **110**, 1295–1302.

Swann, K. (1992) Different triggers for calcium oscillations in mouse eggs involve a ryanodine-sensitive calcium store. *Biochem. J.*, **287** (Pt 1), 79–84.

Talmor, A., Kinsey, W.H. and Shalgi, R. (1998) Expression and immunolocalization of p59c-fyn tyrosine kinase in rat eggs. *Dev. Biol.*, **194**, 38–46.

Tarin, J.J., Perez-Albala, S., Aguilar, A., Minarro, J., Hermenegildo, C. and Cano, A. (1999) Long-term effects of postovulatory aging of mouse oocytes on offspring: a two-generational study. *Biol. Reprod.*, **61**, 1347–1355.

Tarin, J.J., Perez-Albala, S., Perez-Hoyos, S. and Cano, A. (2002) Postovulatory aging of oocytes decreases reproductive fitness and longevity of offspring. *Biol. Reprod.*, **66**, 495–499.

Terada, Y., Luetjens, C.M., Sutovsky, P. and Schatten, G. (2000) Atypical decondensation of the sperm nucleus, delayed replication of the male genome, and sex chromosome positioning following intracytoplasmic human sperm injection (ICSI) into golden hamster eggs: does ICSI itself introduce chromosomal anomalies? *Fertil. Steril.*, **74**, 454–460.

Tesarik, J., Sousa, M. and Testart, J. (1994) Human oocyte activation after intracytoplasmic sperm injection [published erratum appears in vol. 9, p. 968]. *Hum. Reprod.*, **9**, 511–518.

Tompa, P., Toth-Boconadi, R. and Friedrich, P. (2001) Frequency decoding of fast calcium oscillations by calpain. *Cell Calcium*, **29**, 161–170.

Turner, P.R., Sheetz, M.P. and Jaffe, L.A. (1984) Fertilization increases the polyphosphoinositide content of sea urchin eggs. *Nature*, **310**, 414–415.

Uehara, T. and Yanagimachi, R. (1976) Microsurgical injection of spermatozoa into hamster eggs with subsequent transformation of sperm nuclei into male pronuclei. *Biol. Reprod.*, **15**, 467–470.

Van Blerkom, J. (1981) Structural relationship and posttranslational modification of stage-specific proteins synthesized during early preimplantation development in the mouse. *Proc. Natl Acad. Sci. USA*, **78**, 7629–7633.

Van Steirteghem, A., Bonduelle, M., Hamberger, L., Joris, H., Royere, D. and Tarlatzis, B.C. (1998) Assisted reproduction by intracytoplasmic sperm injection: a survey on the clinical experience in 1994 and the children born after ICSI, carried out until 31 December 1993. ESHRE Task Force on Intracytoplasmic Sperm Injection. European Society for Human Reproduction and Embryology. *Hum. Reprod.*, **13**, 1737–1746.

Verlhac, M.H., de Pennart, H., Maro, B., Cobb, M.H. and Clarke, H.J. (1993) MAP kinase becomes stably activated at metaphase and is associated with microtubule-organizing centers during meiotic maturation of mouse oocytes. *Dev. Biol.*, **158**, 330–340.

Vitullo, A.D. and Ozil, J.P. (1992) Repetitive calcium stimuli drive meiotic resumption and pronuclear development during mouse oocyte activation. *Dev. Biol.*, **151**, 128–136.

Watanabe, N., Vande Woude, G.F., Ikawa, Y. and Sagata, N. (1989) Specific proteolysis of the c-mos proto-oncogene product by calpain on fertilization of *Xenopus* eggs. *Nature*, **342**, 505–511.

Williams, C.J., Schultz, R.M. and Kopf, G.S. (1992) Role of G proteins in mouse egg activation: Stimulatory effects of acetylcholine on the ZP_2 to ZP_{2f} conversion and pronuclear formation in eggs expressing a functional m1 muscarinic receptor. *Dev. Biol.*, **151**, 288–296.

Williams, C.J., Mehlmann, L.M., Jaffe, L.A., Kopf, G.S. and Schultz, R.M. (1998) Evidence that Gq family G proteins do not function in mouse egg activation at fertilization. *Dev. Biol.*, **198**, 116–127.

Winston, N.J. and Maro, B. (1995) Calmodulin-dependent protein kinase II is activated transiently in ethanol-stimulated mouse oocytes. *Dev. Biol.*, **170**, 350–352.

Wolny, Y.M., Fissore, R.A., Wu, H., Reis, M.M., Colombero, L.T., Ergün, B., Rosenwaks, Z. and Palermo, G.D. (1999) Human glucosamine-6-phosphate isomerase, a homologue of hamster oscillin, does not appear to be involved in Ca^{2+} release in mammalian oocytes. *Mol. Reprod. Dev.*, **52**, 277–287.

Wu, A., Sutovsky, P., Dominko, T., Schatten, G., Gong, J. and Oko, R. (2001) PT32, a candidate for a sperm borne-egg activating factor. *J. Androl.,* **22** (Suppl.), 95.

Xu, Z., Kopf, G.S. and Schultz, R.M. (1994) Involvement of inositol 1,4,5-trisphosphate-mediated Ca^{2+} release in early and late events of mouse egg activation. *Development*, **120**, 1851–1859.

Xu, Z., Abbott, A., Kopf, G.S., Schultz, R.M. and Ducibella, T. (1997) Spontaneous activation of ovulated mouse eggs: Time-dependent effects on M-phase exit, cortical granule exocytosis, maternal mRNA recruitment, and IP3-sensitivity. *Biol. Reprod.*, **57**, 743–750.

Yanagida, K., Katayose, H., Hirata, S., Yazawa, H., Hayashi, S. and Sato, A. (2001) Influence of sperm immobilization on onset of Ca(2+) oscillations after ICSI. *Hum. Reprod.*, **16**, 148–152.

Yanagimachi, R. (1994) Mammalian fertilization. In Knobil, E. and Neill, J.D. (eds) *The Physiology of Reproduction.* Raven Press Ltd, New York, USA, 189–317.