Mammalian oocytes undergo significant growth during oogenesis and experience extensive cytoplasmic and nuclear modifications immediately before ovulation in a process commonly referred to as oocyte maturation. These changes are intended to maximize the developmental success after fertilization. Entry of a spermatozoon into the oocyte, which occurs a few hours after ovulation, initiates long-lasting oscillations in the free intracellular calcium ([Ca²⁺]ᵢ) that are responsible for all events of oocyte activation and the initiation of the developmental programme that often culminates in the birth of young. Nevertheless, the cellular and molecular changes that occur during maturation to optimize development are transient, and exhibit rapid deterioration. Moreover, fertilization of oocytes after an extended residence in the oviduct (or in culture) initiates a different developmental programme, one that is characterized by fragmentation, programmed cell death, and abnormal development. Inasmuch as [Ca²⁺]ᵢ oscillations can trigger both developmental programmes in mammalian oocytes, this review addresses one of the mechanism(s) possibly used by spermatozoa to initiate these persistent [Ca²⁺]ᵢ responses, and the cellular and molecular changes that may underlie the postovulatory cellular fragmentation of ageing mammalian oocytes.
place that are intended to initiate and regulate resumption of meiosis, prevent polyspermic fertilization, and promote exit from the MII arrest (reviewed by Wassarman and Albertini, 1994); in other words, these changes make oocytes ‘activation competent’. Re-initiation of meiosis is made possible by the activation of maturation-promoting factor (MPF), which comprises the products of the p34 cdc2 and cyclin genes (Draetta and Beach, 1988). The activation of MPF is responsible for inducing germinal vesicle breakdown and promoting progression into meiosis II (Dekel, 1996). In addition to MPF, activation of mitogen-activated protein kinase (MAPK) plays a role in regulating the transition from meiosis I to meiosis II and, more importantly, in the MII stage arrest (reviewed by Ferrel, 1999). These kinases, mostly in conjunction with other kinases or phosphatases, may also regulate the completion of ‘cytoplasmic maturation’ that occurs before ovulation.

One of the cytoplasmic functions that is modified during maturation is the ability to release Ca^{2+} (reviewed by Carroll et al., 1996). Sperm-induced [Ca^{2+}]; oscillations are the hallmark of mammalian fertilization and although the mechanism(s) used by the spermatozoan to initiate these oscillations is still under investigation, it has become apparent that the inositol 1,4,5-trisphosphate receptor isoform-1 (IP_{3}R-1) is the channel of choice during fertilization (Miyazaki et al., 1993). In support of this, the sensitivity of IP_{3}R-1, as evidenced by enhanced Ca^{2+} responses to stimulation with IP_{3}, increases gradually during maturation from the GV stage and peaks at MII (Fuijwara et al., 1993; Mehlmann and Kline, 1994; He et al., 1997). Fertilization of oocytes at different stages of maturation induces oscillations only in oocytes that have nearly completed maturation (Fuijwara et al., 1993; Jones et al., 1995), indicating that a maximally sensitized IP_{3}R system is required for the initiation of oscillations in mammalian oocytes.

Several molecular mechanisms may account for the heightened sensitivity of the IP_{3}R-1 at MII. First, a noticeable albeit not overwhelming increase in the density of these receptors has been observed during maturation in oocytes of mammalian and non-mammalian species (Mehlmann et al., 1996; He et al., 1997; Kume et al., 1997). Second, the phosphorylation status of the receptor may change during maturation, resulting in enhanced conductivity of the receptor. The IP_{3}R-1 has multiple phosphorylation consensus sequences and phosphorylation of selected sites affects the conductivity of the channel (Patel et al., 1999), although the phosphorylation status of the IP_{3}R-1 in mammalian oocytes and zygotes has not been investigated. In addition, IP_{3}R forms complexes with other proteins and this, in turn, may also alter the conductivity of the channel (Bultynck et al., 1999). Other changes that may augment IP_{3}R-1-mediated Ca^{2+} release during maturation include increased Ca^{2+} store content and redistribution of the endoplasmic reticulum (ER) (Jones et al., 1995; Shiraishi et al., 1995). Therefore, several changes that occur concomitantly during oocyte maturation are intended to maximize Ca^{2+} release at the time of fertilization, underscoring the significance of this signalling event not only for exit of meiosis, but also for increased developmental success of early embryos. On the other hand, prolonged residence in the oviduct without fertilization results in gradual inactivation of the Ca^{2+} release mechanisms, and this may be responsible, at least in part, for the fragmentation and poor developmental success of zygotes that result from delayed fertilization.

**Oocyte activation: signalling mechanism for sperm-induced [Ca^{2+}]; oscillations**

The signalling mechanism used by the spermatozoa to initiate and maintain [Ca^{2+}]; oscillations has not been elucidated, although several hypotheses have been proposed (Schultz and Kopf, 1995; Stricker, 1999); the evidence for and against each hypothesis has been reviewed recently and therefore will not be discussed here (Swann and Lai, 1997; Swann et al., 2001). Instead, the focus will be on some of the less thoroughly reviewed aspects of the ‘Fusion/Sperm Factor (SF)’ hypothesis. This hypothesis proposes that a factor from the spermatozoan initiates [Ca^{2+}]; oscillations when delivered into the ooplasm upon fusion of the gamete membranes. The strongest experimental evidence in support of this hypothesis emanates from the ability of spermatozoa to initiate [Ca^{2+}]; oscillations, when injected directly into the ooplasm, despite bypassing the presumably required interactions of the gamete membranes (Tesarik et al., 1994; Nakano et al., 1997). Likewise, the demonstrated ability of sperm fraction or factor preparations to initiate Ca^{2+} release when they are injected into oocytes of several mammalian species provides additional support for this hypothesis (Wu et al., 1997; Swann et al., 2001). Nevertheless, the lack of success in the isolation and characterization of the sperm active molecule(s) has delayed progress. The following discussion will focus on some of the outstanding issues such as the number of active SF molecules, the location of SF, and the regulation of its release.

**How many sperm factors?**

Injection of SFs into mammalian oocytes can initiate, and in many cases replicate, the Ca^{2+} responses induced by fertilization. In most studies the injected SFs were obtained after sonication of spermatozoa or after cycles of freezing and thawing, and the processed supernatants were denominated soluble or cytosolic SFs, since these lysis procedures are generally expected to release most of the cytosolic components of the cell. However, given the special configuration of sperm cells and their lack of obvious cytoplasm, it is presently unknown which of the sperm domains contribute proteins to these soluble fractions. Moreover, it has become readily apparent that the soluble sperm proteins are not the only fractions able to trigger Ca^{2+} release. Results from experiments using the intracytoplasmic sperm injection (ICSI) technique, which makes
possible the injection of whole spermatozoa into oocytes, showed that injection of demembranated sperm heads, which are consequently devoid of soluble SFs, is still able to induce Ca$^{2+}$ release (Perry et al., 2000). These results were interpreted to mean that the spermatozoa may be equipped with more than one SF, and that the soluble SF may not be involved in the persistence of oscillations during fertilization as these fractions are likely to be lost before gamete fusion. This interpretation may be premature for several reasons. First, the protein compositions of the soluble and the less soluble SFs have not been compared extensively. Second, these experiments were carried out exclusively using mouse spermatozoa that are known to contain lower soluble Ca$^{2+}$ activity than spermatozoa from hamsters or pigs (Swann et al., 2001; M. Kurokawa, H. Wu and R. A. Fissore, unpublished), which have been used mainly for the preparation of soluble SFs. Remarkably, our unpublished chromatographic fractionation studies of soluble and less soluble (Triton X-resistant) pig SFs using Superose 12 and hydroxyapatite columns reveal that the Ca$^{2+}$ active molecules from both SFs exhibit very similar elution profiles in both columns, indicating that the same Ca$^{2+}$ active molecule(s) may be present in both soluble and less soluble fractions. Whether the putative molecule has diverse locations in the spermatozoon, or may be associated with different proteins that in turn regulate or modify its release remains to be elucidated.

Where is the sperm factor(s)?

Although the results obtained using sperm heads demembranated with Triton X-100 underscored the possible functional significance of the detergent-resistant SF fraction, they do not reveal the sperm structure(s) that harbours the Ca$^{2+}$-active molecule. However, these studies indicate that the theca, the sperm perinuclear material, is the structure most likely to carry the Ca$^{2+}$-releasing molecule(s). This interpretation is based on the finding that after Triton X-100 treatment, the theca is the only structure that remains associated with the sperm nucleus (Kimura et al., 1998; Perry et al., 1999). In support of this notion, removal of the sperm theca using SDS and trypsin was shown to eliminate the ability of mouse sperm heads to activate oocytes after ICSI (Kimura et al., 1998). Nonetheless, several questions cannot be resolved by the experimental approach just described. First, a significant amount of soluble SF is released during treatment with Triton X-100, and the location of such SF cannot be determined. Second, whether or not the theca-associated Ca$^{2+}$ activity is removed, or simply inactivated, by the trypsin and SDS treatments was not ascertained because the Ca$^{2+}$ activity was never recovered after the treatments. Finally, it remains to be demonstrated whether the theca may serve as an anchor from which the release of the factor takes place, or whether the active molecule(s) of the SF is an integral component of the theca. These questions are easily testable and the answers should contribute to the elucidation of the location of the SFs in the spermatozoon as well as the mode of release of the factor.

Mode of release of sperm factor

How the Ca$^{2+}$-active component of the spermatozoon is released and the oocyte conditions that promote or regulate the release are presently not known. For instance, is the factor gradually released or, alternatively, does it move away from the sperm nucleus soon after fusion? Evidence in support of the latter possibility is provided by the finding that soon after entry of the spermatozoon into the oocyte, the increase in [Ca$^{2+}$], appears to originate away from location of entry of the spermatozoon and opposite to the animal pole (Kline et al., 1999; Deguchi et al., 2000). Likewise, the demonstration that fusion of a cytoplasm from a telophase-stage fertilized mouse oocyte to an unfertilized oocyte can induce activation indicates that the factor has a cytoplasmic location, although [Ca$^{2+}$], oscillations were not measured in this study (Ogonuki et al., 2001). As suggestive as these studies are of an early cytosolic location of the factor, it is plausible that a fraction of the factor remains associated with the sperm nucleus several hours after sperm entry. What is more, it is possible that the Triton X-100-resistant SF fraction remains associated with the nucleus for several hours, and is responsible for the long duration of the oscillations, especially in large domestic species in which oscillations are known to last in excess of 10 h (Fig. 1). Nevertheless, despite these appealing models of release, basic functional and molecular evidence is needed to elucidate the regulation of release of the factor.

Although the fate of the Ca$^{2+}$-active molecule of the spermatozoon within the first few hours after fusion is presently unknown, it appears that by the time of pronuclear formation, at least a portion of it is associated with the pronuclear structures. Early studies of mouse zygotes by Kono et al. (1995) reported that transfer of male or female pronuclei into MII oocytes was able to reinitiate oscillations, whereas fusion of comparably sized cytoplasts was unable to do so. Notably, studies involving injection of physiological amounts of pig SF into mouse oocytes showed that although these fractions can initiate oscillations efficiently, they are not as proficient at associating with the pronuclei. For example, in oocytes fertilized by ICSI, pronuclear envelope breakdown, either spontaneous or induced by exposure to okadaic acid, was accompanied by an increase in [Ca$^{2+}$], whereas SF-generated zygotes failed to show such a response (Gordo et al., 2002a). In addition, oscillations ceased earlier in these oocytes compared with those fertilized normally, even when meiotic exit was inhibited by the addition of colcemid (Gordo et al., 2002a). Thus, the association of the Ca$^{2+}$-active molecule of spermatozoa with pronuclear structures may reflect the presence of an intact SF molecule(s), or SF molecules associated with the correct partners. Such an association of SF with pronuclear binding partners may affect the stability of the factor. Elucidation of the domain(s) or molecules that mediate the association or targeting of SF would be of interest.
It is important to note that notwithstanding the success of ICSI in several mammalian species, its effectiveness is far from acceptable in large domestic species (Horiuchi and Numabe, 1999; Suttner et al., 2000). The most important limitation appears to be an inability of injected spermatozoa to deliver an appropriate activation stimulus in the oocytes of these species. Therefore, although the Fusion/SF hypothesis may represent the mechanism responsible for oscillations in mammalian species, we are far from understanding fundamental aspects of its mode of release, location and activation, and must contemplate the possibility of significant species-specific variability in mammals.

Postovulatory oocyte ageing: a prelude to fragmentation and cell death

Oocyte maturation is timed in such a manner that sperm entry, activation and developmental competence are maximal within a few hours after ovulation; in mice nearly 100% of oocytes are expected to be fertilized within 6 h of ovulation (Braden and Austin, 1954). Nonetheless, interference with this timing is now occurring with increased frequency, especially in species in which humans intervene to control the number of ovulations or the genetic makeup of the progeny. The end-result is fertilization of ‘aged oocytes’. Zygotes from aged oocytes reportedly show limited developmental success and undergo programmed cell death or apoptosis. Apoptosis, a process of cell selection present in all organisms, mediates the elimination of damaged cells and the strict selection, and consequent attrition, of germ cells that takes place in the gonads before and after birth (reviewed by Dunkel et al., 1997; Morita and Tilly, 1999). The molecules involved in programmed cell death have been extensively reviewed elsewhere (Adams and Cory, 1998; Thornberry and Lazebnik, 1998; Heiden and Thompson, 1999); therefore, we will focus on new evidence involving mechanisms that may underlie apoptosis or fragmentation that ensues after fertilization or activation of ovulated, aged mammalian oocytes.
Fertilization or activation induces a different developmental outcome in aged oocytes

The negative impact of delayed fertilization on reproductive success has been known for several decades, but the stage(s) at which embryonic development is compromised, and the mechanism(s) responsible for the developmental failure, were not apparent from earlier studies. Marston and Chang (1964) noted that postovulatory ageing, in mice, hindered embryo development soon after fertilization as they observed that within 24 h after fertilization, grossly mis-shaped and fragmented zygotes developed when insemination was delayed for more than 12 h after ovulation. Subsequent studies in mice confirmed (Juettgen and Bavister, 1983) and extended these findings by showing that aged oocytes exhibit abnormal morphological characteristics before fertilization, such as the disappearance of the microfilament-rich area over the meiotic spindle, disruption of and abnormal spindle location, and chromatin disorganization (Webb et al., 1986). These changes appear to be the consequence of decreasing MPF and MAPK activities, and seem to develop faster in the oviduct than in vitro (Xu et al., 1997; Abbott et al., 1998). This progressive deterioration leads to oocyte fragmentation even in the absence of fertilization (Takase et al., 1995; Fujino et al., 1996; Tarin et al., 2001). Both spontaneous and fertilization-induced fragmentation may have a genetic component since they appear, at least in mice, to be influenced by the strain(s) under investigation (Warner, et al., 1998; Hawes et al., 2001).

As our understanding of oocyte ageing and its developmental consequences has gradually progressed, insights into the mechanism and role of apoptosis have been revealed in several cellular paradigms. Of particular relevance was the demonstration of the postnatal decline in the female germ cell population is mediated by apoptosis (De Pol et al., 1997; Tilly, 2001). Nevertheless, the molecular mechanism responsible for postovulatory fragmentation of oocytes was not ascertained until Takase et al. (1995) showed that ovulated unfertilized mouse oocytes cultured for extended periods undergo cytoplasmic fragmentation accompanied by DNA fragmentation, as determined by terminal deoxynucleotidyl transferase (TdT) nick-end labelling (TUNEL), both of which are classical signs of programmed cell death. Since then, several studies have extended those results by showing that apoptosis is not only observed after fertilization of aged oocytes, or of oocytes from ageing females, but also after exposure of newly ovulated mouse oocytes to chemical or chemotherapeutic compounds (Weil et al., 1996; Perez et al., 1997, 1999a).

Apoptosis requires the expression and activation of specific genes involved in the execution of the cell suicide programme. For the purpose of this review, suffice it to say that mouse oocytes express most of the genes known to participate in this programme (Jurisicova et al. 1998; Exley et al., 1999; Rucker et al., 2000). Mouse oocytes express several caspases, which are proteases responsible for the dismantling of cells, and several of the anti- and pro-apoptotic members of the Bcl-2 gene family. Pro-apoptotic family members promote the release of cytochrome c, a component of the mitochondrial respiratory chain that induces the activation of caspases when released into the cytosol, whereas anti-apoptotic members block the release of this factor (Adams and Cory, 1998). Results from gene disruption and overexpression studies in the ovary underscore the importance of these molecules in oocyte cell death. Oocytes from mice lacking caspase 2 or Bax, or overexpressing Bcl-2, are resistant to apoptosis induced by exposure to chemotherapeutic agents or that occurs spontaneously after ovulation (Bergeron et al., 1998; Morita et al., 1999; Perez et al., 1999b). Hence, as proposed for somatic cells (Olthuijs et al., 1993), alterations in the amounts or ratios of the protein products of these genes may underlie fragmentation and cell death in aged oocytes too.

Mitochondrial dysfunction in aged oocytes

Mitochondrial dysfunction is likely to play an important role in the predisposition of aged oocytes to spontaneous and fertilization- or activation-induced fragmentation. Mitochondria harbour important anti-apoptotic as well as apoptogenic compounds, and serve as the bioenergetic centre of the cell by generating ATP (Green and Reed, 1998; Wang, 2001). Therefore, disruption of any of the mitochondrial membranes, or of its ability to generate ATP, is likely to have negative consequences on cell proliferation and survival. With respect to the latter, the ATP content of mouse oocytes appears to decrease steadily with time of culture (Chi et al., 1988), and in human oocytes, there may be a close association between developmental competence and intra-oocyte concentrations of ATP (Van Blerkom et al., 1995). Extended culture times also decrease the mitochondrial membrane potential in human oocytes (Wilding et al., 2001), possibly resulting in swelling of the mitochondrial matrix. In somatic cells, mitochondrial swelling precedes the rupture of the outer mitochondrial membrane, making possible the release of cytochrome c and other apoptogenic products (Wang, 2001). Advanced stages of mitochondrial dysfunction are also associated with accumulation of reactive oxygen species, which further compromise cell survival. Importantly, exposure of oocytes to different concentrations of hydrogen peroxide was shown to induce release of cytochrome c and to lower the mitochondrial membrane potential (Liu et al., 2000). Moreover, addition of dithiothreitol, a reducing agent, to the culture medium prevented some of the detrimental effects of oocyte ageing on fertilization and development (Tarin et al., 1998).

Results demonstrating that injection of purified mitochondria from somatic cells inhibits spontaneous postovulatory oocyte fragmentation in certain strains of mice (Perez et al., 2000) are consistent with the involvement of mitochondrial dysfunction in oocyte cell death. Likewise,
the beneficial effects of ‘cytoplasmic transfer’, a process during which cytoplasm from fertile donor oocytes is injected into oocytes from females that have failed to conceive, reportedly to overcome some cases of human infertility, may by explained by the addition of fresh mitochondria during this procedure (Cohen et al., 1998).

Nevertheless, the clinical application of these techniques should proceed with caution until studies are carried out to compare the developmental competence to term of these oocytes, and assess potential long-term detrimental effects of these procedures on the offspring.

**[Ca^{2+}]_{i} oscillations and abnormal development in aged oocytes**

As previously mentioned, mammalian oocytes remain fully developmentally competent for a few hours after ovulation. In some cases, unfertilized aged oocytes undergo spontaneous fragmentation. However, in others, late fertilization results in the initiation of development, but high rates of fragmentation and apoptotic death are observed, and some of the surviving embryos appear to exhibit birth defects (Tarin et al., 1999). We have demonstrated that initiation of the [Ca^{2+}]_{i} signal due to fertilization is capable of inducing fragmentation and activation of caspases in aged oocytes (Gordo et al., 2000), implying that this [Ca^{2+}]_{i} signal may act as the instigator of apoptosis in aged oocytes. In addition, in human oocytes, caspase activity was detected only after fertilization, in fragments, and in embryos of poor morphology (Martinez et al., 2002).

A question that arises from these results is how can the same signal trigger such divergent developmental paths? Changes in Ca^{2+} homeostasis have been associated with cellular necrosis and programmed cell death, although the mechanism(s) by which [Ca^{2+}]_{i} changes induce apoptosis is not entirely clear (Trump and Berezesky, 1995; Berridge et al., 1998). Interestingly, Ca^{2+} responses in aged mouse oocytes are abnormal in several respects (Jones and Whittingham, 1996; Igarashi et al., 1997). For instance, the rate of increase of [Ca^{2+}]_{i} during fertilization or after stimulation by agonists is slower in aged oocytes. Likewise, the amplitude of the responses and the duration of the oscillations are decreased in these oocytes. Collectively, these results indicate altered Ca^{2+} homeostasis in aged oocytes (Takahashi et al., 2000). The molecular basis for this defective Ca^{2+} regulation has not been elucidated, but may involve the aforementioned decrease in ATP in conjunction with reduced Bcl-2 expression in aged oocytes (Gordo et al., 2002b). Low availability of ATP may impair the function of the SERCA pumps, the Ca^{2+}-ATPase pumps responsible for the refilling of the endoplasmic reticulum (ER). Reduced amounts of Bcl-2 may also negatively impact the function of the Ca^{2+} pump, although the role of Bcl-2 in Ca^{2+} homeostasis remains controversial (Diestelhorst and Dubyak, 1998; Pinton et al., 2000). Nonetheless, Kuo et al. (1998) suggest that Bcl-2 may upregulate the expression of SERCA mRNA and may interact directly with the pump, possibly modulating its function. Therefore, malfunction of the SERCA pump may be responsible for the abnormal Ca^{2+} homeostasis in aged oocytes.

Fertilization-associated [Ca^{2+}]_{i} oscillations may signal apoptosis in aged oocytes by inducing several cellular responses that simultaneously, and possibly synergistically, promote programmed cell death. First, the initiation of [Ca^{2+}]_{i} oscillations may result in depletion of Ca^{2+} from the ER, as evidenced by the premature cessation of oscillations in aged oocytes. Low Ca^{2+} in the ER is an effective trigger of cell death in somatic cells (liang et al., 1994; Bian et al., 1997) and also suppresses protein synthesis (Soboloff and Berger, 2002), effects which are likely severely to limit embryo development. Second, there appears to be privileged communication between IP_{3}R-mediated Ca^{2+} release and mitochondrial function (Csordás et al., 1999; Szalai et al., 1999). The mitochondria serve as Ca^{2+} stores in the cell and have been shown to participate in the generation of oscillations in several types of cell, including mouse oocytes (Liu et al., 2001) and human embryos (Souza et al., 1997). Under optimal conditions, each of the IP_{3}R-mediated increases in [Ca^{2+}]_{i} stimulates mitochondrial function and ATP production. However, in the presence of a pro-apoptotic stimulus, IP_{3}R-mediated [Ca^{2+}]_{i} oscillations signal the release of cytochrome c, a decrease in the mitochondrial membrane potential, and activation of caspases (Szalai et al., 1999). Therefore, we propose a model whereby postovulatory ageing of oocytes, which results in less ATP and Bcl-2 and an increase in hydrogen peroxide, act as a pro-apoptotic stimulus and, under these circumstances, fertilization-like [Ca^{2+}]_{i} oscillations trigger cell death (Fig. 2).

[Ca^{2+}]_{i} oscillations are responsible for promoting progression of the cell cycle in mammalian oocytes and the apoptotic effects of Ca^{2+} release on aged oocytes may also rely, at least in part, on this transition. As mentioned, mammalian oocytes are arrested at MII, a stage that appears to confer resistance to apoptosis. For instance, mouse MII oocytes are susceptible to staurosporin-induced apoptosis, but at concentrations significantly higher than those required in somatic cells (Weil et al., 1996). Similarly, oocytes from Mos-null mice, which lack MAPK activity and fail to arrest at MII, progress into the cell cycle and undergo high rates of spontaneous fragmentation (Colledge et al., 1994). Aged oocytes also appear to be resistant to fragmentation by [Ca^{2+}]_{i} oscillations when arrested at MII. For example, the initiation of oscillations in aged oocytes treated with colcemid, which induces an arrest of the cell cycle at a mitosis-like stage without interfering with Ca^{2+} responses, failed to promote fragmentation, whereas similarly injected oocytes that were transferred into medium devoid of the drug fragmented shortly thereafter (Table 1). Similar fragmentation was observed in aged oocytes when progression of the cell cycle was promoted by exposure to ionomycin and 6-(dimethylamino)purine (DMAP), a common parthenogenetic activation combination (M. Zhang and R. A. Fissore, unpublished). Interestingly, Xenopus egg
extracts are also significantly more resistant to apoptotic stimuli when prepared from MII-arrested eggs compared with those prepared from interphase stage eggs (Faure et al., 1997). Remarkably, when these extracts are maintained in the laboratory for a few hours, they appear spontaneously to initiate changes consistent with apoptosis, raising the possibility that ‘extract ageing’ also takes place (Smith et al., 2000).

It is possible that critical anti-apoptotic molecules are inactivated or degraded during ageing, and that progression of the cell cycle under these conditions promotes cell death. Toward this end, the decreasing concentrations of MPF and
MAPK that occur during prolonged postovulatory ageing may favour the degradation of anti-apoptotic molecules. Interestingly, in somatic cells, reduced concentrations of MAPK and MPF favour degradation of Bcl-2 and other anti-apoptotic proteins (Boucher et al., 2000; Breitschopf et al., 2000), whereas they do not seem to affect pro-apoptotic proteins (Wada et al., 1998). Remarkably, a similar regulation of anti-apoptotic proteins may occur in effect in oocytes, because reduced amounts of Bcl-2 mRNA and protein, but unchanged amounts of Bax mRNA, have been observed in aged mouse oocytes (Gordo et al., 2002b). In addition, in pig oocytes, fragmentation rates after activation seem to be directly associated with the amount of MPF (Kikuchi et al., 2000).

Progression of the cell cycle in aged oocytes may also promote fragmentation and death by activating apoptotic proteins the functions of which are regulated by phosphorylation. For instance, Bad, one of the pro-apoptotic members of the Bcl-2 family of proteins, is primarily regulated by phosphorylation and its translocation to the ER occurs only in its dephosphorylated state. Calcinurin, a Ca2+-dependent phosphatase, dephosphorylates Bad (Wang et al., 1999), and translocated Bad promotes cell death by binding to and inactivating the anti-apoptotic members of the Bcl-2 family (Zha et al., 1996). Therefore, progression of the cell cycle and the consequent changes in the phosphorylation status of the oocyte may activate a molecule(s) such as Bad and, in aged mouse oocytes, which exhibit reduced amounts of anti-apoptotic proteins, fragmentation and cell death may ensue.

It is worth noting that molecular defects other than those outlined here may account for the fragmentation and developmental incompetence of fertilized aged oocytes. Nonetheless, abnormal Ca2+ homeostasis, decreased amounts of anti-apoptotic proteins, and exit of meiosis are likely to play an important role in limiting the developmental potential of aged oocytes.

Conclusions

The initiation of development at fertilization is a tightly controlled process that relies on the structural and molecular integrity of the oocyte and the fertilizing spermatozoon. Under optimal conditions, fertilization induces a series of [Ca2+] wave oscillations that initiate radical alterations in the cellular and molecular organization of the oocyte that are critical for the progression of the embryonic programme. It follows that any changes in this structure that may compromise development, such as alterations induced by ageing, should signal an arrest of development. Oocytes have evolved an elaborate developmental checkpoint whereby the same Ca2+ signal is capable of promoting activation or apoptosis, depending on the cellular and molecular integrity of the oocyte. It is critical, therefore, that we further advance our understanding of the pathways that regulate Ca2+ release and the mechanisms by which it functions in divergent developmental pathways. Clarification of these pathways may have significant implications in improving the developmental outcome of embryos generated in vitro and in vivo in humans and animals of agricultural significance.

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