Spatiotemporal dynamics of intracellular calcium in the mouse egg injected with a spermatozoon

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Oscillatory rises in intracellular Ca2+ concentration ([Ca2+]i) are the pivotal signal in the fertilization of mammalian eggs. The spatiotemporal dynamics of [Ca2+]i, rises in mouse eggs subjected to intracytoplasmic sperm injection (ICSI) were analysed by Ca2+ imaging and compared with those subjected to in-vitro fertilization (IVF). The first Ca2+ transient occurred 15–30 min after ICSI in most eggs, and was followed by Ca2+ oscillations which lasted for at least 6 h at intervals of ~10 min. The pattern of Ca2+ oscillations, an initial relatively larger Ca2+ transient followed by smaller Ca2+ transients, was similar to that at fertilization. Confocal Ca2+ imaging during early Ca2+ transients showed that, in fertilized eggs, [Ca2+]i increased in a wave which started from the sperm attachment site and propagated across the egg cytoplasm. In eggs subjected to ICSI, [Ca2+]i increased gradually and then a Ca2+ spike was generated when [Ca2+]i reached a certain level. The [Ca2+]i rise occurred in the whole egg, associated with neither a wave nor significant heterogeneity between the cortical and central regions. It is suggested that cytosolic factor(s) may leak from the injected spermatozoon, diffuse slowly in the egg cytoplasm, and then cause a synchronous Ca2+ release from intracellular Ca2+ stores.

Key words: mouse egg/intracytoplasmic sperm injection/intracellular calcium concentration/calcium oscillations

Introduction

Intracytoplasmic sperm injection (ICSI) is not only the current most powerful therapy for male infertility but also an interesting subject in basic research on the mechanism of fertilization (Taylor, 1994; Tesarik, 1994). Tesarik et al. (1994) first showed that human eggs injected with spermatozoa exhibit repetitive transient rises in intracellular Ca2+ concentrations ([Ca2+]i). Ca2+ oscillations which are the pivotal signal leading to egg activation in in-vitro fertilization (IVF) of mammals (Kline and Kline, 1992; Miyazaki et al., 1993), although sperm–egg surface interaction is bypassed by ICSI. Interestingly, Ca2+ oscillations begin 4–10 h after the injection of a spermatozoon (Tesarik et al., 1994), while the Ca2+ responses are quickly generated by sperm–egg fusion in IVF as shown in zona-free hamster eggs (Miyazaki and Igusa, 1981) and human eggs (Sousa et al., 1996a,b). Recently, soluble sperm extracts from the hamster (Swann, 1994), boar (Wu et al., 1997), and human spermatozoa (Sousa et al., 1996c; Palermo et al., 1997) have been shown to induce Ca2+ oscillations in eggs of the mouse as well as in the same species. Parrington et al. (1996) have purified the Ca2+ oscillation-inducing protein, oscillin, from hamster spermatozooa, and demonstrated its presence at the equatorial segment region of hamster, boar, and human spermatozoa. It is, therefore, postulated that cytosolic sperm factors such as oscillin may be released from the injected spermatozoon into the egg cytoplasm when the sperm plasma membrane is disintegrated.

Studies on ICSI using experimental animals are of great significance not only for improvements of ICSI and related methods but also for elucidating the mechanism of egg activation, since the use of human eggs involves ethical and technical limitations. In practice, the development of mouse embryos to offspring has been shown to be possible, originating from mouse eggs injected with spermatozoa (Kimura and Yanagimachi, 1995a), round spermatids (Kimura and Yanagimachi, 1995b), or secondary spermatocytes (Kimura and Yanagimachi, 1995c). Further studies can be performed in the mouse, e.g., using knock-out mice for genes encoding key molecules. Therefore, the basic characterization of events that occur in eggs or embryos after the ICSI procedure is of central importance. The present study aimed to investigate spatial and temporal aspects of [Ca2+]i rises in mouse eggs subjected to ICSI. We were able to record, with high probability, the initial Ca2+ response and succeeding long-lasting Ca2+ oscillations, similar to those seen in IVF, using a conventional Ca2+ imaging method with fura-2. We also analysed the spatial distribution of [Ca2+]i rises in the early Ca2+ responses using confocal laser scanning microscopy.
Preparation of gametes for ICSI

B6D2F1 mice were used for the majority of experiments, while ddy mice were used in others. M2 medium (Fulton and Whittingham, 1978) was used for manipulation of gametes in vitro. To obtain gametes, mice were rapidly killed by cervical dislocation. Mature eggs were obtained from female mice (7–11 weeks old) in which ovulation had been stimulated by i.p. injection of 10 IU pregnant mare’s serum gonadotrophin (PMSG; Teikoku Hormone Mfg., Tokyo, Japan) followed by administration of 10 IU of human chorionic gonadotrophin (HCG; Mochida Pharmaceutical Co Ltd, Tokyo, Japan) 48 h later. Eggs at the metaphase II stage were collected from oviducts 16 h after HCG injection (Kimura and Yanagimachi, 1995a), and freed from cumulus cells by treatment with 0.05% hyaluronidase (P-L Biochemicals, Milwaukee, WI, USA) in M2 medium for 2–3 min. For Ca\(^{2+}\) imaging (see later section), zona-intact eggs were loaded with the Ca\(^{2+}\)-sensitive fluorescent dye fura-2 acetoxyethyl ester (fura-2 AM; Molecular Probes Inc, Eugene, OR, USA). The stock solution was 2 mM fura-2 AM in dimethyl sulphoxide, freeze-stored and diluted to 10 \(\mu\)M in M2 medium before use. Eggs were incubated in the fura-2 containing medium for 20 min at 37°C and then rinsed thoroughly using a glass capillary. For ICSI, the eggs were transferred to a 5 \(\mu\)l drop of M2 medium in a 35 mm shallow plastic dish and covered with paraffin oil. The dish was placed on a cooling plate (Kitasato Supply, Tokyo, Japan) which was set on the stage of an phase-contrast inverted microscope (TMD; Nikon, Tokyo, Japan).

Spermatozoa obtained from the cauda epididymides of male mice (11–15 weeks old) were dispersed in M2 medium and kept there for 30 min or more at 37°C. The spermatozoa that swam up in the medium were collected and transferred to a 2 \(\mu\)l drop of M2 medium containing 5% polyvinylpyrrolidone (PVP; Sigma, St Louis, MO, USA) (Kimura and Yanagimachi, 1995a), which was positioned near the drop containing eggs.

In-vitro fertilization

For IVF, the zona pellucida surrounding the egg was dissolved by applying acid Tyrode’s solution (Hogan et al., 1986) for a short time (20–30 s) and removed mechanically by pipetting (Swann, 1994). This is a mild method usually used for calcium measurements in mouse eggs, manual dissection of the zona not being possible. The zona-free eggs were loaded with fura-2 in the same way as described above, and transferred to a 400 \(\mu\)l drop of M2 medium in a plastic dish for Ca\(^{2+}\) imaging (see later) and placed on the microscope stage. Spermatozoa were allowed to undergo capacitation and the acrosome reaction by incubation in a modified Tyrode’s solution containing 20% human serum and 1% bovine adrenal gland extract at 37°C for 4–5 h (Miyazaki et al., 1986). A small amount of sperm suspension was added to the drop containing the experimental eggs (final sperm concentration, 2000–4000 spermatozoa/ml). IVF was performed at 30°C, as sperm motility is markedly reduced at lower temperatures.

ICSI

The method of ICSI was basically that of Kimura and Yanagimachi (1995a). The glass micropipette for sperm injection had an inner diameter of 5 \(\mu\)m at its tip. The tip was flattened, but its wall was thinned by immersing in a 25% solution of hydrofluoric acid which was drawn in and blown out of the pipette several times. To control suction and ejection procedures, a small volume of mercury was placed in the proximal end of the pipette, which was then filled with water and connected to a microinjector (IM-4B; Narishige, Tokyo, Japan). An active spermatozoon was sucked into the pipette and then immobilized by gently pipetting two to three times.

The injection pipette containing a spermatozoon was advanced to the egg which was attached to a holding pipette, using a piezoelectric micromanipulator (PMAS-CT140, Prima Meat Packers, Tsuchiura, Japan). After penetrating the zona pellucida by application of several piezo pulses, the solution in the tip of the pipette was ejected in such a way that the spermatozoon was located very close to the outlet. The pipette was advanced quickly until its tip almost reached the opposite side of the egg, and then the oolemma was punctured by a few piezo pulses. The spermatozoon was ejected deep into the cytoplasm, together with ~6 \(\mu\)l medium. Then the pipette was gently withdrawn. Cytoplasmic aspiration into the pipette, used in human eggs (Tesarik and Sousa, 1995), was not applied because the procedure may affect the spatial distribution of Ca\(^{2+}\) release from the endoplasmic reticulum (ER) in the cytoplasm. The egg was kept for 5 min in the same drop, and then transferred to another dish for Ca\(^{2+}\) imaging. All ICSI procedures and subsequent measurements were carried out at 18–20°C to avoid disintegration of the eggs (Kimura and Yanagimachi, 1995a).

Measurement of [Ca\(^{2+}\)\_i]

Increases in [Ca\(^{2+}\_i\)] were recorded by a conventional Ca\(^{2+}\) imaging method with an image processor (Argus 200; Hamamatsu Photonics, Hamamatsu, Japan). The experimental dish was punctured in the base to make a hole of 13 mm diameter for the passage of UV light. The hole was closed with a cover slip (25×25 mm) from underneath using dental wax. The attached coverslip was coated with poly-L-lysine (50 \(\mu\)g/ml; Sigma) to facilitate adhesion of the eggs. These modified dishes were used for Ca\(^{2+}\) imaging of eggs at IVF or after ICSI. Neither the wax nor the poly-L-lysine affects Ca\(^{2+}\) dynamics (Shiraishi et al., 1996). The dish was mounted on the stage of the inverted microscope.

For excitation of fura-2 fluorescence, UV light of 340 nm or 380 nm wavelength (UV\(_{340}\) or UV\(_{380}\)) was produced by a xenon lamp and 340 ± 10 or 380 ± 10 nm narrow bandpass filter, and applied to the egg through a \(\times 40\) objective lens (Fluor 40; Nikon). Emission fluorescence (F) passed to a silicon intensifier target camera through a 510 ± 10 nm bandpass filter. Ca\(^{2+}\) images were sampled at 5 or 15 s intervals by applying UV\(_{340}\) for 0.25 s followed 0.8 s later by UV\(_{380}\) for 0.25 s. Data sets were stored on the hard disk of the computer as 8-bit digital images, and processed to calculate the ratio \(R = F\(_{340}\)/F\(_{380}\) later. A calibration curve between \(R\) and [Ca\(^{2+}\_i\)] was obtained by measuring Rs of Ca\(^{2+}\)-EDTAOH \([N-(2-hydroxy-ethyl) ethylenedinitri-triacetic acid] buffer solutions.

Ca\(^{2+}\) imaging with confocal microscopy

Some sperm-injected eggs were investigated with confocal laser scanning microscopy (CLSM; LSM310, Carl Zeiss, Oberkochen, Germany). Only B6D2F1 eggs were used, since they were less susceptible to damage by ICSI and subsequent exposure to laser light, compared with the ddy eggs. For confocal Ca\(^{2+}\) imaging at IVF, eggs were loaded with 1 \(\mu\)M Calcium Green-1 AM (Molecular Probes) for 20 min at 37°C. The stock solution was 10 mM Calcium Green-1 AM in dimethyl sulphoxide, freeze-stored and diluted to 1 \(\mu\)M in M2 medium before use. For confocal Ca\(^{2+}\) imaging after ICSI, eggs were injected with Calcium Green-1 together with a spermatozoon through the same pipette (0.5 mM in the pipette and ~30 \(\mu\)M in the egg). The stock solution was 2 mM Calcium Green-1 in 10 mM HEPES-KOH solution (pH 7.3), freeze-stored and diluted to 0.5 mM in M2 medium before use. The experimental dish was the same as that for conventional Ca\(^{2+}\) imaging. An Achroplan \(\times 40\) objective water immersion lens was used (NA 0.75; Carl Zeiss). The dye was activated by 488 nm argon laser. Emission fluorescence was
passaged through a bandpass filter of 540 ± 25 nm and detected by a photomultiplier. Images of an egg (256×256 pixel 8-bit digital images) were acquired every 2 s from the focal plane where the head of the injected spermatozoon was recognized. At the same time, differential interference contrast (DIC) images were obtained from the same focal plane, using transmitted light and another photomultiplier. More details are given in a previous paper (Shirakawa and Miyazaki, 1996).

Results

Ca²⁺ oscillations within 1 h following ICSI

Changes in [Ca²⁺], were recorded by a conventional Ca²⁺ imaging method with fura-2 at intervals of 5 or 15 s for ~1 h, starting several minutes after injection of a spermatozoon. A large increase in [Ca²⁺], up to ~1 µM was produced during the injection procedure (Figures 1A and 2A), probably because a small amount of Ca²⁺ contained in the M2 medium was injected into the egg and/or the egg plasma membrane was slightly injured by micropipette penetration and injection. Small oscillations were often superimposed on the [Ca²⁺], rise (Figures 1A and 2A). Subsequently, [Ca²⁺], declined to a steady level of ~200 nM in ~10 min (Figures 1A and 2A), probably due to Ca²⁺-buffering activity of the cell and/or recovery from cell damage. The basal [Ca²⁺], was gradually lowered to 100 nM (Figures 1A and 2A).

Ca²⁺ oscillations were recorded within 1 h after ICSI in 11 of 20 eggs (four out of eight B6D2F1 eggs and seven out of 12 ddy eggs). Eight out of nine eggs in which Ca²⁺ oscillations were not recorded disintegrated during [Ca²⁺], measurement, suggesting that Ca²⁺ oscillations did not occur because eggs had been damaged by the injection procedure. Thus, Ca²⁺ oscillations occurred within 1 h after ICSI at high probability rate (11/12), if injection was performed with minor injury to the cell.

The pattern of Ca²⁺ oscillations was similar in B6D2F1 and ddy eggs. Figure 1 shows Ca²⁺ oscillations in a ddy mouse egg as an example, as they were typical and were recorded up to 6.5 h after ICSI. A relatively large Ca²⁺ transient appeared within 25 min after injection of a spermatozoon (arrow in Figure 1A; referred to as the first Ca²⁺ transient). Its peak [Ca²⁺], was ~800 nM and duration was ~4 min. Succeeding Ca²⁺ transients occurred at increasing intervals from 7 to 15 min. Their peak [Ca²⁺], was significantly smaller (400–500 nM) and duration was shorter (1.5–2 min), compared with those of the first Ca²⁺ transient. The Ca²⁺ oscillation was caused by the injected spermatozoon, since injection of M2 medium alone produced an artefact [Ca²⁺], rise but never induced Ca²⁺ oscillations later (Figure 2A).

Figure 1B shows Ca²⁺ oscillations at a later stage in the same egg from which Figure 1A was obtained. After finishing the [Ca²⁺], measurement shown in Figure 1A, the egg was kept at 37°C in a CO₂ incubator for 145 min and then the [Ca²⁺], measurement shown in Figure 1B was resumed. There was another pause of 90 min between the measurements shown in Figure 1B and 1C. Repetitive Ca²⁺ transients continued to occur at a fairly constant intervals of ~10 min for >6 h. Their peak [Ca²⁺], remained at 400–500 nM, although the duration was reduced progressively to ~1 min.

Fertilization of the sperm-injected eggs was confirmed by the formation of the male and female pronuclei in all four B6D2F1 eggs and in four out of seven ddy eggs in which Ca²⁺ oscillations were recorded. Other eggs disintegrated during incubation.

Ca²⁺ oscillations at IVF

Ca²⁺ oscillations at IVF of zona-free eggs were recorded in 30 B6D2F1 eggs and 10 ddy eggs, for 60–80 min in most cases. The patterns of [Ca²⁺], oscillations were similar in both types of eggs. Figure 2B shows an example of Ca²⁺ oscillations at IVF in a B6D2F1 egg. The first Ca²⁺ transient associated
with small \(\mathrm{Ca}^{2+}\) oscillations was greater in amplitude and longer in duration, compared with the succeeding \(\mathrm{Ca}^{2+}\) transients. Thus the pattern of early \(\mathrm{Ca}^{2+}\) oscillations, i.e. the first large \(\mathrm{Ca}^{2+}\) transient followed by smaller \(\mathrm{Ca}^{2+}\) transients was common in IVF and ICSI. Later \(\mathrm{Ca}^{2+}\) transients occurred at fairly constant intervals of \(\approx 10\) min, associated with a gradual decline in amplitude. The \(\mathrm{Ca}^{2+}\) oscillations continued to occur for at least 3 h during recording in two B6D2F1 and two ddy eggs. The interval of the long-lasting \(\mathrm{Ca}^{2+}\) oscillations was 7–10 min, comparable with those following ICSI.

**Spatial dynamics of \([\mathrm{Ca}^{2+}]_i\) rises at IVF**

At IVF, mammalian eggs exhibit \(\mathrm{Ca}^{2+}\) waves starting from the site of sperm attachment and propagating across the egg, as clearly shown in hamster eggs (Miyazaki et al., 1986; 1993). The spatial distribution of \([\mathrm{Ca}^{2+}]_i\), rises in fertilized eggs \((n = 5)\) was analysed with CLSM in the focal plane where the sperm head was recognized. Figure 3A shows \(\mathrm{Ca}^{2+}\) images in the rising phase of the first \(\mathrm{Ca}^{2+}\) transient in an egg at fertilization (during the time indicated by a horizontal bar in Figure 4). Images were acquired every 2 s, and spatial

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**Figure 3.** Sequential confocal images of fluorescence in the rising phase of the first \(\mathrm{Ca}^{2+}\) transient at fertilization (A) (the egg for Figure 4), the first (B) and second (C) \(\mathrm{Ca}^{2+}\) transients after intracytoplasmic sperm injection (ICSI; the egg for Figure 5). B6D2F1 mouse eggs were used. Images were acquired every 2 s in the focal plane in which the sperm head was seen. The images were obtained during the time indicated by horizontal bars in Figures 4 and 5. A bright field image of the egg and sperm head (arrow) is shown top left in A and B. A colour scale indicating the fluorescence intensity is shown bottom right.
Ca$^{2+}$ responses to ICSI

**Figure 4.** (A) changes in the fluorescence intensity (arbitrary unit) during the first Ca$^{2+}$ transient at fertilization, measured at three sites indicated in the inset. (B) the rising phase of the first Ca$^{2+}$ transient shown in A, presented on an expanded time scale. (C) changes in the ratio of fluorescence intensity at three cortical sites to that at the central region (see inset). Horizontal bars indicate the time during which images of Figure 3A were acquired.

Spatial dynamics of [Ca$^{2+}$], rises after ICSI

The spatial distribution of [Ca$^{2+}$], rises in sperm-injected B6D2F1 eggs ($n = 15$) was analysed. Figure 5A shows changes of F in the first and second Ca$^{2+}$ transients in an egg injected with a spermatozoon, measured in the three sites 1, 2, and 3 that are indicated in the inset. The injected sperm head was visible in the deep cytoplasm, stained by Calcium Green-1 (Figure 3B and 3C; see Discussion). The basal level of F$_c$ was only slightly lower than that of F$_p$. Changes in F showed that [Ca$^{2+}$], increased with a slow time course at both the peripheral and central regions (Figure 5A). Subsequently, a rapid large [Ca$^{2+}$], rise was generated when [Ca$^{2+}$], reached a certain critical level. In the rapid rising phase of the first Ca$^{2+}$ transient, [Ca$^{2+}$], increased in a wide area of the egg cytoplasm, but not in a wave starting from a specific site, such as the vicinity of the injected sperm head (Figure 3B). In the 15 eggs examined, the [Ca$^{2+}$], rise was never initiated from the vicinity of the injected spermatozoon. There seemed to be a tendency for the [Ca$^{2+}$], rise to be earlier and greater in the peripheral region than that in the central region (Figure 5B, left). However, the ratio analysis of F at the peripheral and central regions (s1/s2 and s3/s2) did not indicate any difference differences in [Ca$^{2+}$], were emphasized by pseudocolours. A [Ca$^{2+}$] rise began near the sperm attachment site and propagated to the opposite site in 2–4 s (Figure 3A). In Figure 4A, changes in fluorescence intensity of injected Calcium Green-1 was measured in the same egg at three sites: s1, the sperm attachment site; s2, central site; s3, antipode site. Figure 4B shows the time lag of the [Ca$^{2+}$] rise at the three sites during propagation of the Ca$^{2+}$ wave, with the expanded record along the time axis. From Ca$^{2+}$ images, the wave appeared to propagate predominantly in the peripheral region of the egg cytoplasm rather than the central region (Figure 3A). However, the fluorescence intensity (F) was always slightly higher in the peripheral cytoplasm (F$_p$) than that in the central cytoplasm (F$_c$), even at the resting state before adding spermatozoa (see Discussion). The difference presumably resulted in a little higher increase in F$_p$. In Figure 4C, the ratio F$_p$/F$_c$ was calculated, taking F$_p$ at two sites 90° from the sperm attachment site (s4 and s5). The ratio s4/s2 and s5/s2 did not substantially increase during the rising phase of the first Ca$^{2+}$ transient, whereas the ratio s1/s2 (sperm attachment site/central region) increased clearly. Thus, preferential propagation of the Ca$^{2+}$ wave in the peripheral cytoplasm was not significantly detected.

Spatial dynamics of [Ca$^{2+}$], rises after ICSI
in the \([\text{Ca}^{2+}]\), rise between the peripheral and cortical cytoplasm (Figure 5C, left). A synchronous \([\text{Ca}^{2+}]\), rise over the egg cytoplasm also occurred in the second \(\text{Ca}^{2+}\) transient (Figures 5B and 5C, right).

**Discussion**

In the present study, the initiation of \(\text{Ca}^{2+}\) oscillations after ICSI were recorded in mouse eggs and spatiotemporal aspects of early \(\text{Ca}^{2+}\) transients were analysed by \(\text{Ca}^{2+}\) imaging. The first \(\text{Ca}^{2+}\) transient occurred within 1 h (15–30 min in most eggs) after injection of a spermatozoon, and was characterized by its larger amplitude and longer duration, compared with later \(\text{Ca}^{2+}\) transients. The time lag for the occurrence of \(\text{Ca}^{2+}\) oscillations after ICSI was much shorter than that in human eggs (Tesarik et al., 1994; Tesarik and Sousa, 1995; Tesarik et al., 1995). For the induction of \(\text{Ca}^{2+}\) oscillations, it can be supposed that cytosolic sperm factors such as oscillin (Parrington et al., 1996) or other factors (Wilding et al., 1997) are introduced into the egg cytoplasm after the plasma membrane of the injected spermatozoon has disintegrated. In the present experimental conditions, the spermatozoon seemed to be partially permeabilized by the pipetting procedure used for immobilization before injection, with an injection pipette whose inner diameter had to be <5 \(\mu\)m for penetration into fragile mouse eggs. Membrane damage of the injected spermatozoon was suggested by the finding that the sperm head often showed fluorescence (Figure 3B and 3C), although the spermatozoon had been placed in medium containing the membrane-impermeable type of Calcium Green-1. Cytosolic sperm proteins may be retained by mild binding to some intracellular structures and released after a time lag, even when the plasma membrane was partially permeabilized to the extent which allowed the fluorescent dye to diffuse into the sperm cytoplasm. The present experimental conditions were thought to allow the cytosolic sperm factor to readily diffuse out to the egg cytoplasm, leading to \(\text{Ca}^{2+}\) oscillations with the time lag of 15–30 min. The immobilization procedure applied to the spermatozoon seems to provide an advantage of earlier activation of the egg after ICSI.

The spatial pattern of \([\text{Ca}^{2+}]\), rise is likely to depend on the distribution of the ER and \(\text{Ca}^{2+}\) release channels and the sensitivity of these channels (Sousa et al., 1996a,b). \(\text{Ca}^{2+}\) oscillations at fertilization of mouse eggs are mainly mediated by the inositol 1,4,5-trisphosphate receptor (IP\(_3\)R), as they are completely blocked by a monoclonal antibody against IP\(_3\)R (Miyazaki et al., 1993). In mouse eggs, clusters of IP\(_3\)Rs in the deep cytoplasm act as release channels in the ER membrane of the egg and that the ER is distributed in the deep cytoplasm where the sperm (head) was injected, we could expect a \(\text{Ca}^{2+}\) wave starting from the vicinity of the sperm head and propagating in all directions. Such a \(\text{Ca}^{2+}\) wave, however, was not observed (Figures 3B and 3C). \([\text{Ca}^{2+}]\), increased gradually without any particular spatial heterogeneity such as a difference between the cortical and central cytoplasm (Figure 5C). A spatial gradient in \([\text{Ca}^{2+}]\), if any, might be not detected during such a slow and small \([\text{Ca}^{2+}]\), rise. In the rapid rising phase of the first and second \(\text{Ca}^{2+}\) transients, the \([\text{Ca}^{2+}]\), rise was globally synchronous in the whole egg, unlike later \(\text{Ca}^{2+}\) transients of constant \(\text{Ca}^{2+}\) oscillations in human eggs after ICSI in which the \([\text{Ca}^{2+}]\), rise in the cortical region precedes that in the central region (Tesarik et al., 1995).

In confocal microscopy, the fluorescence intensity in the peripheral region of the egg cytoplasm tended to be higher than in the central region. This is partly because the egg is a large spherical cell and the thickness of the egg cytoplasm through which laser light passes and focuses on a given horizontal plane is greater in the central region than in the peripheral region. In the central region, therefore, both laser light and emission light are more susceptible to absorption and scattering, resulting in the lower fluorescence intensity in the central region, particularly near the equatorial plane. This inherent non-uniform fluorescence intensity has to be taken into account when the spatial distribution of a \([\text{Ca}^{2+}]\), rise is analyzed.

In mouse eggs subjected to ICSI, it could be deduced that sperm factors may diffuse slowly from the injected sperm head throughout the egg cytoplasm during the latent period (15–20 min) and cause sensitization of the \(\text{Ca}^{2+}\) release channels to \(\text{Ca}^{2+}\). \(\text{Ca}^{2+}\) seems to be released little by little, resulting in a gradual increase in \([\text{Ca}^{2+}]\). Then a rapid explosive \(\text{Ca}^{2+}\) release seems to occur when \([\text{Ca}^{2+}]\), reaches a certain level, based on the \(\text{Ca}^{2+}\)-dependent \(\text{Ca}^{2+}\) release through IP\(_3\)Rs (Iino and Endo, 1992). Thus, a \(\text{Ca}^{2+}\) spike is generated. On the other hand, injection of the cytosolic sperm factor into mouse eggs has shown that a \(\text{Ca}^{2+}\) wave begins from the cortex at the injection site in the first \(\text{Ca}^{2+}\) transient and that the next \(\text{Ca}^{2+}\) transient can occur at a distinct cortical area (Carroll et al., 1994). Injection of the sperm factor is rapid and will cause a substantial amount to be driven rapidly to the egg’s cortex far from the injection site. Under such conditions, an explosive \(\text{Ca}^{2+}\) release may be induced by the sperm factor in the cortical region more easily than in the deep cytoplasm.

In fertilized mouse eggs, \(\text{Ca}^{2+}\) oscillations last for several hours and cease at the stage of pronucleus formation (Jones et al., 1995). In mouse eggs subjected to ICSI, repetitive \(\text{Ca}^{2+}\) transients occurred with constant amplitudes and intervals for at least 6 h (Figure 1), although \([\text{Ca}^{2+}]\), measurement was not continued up to the cessation of \(\text{Ca}^{2+}\) oscillations. Thus, it

The mechanism and site of action of oscillin to induce \(\text{Ca}^{2+}\) oscillations are still unknown, although injection of the cytosolic sperm extract remarkably sensitizes \(\text{Ca}^{2+}\)-induced \(\text{Ca}^{2+}\) release in the egg (Swann, 1994) similar to fertilized eggs (Igusa and Miyazaki, 1983). If we suppose that oscillin acts on \(\text{Ca}^{2+}\) release channels in the ER membrane of the egg and that the ER is distributed in the deep cytoplasm where the sperm (head) was injected, we could expect a \(\text{Ca}^{2+}\) wave starting from the vicinity of the sperm head and propagating in all directions. Such a \(\text{Ca}^{2+}\) wave, however, was not observed (Figures 3B and 3C). \([\text{Ca}^{2+}]\), increased gradually without any particular spatial heterogeneity such as a difference between the cortical and central cytoplasm (Figure 5C). A spatial gradient in \([\text{Ca}^{2+}]\), if any, might be not detected during such a slow and small \([\text{Ca}^{2+}]\), rise. In the rapid rising phase of the first and second \(\text{Ca}^{2+}\) transients, the \([\text{Ca}^{2+}]\), rise was globally synchronous in the whole egg, unlike later \(\text{Ca}^{2+}\) transients of constant \(\text{Ca}^{2+}\) oscillations in human eggs after ICSI in which the \([\text{Ca}^{2+}]\), rise in the cortical region precedes that in the central region (Tesarik et al., 1995).

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was proved that the mouse eggs treated with ICSI can undergo a developmental process basically similar to normal fertilization, except for a delay of ~30 min prior to the start of Ca\(^{2+}\) oscillations.

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### References


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