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Inositol-1,4,5-Trisphosphate Receptor-1 and -3 and Ryanodine Receptor-3 May Increase Ooplasmic Ca^{2+} During Quail Egg Activation

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We previously reported that egg activation in Japanese quail is driven by two distinct types of intracellular Ca^{2+} ($[Ca^{2+}]i$): transient elevations in $[Ca^{2+}]i$ induced by phospholipase Czeta 1 (PLCZ1) and long-lasting spiral-like Ca^{2+} oscillations by citrate synthase (CS) and aconitate hydratase 2 (ACO2). Although the blockade of inositol 1,4,5-trisphosphate receptors (ITPRs) before microinjections of *PLCZ1*, *CS*, and *ACO2* cRNAs only prevented transient increases in $[Ca^{2+}]i$, a microinjection of an agonist of ryanodine receptors (RYRs) induced spiral-like Ca^{2+} oscillations, indicating the involvement of both ITPRs and RYRs in these events. In this study, we investigated the isoforms of ITPRs and RYRs responsible for the expression of the two types of $[Ca^{2+}]i$ increases. RT-PCR and western blot analyses revealed that ITPR1, ITPR3, and RYR3 were expressed in ovulated eggs. These proteins were degraded 3 h after the microinjection of *PLCZ1*, *CS*, and *ACO2* cRNAs, which is the time at which egg activation was complete. However, degradation of ITPR1 and ITPR3, but not RYR3, was initiated 30 min after a single injection of *PLCZ1* cRNA, corresponding to the time of the initial Ca^{2+} wave termination. In contrast, RYR3 degradation was observed 3 h after the microinjection of *CS* and *ACO2* cRNAs. These results indicate that ITPRs and RYR3 differentially mediate increases in $[Ca^{2+}]i$ during egg activation in Japanese quail, and that downregulation of ITPRs and RYR3-mediated events terminate the initial Ca^{2+} wave and spiral-like Ca^{2+} oscillations, respectively.

Key words: egg activation, inositol 1,4,5-trisphosphate receptor, intracellular Ca^{2+}, Japanese quail, ryanodine receptor

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Introduction

In all vertebrates examined to date, the fertilizing sperm immediately induces an increase in intracellular Ca^{2+} concentrations ($[Ca^{2+}]i$) after sperm-egg fusion (Stricker, 1999; Runft *et al*., 2002). This $[Ca^{2+}]i$ increase evokes a series of events to cause egg activation, such as the resumption of egg meiosis, exocytosis of cortical granules, maternal protein synthesis, and pronuclear formation, thereby leading to initiation of the first zygotic cell cycle program (Miyazaki *et al*., 1993; Stricker, 1999; Ducibella *et al*., 2002; Runft *et al*., 2002).

Although an intracellular $[Ca^{2+}]i$ increase in fertilizing eggs is a universally conserved phenomenon in animals, the shape and pattern of the $[Ca^{2+}]i$ response vary widely among species (Stricker, 1999). A technique used for *in vitro* fertilization in sea urchin and frog revealed a single $[Ca^{2+}]i$ increase from the sperm entry site that propagated throughout the egg within 5 min (Stricker, 1999). In mammalian eggs, the initial elevation in $[Ca^{2+}]i$ is followed by periodic oscillatory increases that spike every 5−15 min and repetitive oscillations that continue until at least the pronuclei are formed (Ca^{2+} oscillation; Miyazaki *et al*., 1993; Jones *et al*., 1995; Nakada *et al*., 1995). In physiologically polyspermic species such as the newt, 2−20 sperms successively enter at different points on the egg surface, with sequential increases in $[Ca^{2+}]i$ occurring at each sperm entry site as small waves; however, each Ca^{2+} wave does not reach the opposite site of the egg (Harada *et al*., 2007, 2011; Iwao, 2012). Therefore, multiple

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Ca^{2+} waves induced by all fertilizing sperm appear to be important for propagation over the entire egg to result in complete egg activation.

Birds also exhibit physiological polyspermy during fertilization (Harper, 1904; Patterson, 1910; Fofanova, 1965; Nakanishi *et al*., 1990; Waddington *et al*., 1998). In the Japanese quail, 100−200 sperm successively enter the egg cytoplasm during fertilization, a number that is markedly higher than that in newt and even other avian species (Mizushima, 2017). We previously reported a unique pattern of increase in $[Ca^{2+}]i$ in quail eggs following microinjection of 2 ng of sperm protein extract, which is equivalent to 200 sperm (SE) (Mizushima *et al*., 2014). SE evoked two phases of $[Ca^{2+}]i$ changes: an initial transient increase in $[Ca^{2+}]i$ followed by multiple long-lasting spiral-like signals. A transient Ca^{2+} wave was initiated at the injection site of the germinal disc immediately after SE injection and spread concentrically into the egg cytoplasm. A spiral-like Ca^{2+} signal then occurred at the injection site 10−15 min after microinjection and continued for at least 1 h. We also demonstrated that the initial transient Ca^{2+} wave was required for the resumption of second meiosis, whereas induction of the spiral-like Ca^{2+} signal appeared to be necessary for ensuring the completion of all events to accelerate the cell cycle progression of initial and early cleavage (Mizushima *et al*., 2014). Furthermore, removing extracellular Ca^{2+} by adding a Ca^{2+} chelator did not affect the induction of an increase in $[Ca^{2+}]i$, indicating that the main sources of Ca^{2+} during egg activation are egg organelles. Although we reported, for the first time, an increase in $[Ca^{2+}]i$ in avian eggs during fertilization, the underlying cellular and molecular mechanisms have not yet been elucidated in detail.

Inositol 1,4,5-trisphosphate receptor type 1 (*ITPR1*) is mainly responsible for the $[Ca^{2+}]i$ increase associated with fertilization in mammals (Miyazaki *et al*., 1992; Fissore *et al*., 1995; Jones and Whittingham, 1996; Lee *et al*., 2010; Ito *et al*., 2011). The essential role of ITPR1 in fertilization was confirmed using functional-blocking antibodies, with antibody injection precluding the $[Ca^{2+}]i$ increase triggered by sperm penetration. Following fertilization, ITPR1 was progressively degraded, which corresponded to the termination of sperm-initiated Ca^{2+} oscillations at the interphase stage (He *et al*., 1997; Brind *et al*., 2000; Jellerette *et al*., 2000; Malcuit *et al*., 2005). This down-regulation was induced by ubiquitination and subsequent degradation by the proteasome, which desensitized ITPR1 (Brind *et al*., 2000). Gating of ITPR1 and Ca^{2+} release requires binding of IP_3, a product of the hydrolysis of phosphatidylinositol 4,5-bisphosphate by phospholipase C (PLC). Phospholipase Czeta1 (*PLCZ1*) was originally isolated as a sperm-specific isoform from mice, and microinjection of its complementary RNA (cRNA) or recombinant protein into the mouse egg elicited Ca^{2+} oscillations similar to those observed during fertilization. Therefore, PLCZ1 may be responsible for the production of IP_3 (Saunders *et al*., 2002). Previous studies on PLCZ1 demonstrated its ability to induce $[Ca^{2+}]i$ increases across mammalian, chicken, and fish species (Cox *et al*., 2002; Kouchi *et al*., 2005; Yoneda *et al*., 2006; Yoon and Fissore, 2007; Coward *et al*., 2005, 2011).

However, we discovered that *PLCZ1* was responsible for the induction of the initial transient Ca^{2+} increase in Japanese quail, whereas citrate synthase (*CS*) and aconitate hydratase 2 (*ACO2*) were needed for long-lasting spiral-like Ca^{2+} oscillations. Heparin and 2-aminoethoxydiphenyl borate, antagonists of ITPRs, precluded the initial Ca^{2+} wave but not the long-lasting spiral-like Ca^{2+} signal (Mizushima *et al*., 2014). These findings suggest that CS- and ACO2-induced spiral-like Ca^{2+} oscillations are generated by cellular events that differ from the PLCZ1-induced IP_3 production pathway. In addition, microinjection of cyclic adenosine diphosphate-ribose, an activator of ryanodine receptors (RYRs), induced similar spiral-like Ca^{2+} signal patterns in eggs, which may be mediated via RYRs. Although three separate isoforms of ITPRs (ITPR1, ITPR2, and ITPR3) and RYRs (RYR1, RYR2, and RYR3), which are encoded by different genes, have been isolated from birds (Percival *et al*., 1994; Guillemette *et al*., 2005), the type of receptor responsible for the $[Ca^{2+}]i$ increase during egg activation in Japanese quail remains unclear. Therefore, this study was conducted to identify the maternal isoforms of ITPRs and RYRs expressed in eggs and investigate the downregulation of ITPRs and RYRs after microinjections of sperm-borne egg-activating factors to reveal their involvement in quail egg activation.

Materials and Methods

Animals

Male and female Japanese quail, *Coturnix japonica*, 8−20 weeks of age (Motoki Corporation, Saitama, Japan), were maintained individually under a photoperiod of 14 h light:10 h dark (lights on at 05:00) with *ad libitum* access to water and a commercial diet (Muroran Uzuraen, Muroran, Japan). All experimental procedures for the care and use of animals were approved by the Animal Care and Use Committee of Hokkaido University (approval number 14-0135).

Microinjections of PLCZ1, CS, and ACO2 cRNAs

To prepare *PLCZ1*, *CS*, and *ACO2* cRNAs, the PCR products of quail *PLCZ1*, *CS*, and *ACO2* cloned into the pGEM-T easy vector (Mizushima *et al*., 2014) were subcloned into pTNT plasmids (Promega, Madison, WA, USA), which were then subjected to RNA synthesis using a Ribomax RNA synthesis system (Promega). RNA concentrations were measured using a spectrophotometer (NanoDrop 8000, Thermo Fisher Scientific, Waltham, MA, USA).

Unfertilized eggs were recovered from the anterior magnum within 2 h of egg oviposition (Mizushima *et al*., 2014), and each egg was microinjected with *PLCZ1* (60 ng/μL), *CS* (100 ng/μL), and *ACO2* (100 ng/μL) cRNAs and then cultured *in vitro* for 30 min or 3 h. All procedures used for microinjections and *in vitro* cultures were performed as described by Mizushima *et al*. (2014) and Ono *et al*. (1994), respectively.

mRNA Expression Analysis of ITPRs and RYRs

Total RNA was extracted from tissues such as the ovary, whole brain, heart, liver, stage X blastoderms isolated from freshly laid eggs, or the germinal discs of eggs collected from the infundibulum 30 min after predicted ovulation; 0.2 μg of

Table 1. **Oligonucleotide primers used for RT-PCR**

Gene	Forward primer, 5′ → 3′	Reverse primer, 5′ → 3′	Accession number
ITPR1	GGTAAACCCTGACTATGAGG	GTAATCCTGCTGAGAATGCC	AB_839359
ITPR2	GCTCAGATATTCGGGATCCT	ACTTCCTCTTCATCAATGTC	AB_839360
ITPR3	AGGAGCTGTCAGACCAGAAG	ACCTCCTCCTCTGAGTACTC	AB_839361
RYR1	GCTGACCGAGAAGAGCAAGT	TCGAAGAACCTACAGACCCCA	XM_032441838.1
RYR2	AAGTCACAGGATCCCAACGC	TGAGCCAGACTCTGTTGGTTA	XM_032443314.1
RYR3	TCGTAGAGAGAAAACGTGCTCC	AGTGTCTGCATGAAGGAGGC	XM_032444977.1
TUBG	ATGCCGCGGGAGATCATCAC	TCACTGCTCCTGTGTGCCCC	XM_015886034.2

total RNA was reverse-transcribed using a ReverTra Ace kit (TOYOBO, Osaka, Japan). The sample volume was 10 µL. One microliter of cDNA from germinal disc samples was amplified using gene-specific primers for the *ITPR1*, *2*, and *3* and *RYR1*, *2*, and *3* genes, and the γ-tubulin (*TUBG*) gene was amplified as an internal control for cDNA (Table 1). The specificity of PCR was confirmed by sequence analysis.

Western Blot Analysis of ITPRs and RYRs

Germinal discs were collected according to the method described by Mizushima *et al*. (2009) and dissolved in intracellular-like medium (120 mM KCl, 0.1 mM EGTA, 10 mM Na-β-glycerophosphate, 0.2 mM PMSF, 1 mM DTT, and 20 mM HEPES–NaOH, pH 7.5) by homogenization and sonication. The supernatant was collected by centrifugation at 10,000 ×*g* for 10 min. Each extract was heated at 70℃ for 5 min, and 20 µg protein per lane was resolved by SDS-PAGE (Laemmli, 1970) on a 6% polyacrylamide gel and transferred onto a polyvinylidene fluoride membrane (Millipore, Bedford, MA, USA). Following transfer and blocking for 30 min using a detector block (SeraCare Life Sciences, Milford, MA, USA), the membrane was incubated at 4℃ overnight with a rabbit anti-rat ITPR1 antibody (Alomone Labs Ltd., Jerusalem, Israel), rabbit anti-human ITPR3 antibody (LifeSpan BioSciences, Inc., Seattle, WA, USA), or mouse anti-chicken RYR antibody (GeneTex, Inc., Irvine, CA). The membrane was then incubated at 4℃ for 1 h with a goat anti-rabbit or a donkey anti-mouse secondary antibody coupled with horseradish peroxidase (Millipore). An anti-chicken γ-tubulin monoclonal antibody (Sigma-Aldrich, St. Louis, MO, USA) was used to detect TUBG after separating the proteins on a 12% polyacrylamide gel. Immunoreactivity was detected using Immobilon Western Detection Reagent (Millipore) and LAS 3000 (GE Healthcare, Little Chalfont, UK). Visualized blots were digitized using ImageJ 1.48v software (NIH, Bethesda, MD, USA).

Statistical Analysis

Protein expression levels were normalized relative to those of the TUBG protein as an internal control. Data were expressed as the mean±standard deviation and analyzed for significant differences by ANOVA. Means were compared using Tukey's test. Differences were considered to be significant at $P<0.05$.

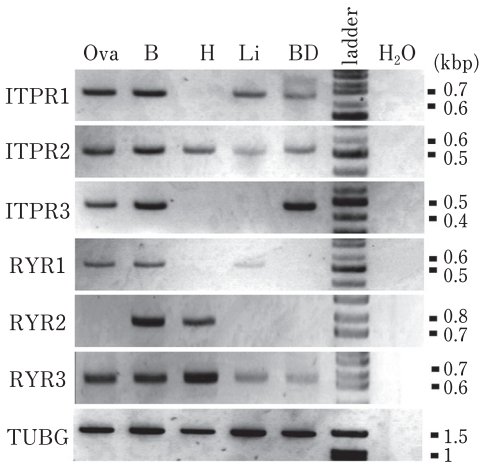

Fig. 1. **Tissue distribution of mRNAs for *ITPR*s and *RYR*s.** Two hundred nanograms of total RNA extracts from the ovary (Ova), brain (B), heart (H), liver (Li), or blastoderm (BD) were subjected to RT-PCR using specific primers. As a negative control, distilled water (H_2O) was used. A representative gel of three independent experiments is shown.

Results

Expression Profiles of ITPRs and RYRs in Quail Tissues

To investigate the presence of all *ITPR* and *RYR* transcripts in quail, we initially analyzed mRNAs isolated from various tissues, as tissue-specific expression of each *ITPR* and *RYR* isoform has been reported in mammals (Newton *et al*., 1994; Giannini *et al*., 1995). Fig. 1 shows the mRNA expression levels of ITPRs and RYRs in the ovary, brain, heart, liver, and blastoderm of quail determined by RT-PCR. The PCR products of *ITPR1* and *ITPR2* were detected in all tissues, except for *ITPR1* in the heart. In contrast, the PCR products of *ITPR3* were only detected in the ovary, brain, and blastoderm. Analyses of the mRNA distribution of *RYR1* and *RYR2* revealed the expression of the former in the ovary, brain, and liver and of the latter in only the brain and heart. In contrast, the mRNA expression of *RYR3* was detected in all tissues. These results demonstrate that multiple types of *ITPR*s and *RYR*s were co-expressed in most tissues, and their expression profiles were similar to those of murine tissues (Newton *et al*., 1994; Giannini *et al*., 1995). Collectively, these results indicate the presence of all transcripts encoding three distinct

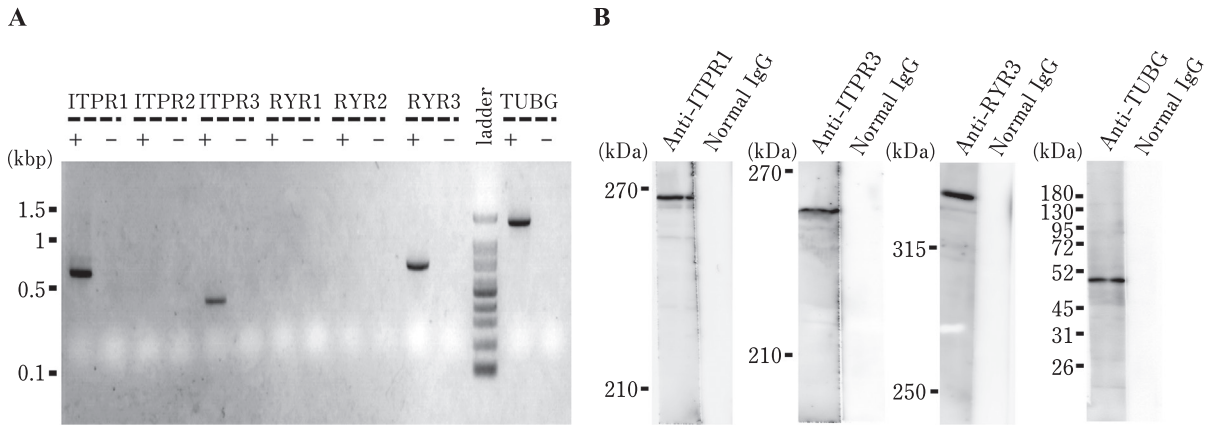

Fig. 2. **mRNA and protein expression of *ITPR*s and *RYR*s in quail eggs.** (A) mRNA expression of *ITPR*s and *RYR*s in quail eggs. Two hundred nanograms of total RNA extracts from the germinal discs of eggs collected from the infundibulum 30 min after predicted ovulation were subjected to RT-PCR using specific primers. As a non-RT control, mRNA from the germinal disc was treated in the same manner, except that reverse transcriptases (−) were omitted. A representative gel of three independent experiments is shown. (B) Protein expression of ITPR1, ITPR3, and RYR3 in quail eggs. Twenty micrograms of protein extracts from the germinal disc were separated by SDS-PAGE, transferred onto polyvinylidene fluoride membranes, and detected with anti-ITPR1, ITPR3, and RYR3 antibodies or control normal rabbit/mouse IgG.

isoforms of *bona fide ITPR*s and *RYR*s in quail.

Expression of ITPRs and RYRs in Quail Eggs

Figure 2A shows the mRNA expression of the *ITPR* and *RYR* isoforms in the germinal discs of ovulated eggs detected by RT-PCR. The PCR products of *ITPR1*, *ITPR3*, and *RYR3* showed the predicted sizes, whereas those of *ITPR2*, *RYR1*, and *RYR2* were below the detection limit. Western blot analysis showed that anti-ITPR1, anti-ITPR3, and anti-RYR3 reacted with bands at approximately 270, 250, and 500 kDa, respectively (Fig. 2B). The bands did not react with the normal rabbit or mouse IgG bands. These results indicate that the ITPR1, ITPR3, and RYR3 proteins are present in ovulated eggs.

Down-regulation of ITPRs and RYRs After a Microinjection of Sperm-borne Egg-activating Factors

To investigate the involvement of ITPR1, ITPR3, and RYR3 in the generation of the initial Ca^{2+} wave and spiral-like Ca^{2+} oscillations, we examined the degradation of these proteins after microinjections of sperm-borne egg-activating factors. As shown in Fig. 3A and 3C, the intensities of the bands for the ITPR1 and ITPR3 proteins were both significantly weaker following triple injection of *PLCZ1*, *CS*, and *ACO2* cRNA than those observed in eggs injected with solvent 30 min after microinjection. However, RYR3 was not degraded (Fig. 3A and 3C). In addition, similar degradation of the ITPR1 and ITPR3 proteins was observed 30 min after single injection of *PLCZ1* cRNA (Fig. 3B and 3C). In contrast, when the eggs were microinjected with *CS* and *ACO2* cRNAs without *PLCZ1* cRNA, RYR3 protein expression as well as ITPR1 and ITPR3 protein levels did not decrease after 30 min of incubation (Fig. 3B and 3E). Time-course studies indicated that RYR3 was degraded by *CS* and *ACO2* cRNAs with or without *PLCZ1* cRNA 3 h after injection (Fig. 3D–F). In contrast, neither ITPR1 nor ITPR3 protein degradation occurred following microinjections of *CS* and *ACO2* cRNAs, even after 3 h (Fig. 3E and 3F).

Discussion

In the present study, we demonstrated for the first time that ITPR1 and ITPR3 are expressed at both the mRNA and protein levels in quail eggs. In mice, although all *ITPR* isoform mRNAs were expressed in the egg, the ITPR1 and ITPR2 proteins were predominant (Fissore *et al*., 1999). The ITPR3 protein was present to a lesser extent, if any, because it was not observed by immunochemistry and its detection by Western blotting required more than 1000 eggs (Fissore *et al*., 1999). ITPR1 was localized in the periphery of ovulated MII mouse eggs, whereas ITPR2 was restricted to the cortical vesicle. The cortical vesicle plays a pivotal role in responding to the polyspermy block by releasing its contents outwards to make the zona pellucida refractory to the binding and fusion of a second sperm (the zona reaction). Therefore, ITPR2 may not release the large amount of Ca^{2+} required to trigger Ca^{2+} oscillations but may amplify the signaling events required for the polyspermy block in mouse eggs. In contrast, neither a membrane block nor intracellular organelles similar to the cortical vesicle have been detected in polyspermic eggs, such as in birds (Mizushima, 2017). In addition, an interesting feature of the ITPR2 mRNA expression pattern in the present study is its absence in quail eggs, although ITPR2 mRNA was expressed in all tissues studied. These results support the hypothesis that ITPR2 may be involved in the polyspermy

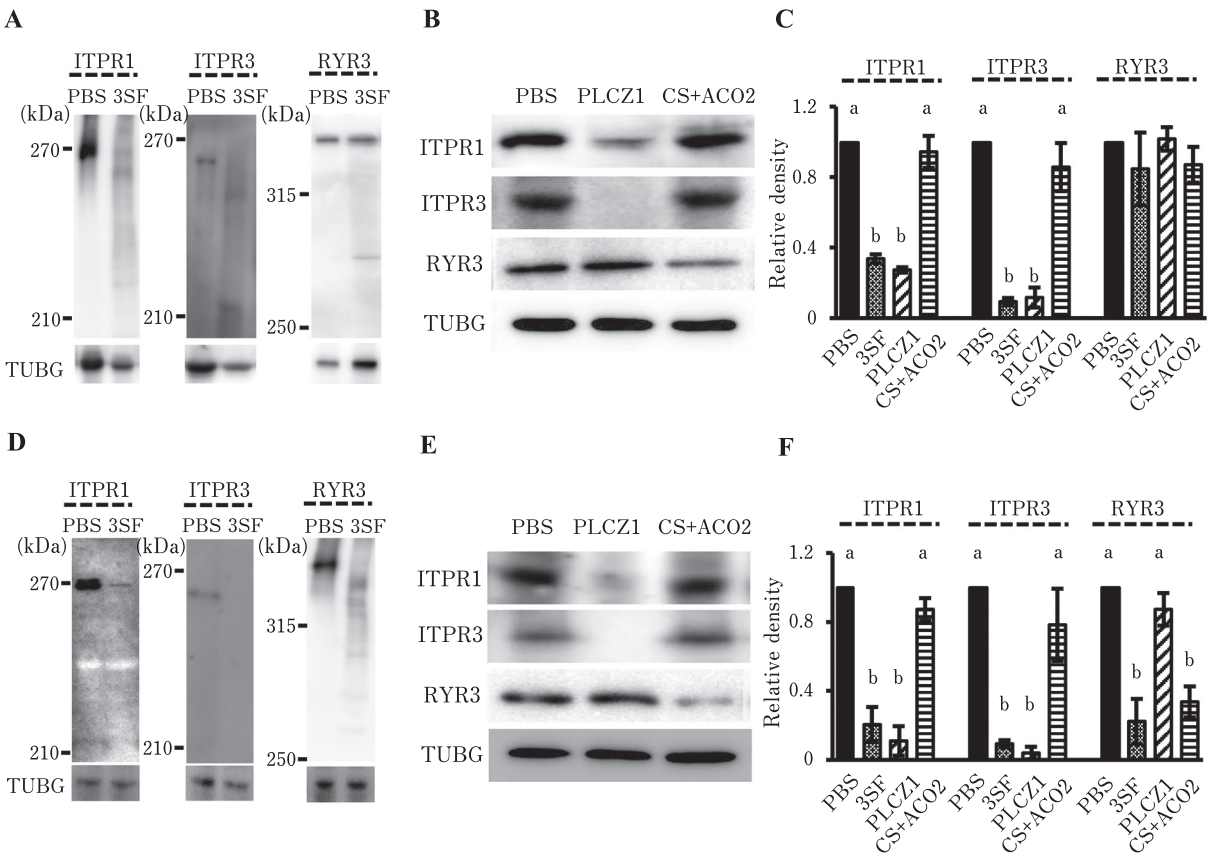

Fig. 3. **Changes in ITPR1, ITPR3, and RYR3 protein expression levels in quail eggs activated by injections of *PLCZ1*, *CS*, and *ACO2* cRNAs.** (A and D) Western blotting analysis 30 min (A) and 3 h (D) after microinjection of a mixture of cRNAs of 3 sperm factors (3SF) (*PLCZ1*, *CS*, and *ACO2*). (B and E) Western blotting analysis 30 min (A) and 3 h (D) after microinjection of *PLCZ1* cRNA alone or *CS* and *ACO2* cRNA. (C and F) Quantification of immunoreactivity 30 min (C) and 3 h (F) after the cRNA microinjection. Band intensities were quantified and expressed as the means±standard deviations of three independent experiments. Values with different letters are significantly different ($P<0.01$).

block, a process that does not occur in avian eggs in which polyspermic fertilization takes place.

In mammalian eggs, 70−80% of the ITPR1 protein was degraded when the eggs were microinjected with *PLCZ1* cRNA or sperm (Malcuit *et al*., 2005). Additionally, microinjection of the anti-ITPR1 antibody completely inhibited fertilization-associated increases in $[Ca^{2+}]i$, indicating the role of ITPR1 in the release of most of the Ca^{2+} from intracellular Ca^{2+} stores during egg activation. In contrast to mouse eggs, ITPR3 was expressed at significant levels in quail eggs, and ITPR1 and ITPR3 were both progressively degraded 30 min after the microinjection of *PLCZ1* cRNA. It is important to note that the timing of ITPR degradation synchronized with that of the termination of the initial transient $[Ca^{2+}]i$ increase (Mizushima *et al*., 2014). These results strongly support that gating of both ITPR1 and ITPR3 in response to sperm entry is involved in inducing the initial $[Ca^{2+}]i$ increase. Microinjection of mouse *PLCZ1* cRNA, which triggered Ca^{2+} oscillations in mouse eggs, only generated a Ca^{2+} wave and not mammalian-like Ca^{2+} oscillations in quail eggs (data not shown). A previous study reported that IP_3-activated Ca^{2+} signals differ in somatic cells expressing different ITPR isoforms (Ehrlich and Watras, 1988; Khodakhah and Ogden, 1993; Hajnnoczky and Thomas, 1994). This result implies that the different ITPR types contribute to the distinct regulation of Ca^{2+} release from cells and may have different affinities for IP_3 (Newton *et al*., 1994; Joseph *et al*., 1995). In addition, ITPR isoforms assemble as homo- or heterotetramers to form functional channels; thus, it is reasonable to postulate that the different binding affinities of IP_3 to each channel in mouse and quail eggs contribute to the species-specific patterns of $[Ca^{2+}]i$ increases (Nucifora *et al*., 1996).

Interestingly, RYR3 was downregulated during egg activation in quail. We previously reported that an RYR agonist generated a Ca^{2+} spike similar to that of CS- and ACO2-induced spiral-like oscillations (Mizushima *et al*., 2014).

Collectively, these findings and the present results suggest that the periodic and long-lasting Ca^{2+} spikes are mediated via RYR3. Although the duration of CS- and ACO2-generated spiral-like oscillations has not been examined, intracytoplasmic sperm injection-treated quail eggs initiated the first cleavage 3 h after microinjections, indicating the completion of quail egg activation within 3 h of these injections (Mizushima *et al*, 2014). This assumption is supported by the present results showing that RYR3 was degraded 3 h after microinjection.

RYRs have not been detected in frog and hamster eggs using RYR-specific antibodies (Miyazaki *et al*., 1992; Parys *et al*., 1994). Furthermore, RYR agonists did not induce changes in $[Ca^{2+}]i$, suggesting that RYRs do not play a major role in Ca^{2+} release from intracellular Ca^{2+} stores during egg activation in these species (Miyazaki *et al*., 1992; Nuccitelli *et al*., 1993). Although conflicting findings on the presence of RYR2 and RYR3 in mouse and bovine eggs have been reported by immunological studies (Carroll and Swann, 1992; Swann, 1992; Kline and Kline, 1994; Yue *et al*., 1995 and 1998), evidence suggests that a functional disturbance in ITPR1 inhibits all aspects of egg activation in mice, hamsters, and humans (Miyazaki *et al*., 1992; Xu *et al*., 1994; Goud *et al*., 2002).

In summary, our results suggest that ITPR1, ITPR3, and RYR3 regulate the two distinct Ca^{2+} signals generated by PLCZ1, CS, and ACO2 in Japanese quail. However, as single injection of *CS* or *ACO2* cRNA did not induce significant release of Ca^{2+} from quail eggs (Mizushima *et al*., 2014), further studies are needed to identify the cellular and biochemical components mediating Ca^{2+} release from RYR3 channels.

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Conflicts of Interest

The authors declare no conflict of interest.

References

Brind S, Swann K and Carroll J. Inositol 1,4,5-trisphosphate receptors are downregulated in mouse oocytes in response to sperm or adenophostin A but not to increases in intracellular Ca^{2+} or egg activation. Developmental Biology, 223: 251−265. 2000.

Carroll J and Swann K. Spontaneous cytosolic calcium oscillations driven by inositol trisphosphate occur during in vitro maturation of mouse oocytes. Journal of Biological Chemistry, 267: 11196−11201. 1992.

Cox LJ, Larman MG, Saunders CM, Hashimoto K, Swann K and Lai FA. Sperm phospholipase Cζ from humans and cynomolgus monkeys triggers Ca^{2+} oscillations, activation and development of mouse oocytes. Reproduction, 124: 611−623. 2002.

Coward K, Ponting CP, Chang H-Y, Hibbitt O, Savolainen P, Jones KT and Parrington J. Phospholipase Cζ, the trigger of egg activation in mammals, is present in a non-mammalian species. Reproduction, 130: 157−163. 2005.

Coward K, Ponting CP, Zhang N, Young C, Huang CJ, Chou CM, Kashir J, Rafael A, Fissore RA and Parrington J. Identification and functional analysis of an ovarian form of the egg activation factor phospholipase Czeta (PLCζ) in pufferfish. Molecular Reproduction and Development, 78: 48−56. 2011.

Ducibella T, Huneau D, Angelichio E, Xu Z, Schultz RM, Kopf GS, Fissore R, Madoux S and Ozil JP. Egg-to-embryo transition is driven by differential responses to Ca^{2+} oscillation number. Developmental Biology, 250: 280−291. 2002.

Ehrlich BE and Watras J. Inositol 1,4,5-trisphosphate activates a channel from smooth muscle sarcoplasmic reticulum. Nature, 336: 583−586. 1988.

Fissore RA, Pinto-Correia C and Robl JM. Inositol trisphosphate-induced calcium release in the generation of calcium oscillations in bovine eggs. Biology of Reproduction, 53: 766−774. 1995.

Fissore RA, Longo FJ, Anderson E, Parys JB and Ducibella T. Differential distribution of inositol trisphosphate receptor isoforms in mouse oocytes. Biology of Reproduction, 60: 49−57. 1999.

Fofanova KA. Morphologic data on polyspermy in chickens. Federation proceedings - Translation supplement, 24: 239−247. 1965.

Giannini G, Conti A, Mammarella S, Scrobogna M and Sorrentino V. The ryanodine receptor/calcium channel genes are widely and differentially expressed in murine brain and peripheral tissues. The Journal of Cell Biology, 128: 893−904. 1995.

Goud PT, Foud AP, Leybaert L, Van Oostveldt P, Mikosshiba K, Diamod MP and Dhont M. Inositol 1,4,5-trisphosphate receptor function in human oocyte: calcium responses and oocyte activation-related phenomena induced by photolytic release of $InsP_3$ are blocked by a specific antibody to the type I receptor. Molecular Human Reproduction, 8: 912−918. 2002.

Guillemette J, Caron AZ, Regimbald-Dumas Y, Arguin G, Mignery GA, Boulay G and Guillemette G. Expression of a truncated form of inositol 1,4,5-trisphosphate receptor type III in the cytosol of DT40 triple inositol 1,4,5-trisphosphate receptor-knockout cells. Cell Calcium, 37: 97−104. 2005.

Hajnóczky G and Thomas AP. The inositol trisphosphate calcium channel is inactivated by inositol trisphosphate. Nature, 370: 474−477. 1994.

Harada Y, Matsumoto T, Hirahara S, Nakashima A, Ueno S, Oda S, Miyazaki S and Iwao Y. Characterization of a sperm factor for egg activation at fertilization of the newt *Cynops pyrrhogaster*. Developmental Biology, 306: 797−808. 2007.

Harada Y, Kawazoe M, Eto Y, Ueno S and Iwao Y. The Ca^{2+} increase by the sperm factor in physiologically polyspermic newt fertilization: its signaling mechanism in egg cytoplasm and the species-specificity. Developmental Biology, 351: 266−276. 2011.

Harper EH. The fertilization and early development of the pigeon's egg. American Journal of Anatomy, 3: 349−386. 1904.

He CL, Damiani P, Parys JB and Fissore RA. Calcium, calcium release receptors, and meiotic resumption in bovine oocytes. Biology of Reproduction, 57: 1245−1255. 1997.

Ito J, Yoshida T, Kasai Y, Wakai T, Parys JB, fissure RA and Kashiwazaki N. Phosphorylation of inositol 1,4,5-triphosphate receptor 1 during in vitro maturation of porcine oocytes. Animal Science Journal, 81: 34−41. 2011.

Iwao Y. Egg activation in physiological polyspermy. Reproduction, 144: 11−22. 2012.

Jellerette T, He CL, Wu H, Parys JB and Fissore RA. Downregulation of the inositol 1,4,5-trisphosphate receptor in mouse eggs following fertilization or parthenogenetic activation. Developmental Biology, 223: 238−250. 2000.

Jones KT and Whittingham DG. A comparison of sperm- and IP3-

induced Ca^{2+} release in activated and aging mouse oocytes. Developmental Biology, 178: 229−237. 1996.

Jones KT, Carroll J, Merriman JA, Whittingham DG and Kono T. Repetitive sperm-induced Ca^{2+} transients in mouse oocytes are cell cycle dependent. Development, 121: 3259−3266. 1995.

Joseph SK, Lin C, Pierson S, Thomas AP and Maranto AR. Heteroligomers of type-I and type-III inositol trisphosphate receptors in WB rat liver epithelial cells. Journal of Biological Chemistry, 270: 23310−23316. 1995.

Khodakhah K and Ogden D. Functional heterogeneity of calcium release by inositol trisphosphate in single Purkinje neurons, cultured cerebellar astrocytes, and peripheral tissues. Proceedings of the National Academy of Sciences of the United States of America, 90: 4976−4980. 1993.

Kline JT and Kline D. Regulation of intracellular calcium in the mouse egg: evidence for inositol trisphosphate-induced calcium release, but not calcium-induced calcium release. Biology of Reproduction, 50: 193−203. 1994.

Kouchi Z, Fukami K, Shikano T, Oda S, Nakamura Y, Takenawa T and Miyazaki S. Recombinant phospholipaseCzeta has high Ca^{2+} sensitivity and induces Ca^{2+} oscillations in mouse eggs. Journal of Biological Chemistry, 279: 10408−10412. 2005.

Laemmli UK. Cleavage of structural proteins during the assemble of the head of bacteriophage T4. Nature, 227: 680−685. 1970.

Lee B, Yoon SY, Malcuit C, Parys JB and Fissore RA. Inositol 1,4,5-trisphosphate receptor 1 degradation in mouse eggs and impact on $[Ca^{2+}]$i oscillations. Journal of Cellular Physiology, 222: 238−247. 2010.

Malcuit C, Knott JG, He C, Wainwright T, Parys JB, Robl JM and Fissore RA. Fertilization and inositol 1,4,5-trisphoshpate (IP_3)-induced calcium release in type-1 inositol 1,4,5-trisphoshpate receptor down-regulated bovine eggs. Biology of Reproduction, 73: 2−13. 2005.

Miyazaki S, Yuzaki M, Nakada K, Shirakawa H, Nakanishi S, Nakade S and Mikoshiba K. Block of wave and Ca^{2+} oscillation by antibody to the inositol 1,4,5-trisphosphate receptor in fertilized hamster eggs. Science, 257: 251−254. 1992.

Miyazaki S, Shirakawa H, Nakada K and Honda Y. Essential role of the inotitol 1,4,5-triphosphate/Ca^{2+} release channel in Ca^{2+} waves and Ca^{2+} oscillations at fertilization of mammalian egg. Developmental Biology, 58: 62−78. 1993.

Mizushima S, Takagi S, Ono T, Atsumi Y, Tsukada A, Saito N and Shimada K. Phospholiase Cζ mRNA expression and its potency during spermatogenesis for activation of quail oocyte as a sperm factor. Molecular Reproduction and Development, 76: 1200−1207. 2009.

Mizushima S, Hiyama G, Shiba K, Inaba K, Dohra H, Ono T, Shimada K and Sasanami T. The birth of quail chicks after intracytoplasmic sperm injection. Development, 141: 3799−3806. 2014.

Mizushima S. Fertilization 2: Polyspermic fertilization. Advances in Experimental Medicine and Biology, 1001: 105−123. 2017.

Nakada K, Mizuno J, Shiraishi K, Endo K and Miyazaki S. Initiation, persistence, and cessation of the series of intracellular Ca^{2+} responses during fertilization of bovine eggs. Journal of Reproduction and Development, 41: 77−84. 1995.

Nakanishi A, Utsumi K and Iritani A. Early nuclear events of in vitro fertilization in the domestic fowl (*Gallus domesticus*). Molecular Reproduction and Development, 26: 217−221. 1990.

Newton CL, Mignery GA and Südhof TC. Co-expression in vertebrate tissues and cell lines of multiple inositol 1,4,5-trisphoshpate ($InsP_3$) receptors with distinct affinities for $InsP_3$. Journal of Biological Chemistry, 269: 28613−28619. 1994.

Nucifora Jr FC, Sharp AH, Milgram SL and Ross CA. Inositol 1,4,5-trisphosphate receptors in endocrine cells: localization and association in hetero- and homotetramers. Molecular Biology of the Cell, 7: 949−960. 1996.

Nuccitelli R, Yim DL and Smart T. The sperm-induced Ca^{2+} wave following fertilization of the Xenopus oocyte requires the production of Ins (1,4,5) P3. Developmental Biology, 158: 200−212. 1993.

Ono T, Murakami T, Mochii M, Agata K, Kino K, Otsuka K, Ohta M, Mizutami M, Yoshida M and Eguchi G. A complete culture system for avian transgenesis, supporting quail embryos from the single-cell stage to hatching. Developmental Biology, 161: 126−130. 1994.

Patterson JT. Studies on the early development of the hen's egg. I History of the early cleavage and of the accessory cleavage. Journal of Morphology. 21: 101−134. 1910.

Parys JB, McPherson SM, Mathews L, Campbell KP and Longo FJ. Presence of inositol 1,4,5-trisphosphate receptor, calreticulin, and calsequestrin in oocytes of sea urchins and *Xenopus laevis*. Developmental Biology, 161: 466−476. 1994.

Percival AL, Williams AJ, Kenyon JL, Grinsell MM, Airey JA and Sutko JL. Chicken skeletal muscle ryanodine receptor isoforms: ion channel properties. Biophysical Journal, 67: 1834−1850. 1994.

Saunders CM, Larman MG, Parrington J, Cox LJ, Royse J, Blayney LM, Swann K and Lai FA. PLCζ: a sperm-specific trigger of Ca^{2+} oscillations in eggs and embryo development. Development, 129: 3533−3544. 2002.

Stricker SA. Comparative biology of calcium signaling during fertilization and egg activation in animals. Developmental Biology, 211: 157−176. 1999.

Swann K. Different triggers for calcium oscillations in mouse oocytes involve a ryanodine-sensitive calcium store. Biochemical Journal, 287: 79−84. 1992.

Runft LL, Jaffe LA, and Mehlmann LM. Egg activation at fertilization: Where it all begins. Developmental Biology, 245: 237−254. 2002.

Xu Z, Kopf GS and Schultz RM. Involvement of inositol 1,4,5-trisphosphate-mediated Ca^{2+} release in early and late events of mouse oocyte activation. Development, 120: 1851−1859. 1994.

Yoneda A, Kashima M, Yoshida S, Terada K, Nakagawa S, Sakamoto A, Hayakawa K, Suzuki K, Ueda J and Watanabe T. Molecular cloning, testicular postnatal expression, and oocyte-activating potential of porcine phospholipase Cζ. Reproduction, 132: 393−401. 2006.

Yoon SY and Fissore RA. Release of phospholipase Cζ and $[Ca^{2+}]$i oscillation-inducing activity during mammalian fertilization. Reproduction, 134: 695−704. 2007.

Yue C, White KL, Reed WA and Bunch TD. The existence of inositol 1,4,5-trisphosphate and ryanodine receptors in mature bovine oocytes. Development, 121: 2645−2654. 1995.

Yue C, White KL, Read WA and King E. Localization and regulation of ryanodine receptor in bovine oocyte. Biology of Reproduction, 58: 608−614. 1998.

Waddington D, Gribbin C, Sterling RJ, Sang HM and Perry MM. Chronology of events in the first cell cycle of the polyspermic egg of the domestic fowl (*Gallus domesticus*). International Journal of Developmental Biology, 42: 625−628. 1998.