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Antibody against the actin-binding protein depactin attenuates Ca^{2+} signaling in starfish eggs

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ABSTRACT

Being present in starfish oocytes, the cofilin/ADF (actin-depolymerizing factor) family protein depactin severs actin filaments. Previously, we reported that exogenous cofilin microinjected into starfish eggs significantly augmented the Ca^{2+} release in response to inositol 1,4,5-trisphosphate ($InsP_3$) or fertilizing sperm, raising the possibility that intracellular Ca^{2+} signaling could be modulated by the actin cytoskeleton. In this communication, we have targeted the endogenous depactin by use of the specific antibody that was raised against its actin-binding domain. The anti-depactin antibody microinjected into the starfish oocytes and eggs effectively altered the structure of the actin cytoskeleton, and significantly delayed the meiotic progression induced by 1-methyladenine. When microinjected into the mature eggs, the anti-depactin antibody markedly reduced the amplitude of the Ca^{2+} response in a dose-dependent manner, corroborating the results of our previous study with cofilin. In addition, the eggs microinjected with the anti-depactin antibody displayed reduced rate of successful elevation of the fertilization envelope and an elevated tendency of polyspermic interaction. Taken together, our data suggest that the actin cytoskeleton is implicated not only in meiotic maturation and intracellular Ca^{2+} signaling, but also in the fine regulation of gametes interaction and cortical granules exocytosis.

1. Introduction

In virtually all animal species, fertilized eggs produce a Ca^{2+} wave that starts from the sperm interaction site and travels to the opposite side of the egg [1], which is believed to initiate egg activation and embryonic development [2]. The intracellular Ca^{2+} release and wave propagation in echinoderm eggs can be recapitulated in large part by the use of second messengers such as inositol 1,4,5-trisphosphate ($InsP_3$) and nicotinic acid adenine dinucleotide phosphate (NAADP). The Ca^{2+} wave in the fertilized echinoderm egg is usually preceded by a rapid plasma membrane depolarization leading to Ca^{2+} influx through L-type Ca^{2+} channels [3,4]. Immediately after fertilization, the actin cytoskeleton in echinoderm eggs undergoes accelerated rearrangement, which may play a role in sperm incorporation and subsequent development [5–9]. While it is believed that the intracellular Ca^{2+} increase in the fertilized eggs induces cortical granules exocytosis [10] and the rapid changes of the cortical actin cytoskeleton [11], recent studies in the starfish eggs have suggested that the structural alteration of the actin cytoskeleton itself may in turn modulate the patterns of the intracellular Ca^{2+} signaling, which has been demonstrated in various experimental approaches [12,13].

Previously, we reported that starfish eggs microinjected with *exogenous* cofilin exhibited significantly augmented Ca^{2+} release in response to the fertilizing sperm or to $InsP_3$, rendering a support to the idea that the actin cytoskeleton may be implicated in the intracellular Ca^{2+} signaling [14]. Cofilin is a small (circa 17 kDa) actin-binding protein that binds, twists, and severs the actin filament and thereby plays a pivotal role in remodeling the actin cytoskeleton inside cells [15,16]. One of the earliest members of the cofilin/ADF (actin-depolymerizing factor) family proteins is depactin, which was discovered and purified from the starfish (*Asterias amurensis*) oocytes [17]. The end-label fingerprinting analysis of the cross-linked actin–depactin complex revealed that the N-terminus of depactin directly interacts with actin [18]. The site-directed antibody against the N-terminus of depactin used in the latter study also successfully detected a single band sized 17 kDa in our previous Western blot analysis of *Astropecten aranciacus* oocytes, indicating that depactin is also present in the oocytes of the Mediterranean starfish [14].

In this communication, we have further tested the findings of our previous study by targeting the *endogenous* cofilin-like protein, depactin. We have reasoned that the antibody against the

Abbreviations: GVBD, germinal vesicle breakdown; RFU, relative fluorescence unit; TTP, time to the Ca^{2+} peak.

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actin-binding domain of depactin at the N-terminus could be used as an interfering tool with which to alter the structure of the actin cytoskeleton. Since the microinjected cofilin had significantly increased the intracellular Ca^{2+} release in the fertilized eggs [14], the anti-depactin antibody should reduce the Ca^{2+} response, if the working hypothesis of the actin-based Ca^{2+} modulation is to remain valid. In addition, as the exquisite regulation of the actin cytoskeleton is required for gametes interaction and cortical granule exocytosis [8,19,20], we have also examined if functional interference of the actin–depactin interaction would to lead to deregulation of these physiological processes in starfish eggs.

2. Materials and methods

2.1. Preparation of oocytes

Oocytes were obtained from *A. aranciacus* as described previously [8]. Nearly all oocytes were marked by the presence of the large nucleus (germinal vesicle, GV), and were referred to as 'immature oocytes'. The GV-stage oocytes were treated with 10 μM of 1-methyladenine (1-MA) to induce meiotic maturation, and the GV breakdown (GVBD) was monitored with the Zeiss STEMI-SV11 stereomicroscope.

2.2. Microinjection, caged compounds, calcium imaging, and confocal microscopy

Microinjection of the oocytes and Ca^{2+} imaging were performed as previously described [8]. Calcium-Green and caged $InsP_3$ (Molecular Probes) were prepared in the injection buffer (10 mM Hepes and 100 mM L-Asp, pH 7.0). To activate the caged $InsP_3$, microinjected eggs were irradiated with 330 nm UV light. The quantified Ca^{2+} signal was normalized to the baseline fluorescence (F_0) following the formula $F_{rel} = [F-F_0]/F_0$, where F represents the average fluorescence level of the entire oocyte. In plotting the data, the image frame immediately before the first detectable Ca^{2+} signal was taken as $t = 0$. As described previously [19], F-actin was visualized with Alexa-Fluor-488-phalloidin (50 μM in pipette) in Zeiss LSM 510 META Laser Scanning Confocal Microscope (Jena, Germany).

2.3. Preparation of antibody for microinjection

Purified rabbit polyclonal antibody against the N-terminus of the depactin protein [18] was a generous gift from Dr. I. Mabuchi at the Gakushuin University, Tokyo, Japan. Immunoglobulin purified from the serum of a non-immunized rabbit with the use of Protein A Sepharose CL-4B (GE Healthcare Life Sciences) was taken as the control antibody. The control and anti-depactin antibodies were shifted to the injection buffer using Amicon Ultra Centrifugal Filters 10 K (Millipore), and the concentration was adjusted with the injection buffer before microinjection.

2.4. Statistical analysis

The average and variation of the data were reported as 'mean ± standard deviation (SD)' in all cases. The paired *t*-test and the one-way ANOVA were performed by use of Prism 3.0 (GraphPad Software, La Jolla, USA), and the *P*-values smaller than 0.05 ($P < 0.05$) were considered statistically significant.

3. Results

3.1. Anti-depactin antibody induces structural changes of the actin cytoskeleton in starfish oocytes

To test if the anti-depactin antibody could be used as a tool for modifying the actin cytoskeleton, immature oocytes at the GV-stage were microinjected with the anti-depactin antibody, and the structure of the actin filaments in the live cells was examined with a second microinjection of Alexa 488-phalloidin 30 min later. As expected, we found that the anti-depactin antibody induced appreciable remodeling of the actin cytoskeleton. Whereas the oocytes preinjected with the control antibody displayed the characteristic distribution of actin filaments in the immature oocytes, i.e., relatively loose but intricate scaffolds of actin fibers in the cytoplasm and the tightly knit actin network around the GV and in the subplasmalemmal region with a total absence of the phalloidin-stained F-actin in the GV (Fig. 1A, left), the oocytes preinjected with the anti-depactin antibody exhibited a novel and unusual formation of the actin fibers in the GV with the creation of numerous punctuate actin aggregates in the cytoplasm (Fig. 1A, right). These results indicated that the microinjected anti-depactin antibody interfered with subcellular region-specific actin dynamics, and that the use of the function-blocking antibody could be adopted as a strategy to modify the actin cytoskeleton in oocytes.

3.2. Anti-depactin antibody impedes progression of meiotic maturation of starfish oocytes

As the anti-depactin antibody tended to induce the formation of actin filaments in the GV with concomitant disappearance of the actin fibers around the GV, we examined if these changes might reflect incidental breakdown of the GV as a result of a potential escape from the meiotic arrest. However, the anti-depactin antibody itself did not induce GVDB without 1-MA treatment (data not shown), but instead significantly delayed the meiotic maturation process in the 1-MA-treated oocytes. Plotted on the time scale after the addition of 1-MA, the frequency of the post-GVBD eggs sharply increased between 45 and 60 min in the two groups of oocytes preinjected either with the injection buffer or with the control antibody, displaying the nearly identical trajectories (Fig. 1B). In these control oocytes, the time point at which a half of the cell population manifested clear signs of GVDB ($t_{1/2}$) was a little before 50 min. By contrast, in the oocytes preinjected with the anti-depactin antibody, the steeply rising curve was shifted to the right by 5–10 min, and the extrapolated $t_{1/2}$ was attained at about 57 min. Thus, microinjected into immature oocytes, the anti-depactin antibody did not block GVBD in the given condition, but noticeably delayed its occurrence.

3.3. Anti-depactin antibody inhibits the progress of the oocytes' sensitization to $InsP_3$ during meiotic maturation

The intracellular Ca^{2+}-releasing mechanism of starfish oocytes becomes optimized during meiotic maturation so that the same amount of $InsP_3$ evokes much larger Ca^{2+} release in the mature eggs than in immature oocytes [21]. Since the process of sensitization to $InsP_3$ appears to depend on the actin cytoskeleton [22], we tested if the anti-depactin antibody could interfere with this transition by examining the Ca^{2+} responses to the caged $InsP_3$ that was liberated at various time points of meiotic maturation (Fig. 2). From the comparison of the Ca^{2+} releases just in the control oocytes that were photo-activated before, 40 min after, and 70 min after the addition of 1-MA (Fig. 2A, green curves), it was evident that the sensitization to $InsP_3$ takes place mainly at the later stage

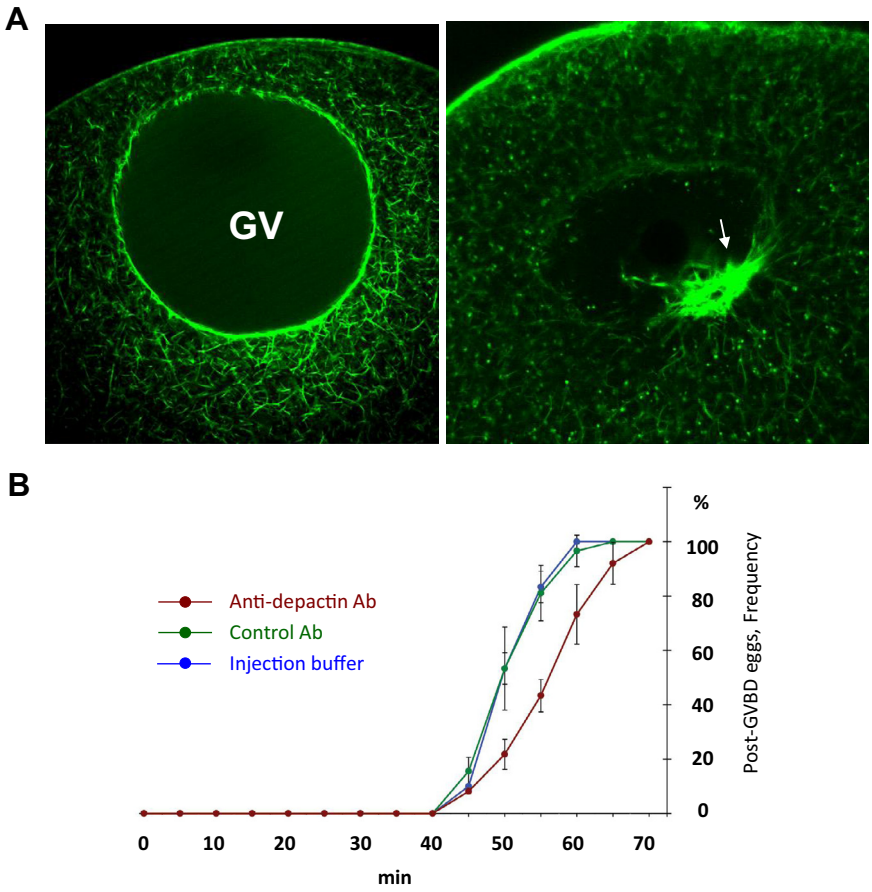
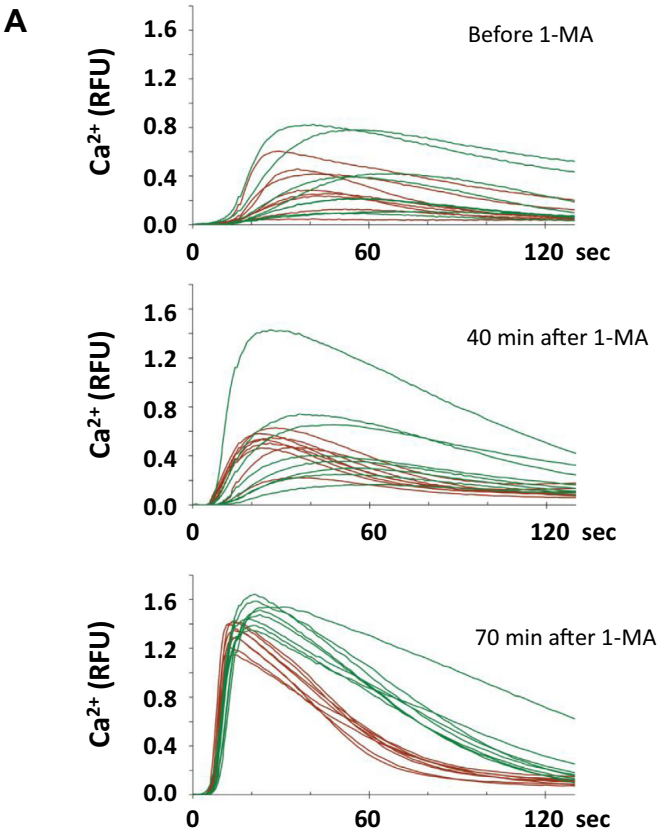


Fig. 1. Functionality of the anti-depactin antibody. (A) Anti-depactin antibody drastically altered the structure of the actin cytoskeleton in *A. aranciacus* oocytes. Oocytes at the germinal vesicle (GV)-stage were microinjected with the control or anti-depactin antibody (9 μg/μl in pipette). After 25 min incubation, the oocytes were microinjected with Alexa 488-phalloidin to visualize F-actin in the live cells. The images captured with confocal microscopy 5 min later indicated that anti-depactin antibody specifically induced formation of actin filaments in the GV (arrow) and created numerous punctuate actin aggregates in the cytoplasm. (B) Anti-depactin antibody significantly delayed the meiotic maturation in starfish oocytes. The GV-stage oocytes microinjected with the injection buffer or either antibody (4 μg/μl) were induced to undergo meiotic maturation by adding 1-MA (10 μM). The frequencies of the oocytes displaying clear signs of GV breakdown in each group was calculated from 20 oocytes at 5 min intervals. The data were pooled from three independent experiments.

of meiotic maturation. Whereas the first 40 min of the 1-MA exposure resulted in only a marginal and non-significant increase in the peak amplitude of the Ca^{2+} release after $InsP_3$ uncaging (0.38 ± 0.29 RFU before 1-MA *versus* 0.54 ± 0.41 RFU at 40 min, $P = 0.4904$), the average peak amplitude at 70 min was nearly a threefold leap (1.49 ± 0.098 RFU) from the response at 40 min ($P < 0.001$, $n = 8$). Compared with these control oocytes, the oocytes preinjected with the anti-depactin antibody exhibited consistently lower Ca^{2+} peaks at each time point (Fig. 2A, the brown curves *versus* the green ones), although the difference was statistically significant only at 70 min (1.31 ± 0.10 RFU *versus* 1.49 ± 0.098 RFU of the control, $P < 0.005$). It is noteworthy that the oocytes preinjected with either antibody were all well past GVDB at 70 min, but this extra time which was difficult to individually control might not have been long enough to restore a full optimization of the intracellular Ca^{2+}-releasing system. Thus, it would be fair to say that the anti-depactin antibody either delayed the progress of the oocytes' sensitization to $InsP_3$, as it did to the timing of GVBD, or might have repressed the Ca^{2+} increase in a more direct way.

3.4. Anti-depactin antibody subtly changes the kinetics of the $InsP_3$-dependent Ca^{2+} release

Previously we reported that microinjection of starfish eggs with human cofilin led to a higher Ca^{2+} release upon $InsP_3$ uncaging, but the Ca^{2+} rise curiously displayed a considerably slower rising kinetics [14]. Conversely, an interesting point we noted here is that the rising kinetics of the Ca^{2+} increase in the oocytes with the anti-depactin antibody was appreciably faster than in the control oocytes, as the Ca^{2+} trajectories of the two groups were clearly separable especially at 70 min (Fig. 2A). This point can also be demonstrated by examining the length of time that is required for the first detectable Ca^{2+} signal to increase and attain its peak value. Thus, the measure of 'the time to the peak (TTP)' could be considered an index of the 'explosive' nature of the cytoplasm as an excitable matrix propagating Ca^{2+} signals. From the comparison among the control oocytes, it was evident that the TTP of the Ca^{2+} release by uncaged $InsP_3$ was not significantly shortened until the meiotic maturation surpassed GVDB, as the oocytes at 40 min into the meiotic maturation (still at the pre-GVBD stage) displayed their average TTP (46.2 ± 11.9 s) not significantly shorter than what it was before the 1-MA exposure (56.9 ± 10.8 s, $n = 8$, $P = 0.1592$). However, the average TTP was remarkably shortened by more than twofold (22.4 ± 3.4 s, $P < 0.01$) when the oocytes became post-GVBD eggs (70 min) and displayed a high level of Ca^{2+} response. Interestingly, despite the lower Ca^{2+} response, we found that the TTP in the oocytes preinjected with the anti-depactin antibody was significantly shorter than that in the control at each time point (Fig. 2B). Thus, the observation that the oocytes with the anti-depactin antibody exhibit lower Ca^{2+} response but significantly faster rising kinetics is somewhat conspicuous in a sense that the oocyte with a higher Ca^{2+} response usually shows faster rising kinetics.



B

	Control Ab	Anti-depactin Ab	Significance
Before 1-MA	56.9 ± 10.8 sec	40.9 ± 7.1 sec	$P < 0.01$
40 min after 1-MA	46.2 ± 11.9 sec	27.5 ± 5.2 sec	$P < 0.005$
70 min after 1-MA	22.4 ± 3.4 sec	13.8 ± 1.2 sec	$P < 0.0001$

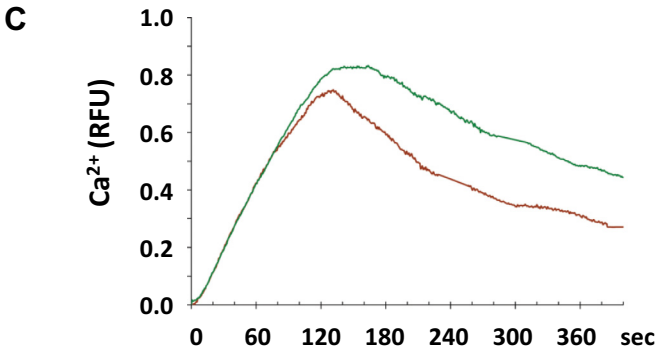


Fig. 2. Effects of the anti-depactin antibody on intracellular Ca^{2+} signaling. (A) Anti-depactin antibody inhibited the progress of the oocytes′ sensitization to $InsP_3$ during meiotic maturation. Oocytes at the GV stage were microinjected with the mixture of Calcium Green and caged $InsP_3$ (250 and 5 μM, respectively), and with either anti-depactin or control antibodies (4 μg/μl). After 20 min, 1-MA was added to induce meiotic maturation. Immediately before (top panel), 40 min after (middle), and 70 min after (bottom panel) the 1-MA addition, the Ca^{2+} increase in response to the uncaged $InsP_3$ (UV illumination, 15 s) was examined as described in Section 2. Ca^{2+} responses in the oocytes matured in the presence of the control and anti-depactin antibodies were depicted in green and brown curves, respectively. (B) The kinetics of the Ca^{2+} rises in response to the uncaged $InsP_3$ was presented in terms of the time required to arrive at the peak. (C) Intracellular Ca^{2+} increases in response to the fertilizing sperm. As described in the Section 2, the Ca^{2+} increases at fertilization were quantified for the oocytes matured in the presence of the microinjected control (green) or anti-depactin antibody (brown curve) for 70 and 80 min, respectively.

One possible explanation is that the liberated $InsP_3$ might have been 'seen' by the receptors slightly faster due to the changed cytoskeletal environment and the reduced diffusion barrier.

3.5. The changes of the actin cytoskeleton may modulate the Ca^{2+} response

As aforementioned, the reduced level of Ca^{2+} release in the eggs matured in the presence of the microinjected anti-depactin antibody may be due to the delayed progression of meiotic maturation during which the intracellular Ca^{2+}-release mechanism is sensitized. However, this may not be entirely a matter of timing. When the eggs matured with the microinjected anti-depactin antibody were given an extra compensatory time of 10 min, the level of the Ca^{2+} release at fertilization (0.75 ± 0.080 RFU, $n = 6$) was still significantly lower than that in the control eggs (0.88 ± 0.029 RFU, $n = 4$, $P < 0.05$) (Fig. 2C). Thus, this result suggests that anti-depactin antibody not only delayed the meiotic progression but may also have inflicted a lasting physical change that contributed to intracellular Ca^{2+} signaling. To examine the effect of the anti-depactin antibody on the Ca^{2+} signaling independent of the issue of meiotic maturation, we have introduced the control and anti-depactin antibodies into the eggs that had been already matured normally, and compared their Ca^{2+} responses to the fertilizing sperm. Similar to the results obtained with immature oocytes (Fig. 1A), this treatment markedly altered the structure of the actin cytoskeleton in the post-GVBD eggs (Fig. 3A). Here again, the intricate scaffolds of the cytoplasmic actin fibers largely disappeared with the concomitant formation of numerous punctuate F-actin aggregates. In addition, the characteristic organization of the subplasmalemmal actin network seen in the control eggs was absent in the eggs microinjected with the anti-depactin antibody (Fig. 3A), suggesting that the nature of the egg surface and the cortex was significantly changed. We found the treatment with the anti-depactin antibody (1.6–9 μg/μl) repress the intracellular Ca^{2+} release at fertilization in a dose-dependent manner (Fig. 3B). When the extent of the repression was compared by normalizing the Ca^{2+} responses with the average amplitude of the Ca^{2+} peaks in the corresponding control eggs, the eggs microinjected with 1.6, 3, and 9 μg/μl of anti-depactin antibodies showed respectively 90.4 ± 12.7 ($n = 8$), 72.8 ± 21.6 ($n = 4$), and 57.6 ± 8.0% ($n = 5$) of the levels in the corresponding controls (Fig. 3B).

3.6. Effects of the anti-depactin antibody on egg–sperm interaction and cortical granules exocytosis

We have examined the physiological consequences of the structural changes of the cortical actin cytoskeleton induced by anti-depactin antibody. At fertilization, a significant sperm–egg collision is marked by a localized Ca^{2+} spot on the egg surface at the very site of the interaction. Monospermic fertilization usually produces a Ca^{2+} wave propagating from a single Ca^{2+} spot, but we have found that the eggs microinjected with the anti-depactin antibody tend to produce supernumerary Ca^{2+} spots, which is an indirect sign of deregulated sperm–egg interaction that may lead to polyspermic fertilization (Fig. 4A). In addition, we found that

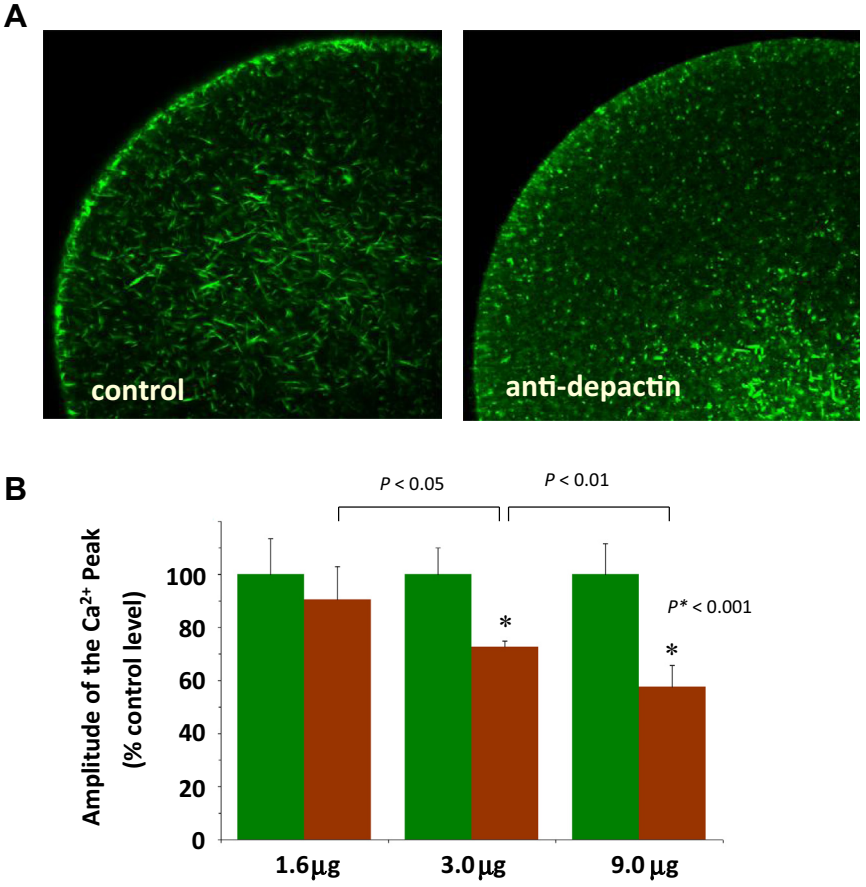


Fig. 3. Anti-depactin antibody represses the Ca^{2+} response in the starfish eggs at fertilization. (A) Structural changes of the actin cytoskeleton by the anti-depactin antibody in the mature eggs. Post-GVBD eggs were microinjected with either the control or anti-depactin antibody (9 μg/μl). After 25 min incubation, F-actin was visualized by a second microinjection with Alexa 488-phalloidin. (B) Dose-dependent effects of the anti-depactin antibody on the Ca^{2+} increase in the starfish eggs at fertilization. Post-GVBD eggs containing calcium dyes were microinjected either with the control or anti-depactin antibody and incubated 30 min before fertilization. The amplitudes of the Ca^{2+} peaks in the eggs with the depactin antibody were normalized with the average amplitude of the Ca^{2+} peaks in the eggs with the equal amount of the control antibody.

the rate of successful and full elevation of the fertilization envelope was significantly decreased in the eggs microinjected with the anti-depactin antibody in comparison with the eggs with the control antibody (Fig. 4B). Taken together, these results corroborated our previous findings that the actin cytoskeleton contributes to the fine regulation of intracellular Ca^{2+} signaling, gametes interaction, and cortical granules exocytosis [8,14,19].

4. Discussion

In this communication, we have demonstrated that the actin cytoskeleton of starfish oocytes and eggs could be markedly reorganized by the microinjection of the anti-depactin antibody. It appeared that sequestration of endogenous depactin in the oocytes shifted the dynamics of the interactions between actin and depactin and produced punctuate F-actin aggregates in the cytoplasm, as well as an unusual formation of the actin fibers in the GV, which normally do not display phalloidin-stainable microfilaments (Fig. 1A) despite its presumed abundance of actin [23]. In line with the findings in *Xenopus* oocytes [24], the alteration of the actin cytoskeleton by the depactin antibody significantly delayed GVBD in starfish oocytes (Fig. 1B). Even after the GVBD, these eggs did not mix well the nucleoplasm with the cytoplasm unlike in the control eggs (data not shown), and exhibited a significantly reduced amount of Ca^{2+} release in response to the uncaged $InsP_3$ (Fig. 2A). Thus, in agreement with the previous finding [22], the actin cytoskeleton appears to play a role in meiotic maturation of the Ca^{2+} releasing mechanism. However, our data in Fig. 3 supports the idea that the actin cytoskeleton may more directly modulate the ongoing physiological process of intracellular Ca^{2+} release from the stores, as the reduced level of Ca^{2+} release at fertilization was observed when the anti-depactin antibody was introduced into the eggs that had been already matured normally. Hence, this result extends further and corroborates our previous observations in which microinjection of cofilin led to the enhancement of the Ca^{2+} release at fertilization [14].

It should be noted that a certain aspect of intracellular Ca^{2+} signaling in starfish eggs, whether it be the magnitude, rising kinetics, or the onset of the Ca^{2+} wave, was significantly altered when the normal dynamics of actin polymerization was interfered with by various methods using latrunculin A [22], actin-depolymerizing protein [14], jasplakinolide and heparin [19], GDPβS [25], and the PIP2-sequestering domain of PLC-δ1 [8]. The list of the experimental paradigm is now extended to the use of a function-blocking antibody. Furthermore, inhibition of actin polymerization with latrunculin A gives rise to a 'spontaneous' increase of intracellular Ca^{2+} and to membrane depolarization with the considerable time lag expected for actin depolymerization [26,27]. The inevitable question is then how the actin cytoskeleton regulates Ca^{2+} signaling. While not much has been proven as an answer, it has been suggested that the actin cytoskeleton may interact with the ion channels and influence their activities by altering their microenvironment [28,29] or by participating in intracellular Ca^{2+} homeostasis as a physical entity of supplemental Ca^{2+} storage [13,30,31]. In addition, actin itself may effect a Ca^{2+} buffer or a diffusion barrier for the Ca^{2+}-inducing second messenger $InsP_3$ [30]. Finally, the results of our study (Fig. 4) further extended the previous findings that interference of the actin dynamics almost always increased the incidents of polyspermy and led to failed exocytosis of cortical

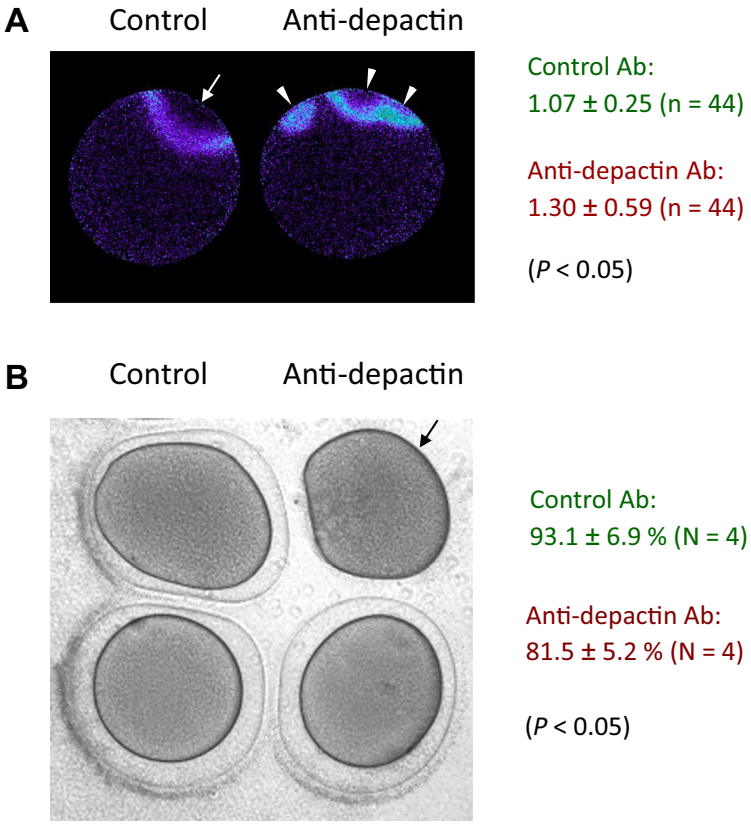


Fig. 4. Effects of the anti-depactin antibody on egg–sperm interaction and cortical granules exocytosis. (A) Sperm-induced Ca^{2+} spots and the initial Ca^{2+} waves in the starfish eggs at fertilization. The average number of the initial Ca^{2+} spots (white arrow and arrowheads) at fertilization was significantly increased in the eggs pre-injected with the anti-depactin antibody. (B) Preinjection of the eggs with the anti-depactin antibody significantly reduced the rate of successful elevation of the fertilization envelope (FE). The egg with failed FE elevation was marked with the black arrow. Data were pooled from four different batches of eggs.

granules [8,19,32]. Thus, exquisite regulation of the actin cytoskeleton in the egg cortex is thought to be essential not only for the modulation of intracellular Ca^{2+} signaling, but also for the vesicular exocytosis and sperm incorporation.

Acknowledgments

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Reference

[1] L.F. Jaffe, Classes and mechanisms of calcium waves, Cell Calcium 14 (1993) 736–745.
[2] R.A. Steinhardt, D. Epel, Activation of sea-urchin eggs by a calcium ionophore, Proc. Natl. Acad. Sci. USA 71 (1974) 1915–1919.
[3] M. Whitaker, R.F. Irvine, Inositol 1,4,5-trisphosphate microinjection activates sea urchin eggs, Nature 312 (1984) 636–639.
[4] L. Santella, D. Lim, F. Moccia, Calcium and fertilization: the beginning of life, Trends Biochem. Sci. 29 (2004) 400–408.
[5] F.J. Longo, Effects of cytochalasin B on sperm–egg interactions, Dev. Biol. 67 (1978) 249–265.
[6] L.G. Tilney, L.A. Jaffe, Actin, microvilli, and the fertilization cone of Sea urchin eggs, J. Cell Biol. 87 (1980) 771–782.
[7] M. Terasaki, Actin filament translocations in sea urchin eggs, Cell Motil. Cytoskeleton 34 (1996) 48–56.
[8] J.T. Chun, A. Puppo, F. Vasilev, et al., The biphasic increase of PIP2 in the fertilized eggs of starfish: new roles in actin polymerization and Ca^{2+} signaling, PLoS One 5 (2010) e14100.
[9] F. Vasilev, J.T. Chun, G. Gragnaniello, et al., Effects of ionomycin on egg activation and early development in starfish, PLoS One 7 (2012) e39231.
[10] V.D. Vacquier, The isolation of intact cortical granules from sea urchin egg: calcium ions trigger granule discharge, Dev. Biol. 43 (1975) 62–74.
[11] A. Arai, K. Kyozuka, T. Nakazawa, Cytoplasmic Ca^{2+} oscillation coordinates the formation of actin filaments in the sea urchin eggs activated with phorbol ester, Cell Motil. Cytoskeleton 42 (1999) 27–35.
[12] J.T. Chun, L. Santella, Roles of the actin-binding proteins in intracellular Ca^{2+} signaling, Acta Physiol. 195 (2009) 61–70.
[13] L. Santella, J.T. Chun, Actin, more than just a housekeeping protein at the scene of fertilization, Sci. China Life Sci. 54 (2011) 733–743.
[14] G.A. Nusco, J.T. Chun, E. Ercolano, et al., Modulation of calcium signaling by the actin-binding protein cofilin, Biochem. Biophys. Res. Commun. 348 (2006) 109–114.
[15] S.K. Maciver, How ADF/cofilin depolymerizes actin filaments, Curr. Opin. Cell Biol. 10 (1998) 140–144.
[16] J.R. Bamburg, Proteins of the ADF/cofilin family: essential regulators of actin dynamics, Annu. Rev. Cell Dev. Biol. 15 (1999) 185–230.
[17] I. Mabuchi, An actin-depolymerizing protein (depactin) from starfish oocytes: properties and interaction with actin, J. Cell Biol. 97 (1983) 1612–1621.
[18] K. Sutoh, I. Mabuchi, End-label fingerprintings show that an N-terminal segment of depactin participates in interaction with actin, Biochemistry 28 (1989) 102–106.
[19] A. Puppo, J.T. Chun, G. Gragnaniello, et al., Alteration of the cortical actin cytoskeleton deregulates Ca^{2+} signaling, monospermic fertilization, and sperm entry, PLoS One 3 (2008) e3588.
[20] K. Kyozuka, J.T. Chun, A. Puppo, et al., Actin cytoskeleton modulates calcium signaling during maturation of starfish oocytes, Dev. Biol. 320 (2008) 426–435.
[21] K. Chiba, R.T. Kado, L.A. Jaffe, Development of calcium release mechanisms during starfish oocyte maturation, Dev. Biol. 140 (1990) 300–306.
[22] D. Lim, E. Ercolano, K. Kyozuka, et al., The M-phase-promoting factor modulates the sensitivity of the Ca^{2+} stores to inositol 1,4,5-trisphosphate via the actin cytoskeleton, J. Biol. Chem. 278 (2003) 42505–42514.
[23] T.G. Clark, R.W. Merriam, Diffusible and bound actin in nuclei of *Xenopus laevis* oocytes, Cell 12 (1977) 883–891.
[24] I. Okada, S. Fujiki, S. Iwase, et al., Stabilization of actin filaments prevents germinal vesicle breakdown and affects microtubule organization in *Xenopus* oocytes, Cytoskeleton 69 (2012) 312–323.
[25] K. Kyozuka, J.T. Chun, A. Puppo, et al., Guanine nucleotides in the meiotic maturation of starfish oocytes: regulation of the actin cytoskeleton and of Ca^{2+} signaling, PLoS One 4 (2009) e6296.
[26] D. Lim, K. Lange, L. Santella, Activation of oocytes by latrunculin A, FASEB J. 16 (2002) 1050–1056.
[27] F. Moccia, Latrunculin A depolarizes starfish oocytes, Comp. Biochem. Physiol., A: Mol. Integr. Physiol. 148 (2007) 845–852.
[28] K. Fukatsu, H. Bannai, S. Zhang, et al., Lateral diffusion of inositol 1,4,5-trisphosphate receptor type 1 is regulated by actin filaments and 4.1 N in neuronal dendrites, J. Biol. Chem. 279 (2004) 48976–48982.
[29] M.R. Turvey, K.E. Fogarty, P. Thorn, Inositol (1,4,5)-trisphosphate receptor links to filamentous actin are important for generating local Ca^{2+} signals in pancreatic acinar cells, J. Cell Sci. 118 (2005) 971–980.
[30] K. Lange, J. Gartzke, F-actin-based Ca signaling – a critical comparison with the current concept of Ca signaling, J. Cell Physiol. 209 (2006) 270–287.
[31] L. Santella, A. Puppo, J.T. Chun, The role of the actin cytoskeleton in calcium signaling in starfish oocytes, Int. J. Dev. Biol. 52 (2008) 571–584.
[32] L. Santella, F. Vasilev, J.T. Chun, Fertilization in echinoderms, Biochem. Biophys. Res. Commun. 425 (2012) 588–594.