Modulation of calcium signalling by the actin-binding protein cofilin

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Abstract

Cofilin is a small protein that belongs to the family of actin-depolymerizing factors (ADF). The main cellular function of cofilin is to change cytoskeletal dynamics and thus to modulate cell motility and cytokinesis. We have recently demonstrated that the actin cytoskeleton is involved in the modulation of Ca²⁺ signalling in starfish oocytes. To extend these observations, we have explored whether cofilin influences Ca²⁺ signalling in the oocytes. Here we show that microinjection of the functionally active cofilin alters the Ca²⁺ signalling mediated by the three major second messengers, InsP₃, NAADP, and cADPr. Cofilin intensifies the Ca²⁺ signals induced by InsP₃ and NAADP, and delays those induced by cADPr. Furthermore, the injection of cofilin increases the Ca²⁺ signals during hormone-induced oocyte maturation and fertilization. The results suggest that the dynamic regulation of F-actin by its binding proteins may play an important role in the modulation of intracellular Ca²⁺ signalling.

Keywords: Actin-depolymerising factor; Inositol 1,4,5-trisphosphate; Cyclic ADP-ribose; Nicotinic acid adenine dinucleotide phosphate; Phosphatidylinositol-4,5-bisphosphate; 1-Methyladenine; Latrunculin A

Starfish oocytes have been an excellent model system in the study of the generation and propagation of the Ca²⁺ signal in the living cell. Ca²⁺ released by three major second messengers, i.e., InsP₃, cADPr, and NAADP [1–6], is critical to the maturation and fertilization of the oocytes. The three major second messengers are believed to play distinct roles in generating Ca²⁺ signals during fertilization of starfish oocytes [7–9]. We have recently demonstrated that Ca²⁺ release from the InsP₃-sensitive stores is enhanced during oocyte maturation [10]. Since the increased Ca²⁺ response to the exogenously added InsP₃ nicely correlated with the dynamic rearrangement of F-actin [10], we have suggested that the asymmetrically distributed cytoskeleton may be involved in the enhanced Ca²⁺ response. Indeed, the addition of the actin-depolymerizing agent latrunculin A (LAT-A) induced a massive release of intracellular Ca²⁺ in mature oocytes, but not in immature oocytes [11]. Hence, the reorganization of F-actin during the maturation process [12] appeared to be somehow involved in the modulation of Ca²⁺ signalling.

Cofilin/ADF is an actin-binding protein that induces microfilament reorganization both in vivo [13,14] and in vitro [15–17]. The binding site for actin in the cofilin/ADF molecule overlaps the domain that binds PIP₂ [18,19]. This dual binding activity suggests the possible involvement of cofilin/ADF in the control of Ca²⁺ release. In the current communication we have explored this possibility. We have observed that exogenously added cofilin increased the intracellular Ca²⁺ release mediated by InsP₃ and NAADP in starfish oocytes. By contrast, it delayed cADPr-induced Ca²⁺ increase. Moreover, cofilin stimulated the intracellular Ca²⁺ release that occurs naturally during oocyte maturation and fertilization.

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Materials and methods

Preparation of oocytes. The Japanese (Asterina pectinifera) and Mediterranean (Astreopetra aurantia) species of starfish were acquired and maintained as previously described [9]. Fully grown immature oocytes arrested at the prophase of the first meiotic division were dissected from the gonads and processed in filtered artificial seawater (ASW: 460 mM NaCl, 10.1 mM KCl, 9.2 mM CaCl₂, 35.9 mM MgCl₂, 17.5 mM MgSO₄, and 2.5 mM NaHCO₃, pH 8.0) as described previously [9]. For fertilization experiment, immature oocytes were first stimulated with 1-MA (10 μM) for 1 h, and then spermatozoa were added to the mature oocytes in ASW.

Actin depolymerization assay. Pyrene-G-actin (monomer) was polymerized to fluorescent pyrene F-actin by using the Actin Polymerization Biochem kit BK003 (Cytoskeleton Inc., USA). Measurement of the fluorescence was performed in a spectrophotometer (Perkin-Elmer) with the excitation/emission wavelength at 365 and 407 nm, respectively.

Microinjection, photoactivation of caged compounds, and Ca²⁺ imaging. Fluorescent calcium dye (Oregon Green 488 BAPTA-1) was coupled to the antibody was extensively rinsed and the blot was incubated in the same binding buffer in the presence of HRP-conjugated anti-rabbit IgG. The quantified Ca²⁺ signal was normalized to the fluorescence. When cofilin was added to the preassembled pyrene F-actin, the fluorescence decreased immediately to the basal level as expected, indicating that the actin filaments were quickly depolymerized (Fig. 1). The cofilin storage buffer used as a control only produced a modest depolymerization, the extent of which was three to four times lower than that induced by cofilin.

Cofilin enhances InsP₃-induced calcium release

Previously, we had demonstrated that the microfilament-disrupting agent LAT-A enhanced the InsP₃-dependent calcium release [10,11]. To extend this finding, we examined whether and how cofilin influences the dynamics of the InP₃-induced Ca²⁺ response. For this, immature oocytes were injected with caged InsP₃ (20 μM) and the Ca²⁺ indicator as described in the Materials and methods section. Five minutes later, when the Ca²⁺ indicator as described in the Materials and methods section. Five minutes later, when the Ca²⁺ indicator was activated, InsP₃ had diffused uniformly inside the cell, InsP₃, was liberated by 25 s of continuous UV irradiation. As shown in Fig. 2A, InsP₃ promoted Ca²⁺ release from intracellular stores at the cell cortex. Then, the cortical Ca²⁺ signal began to spread centripetally until the entire cytoplasm was filled with Ca²⁺ (Fig. 2A). The quantification of the Ca²⁺ signal over the entire oocyte showed that the libera-
Ca\(^{2+}\) signal. For this, control or cofilin-preloaded oocytes were injected with caged NAADP. In the absence of pre-injected cofilin, the photoactivation of NAADP induced a Ca\(^{2+}\) increase which started in the cortical domain of the oocyte (Fig. 3A). Then the Ca\(^{2+}\) wave spread centripetally to the entire oocyte and reached its peak approximately 5 s after the beginning of UV irradiation. In the presence of cofilin, the Ca\(^{2+}\) increase was slightly delayed, as its peak occurred 8 s after the UV irradiation started. As was the case with InsP\(_3\), the peak of the Ca\(^{2+}\) signal was substantially (44\%) enhanced by cofilin injection (Fig. 3B).

**Cofilin strongly delays the onset of the cADPr-dependent Ca\(^{2+}\) signalling**

We then examined the effect of cofilin on the Ca\(^{2+}\) release induced by the third Ca\(^{2+}\)-linked second messenger, cADPr. In the absence of cofilin, the Ca\(^{2+}\) signal generated by uncaged cADPr initiated after 5 s in several distinct spots of the oocyte before spreading to the entire cytoplasm (Fig. 4A). This particular pattern confirmed the previous observation from our laboratory [21]. The peak of the global Ca\(^{2+}\) increase was reached between 10 and 15 s, which is considerably delayed in comparison with InsP\(_3\) (9 s) or NAADP (5 s). In the presence of pre-injected cofilin, the amplitude of the cytoplasmic Ca\(^{2+}\) peak was not changed much, but the
onset and development of Ca\textsuperscript{2+} increase were very much delayed after cADPr uncaging. The peak was reached 30–60 s later than in the control oocytes (Fig. 4B). These results clearly indicate that cofilin, which reorganizes actin filaments, is able to influence the pattern and the amount of Ca\textsuperscript{2+} release in response to all three Ca\textsuperscript{2+}-related second messengers, i.e., InsP\textsubscript{3}, NAADP, and cADPr.

The Ca\textsuperscript{2+} release during oocyte maturation is potentiated by cofilin

To test if cofilin also influences Ca\textsuperscript{2+} signalling during physiological process, control and cofilin-preloaded oocytes were stimulated with the maturation hormone 1-methyladenine. After 2 min of hormone incubation, control oocytes began to produce Ca\textsuperscript{2+} signal at the spot of the vegetal pole (Fig. 5A, arrow), from which it subsequently spread along the cortex toward the animal pole. This process was rather fast: 15–20 s after Ca\textsuperscript{2+} had first appeared in the oocyte, the Ca\textsuperscript{2+} signal swept through the entire cell.

In the presence of injected cofilin, the Ca\textsuperscript{2+} signal started nearly 10 s delayed but from a broad area of the cortex rather than from a single spot (Fig. 5A). As a result, the amount of Ca\textsuperscript{2+} release after hormonal stimulation was more than doubled in the presence of cofilin. When the global Ca\textsuperscript{2+} was quantified, the peak of the Ca\textsuperscript{2+} signal was 105% higher than that of the control oocytes (Fig. 5B). During the propagation of the signal, a gradient of Ca\textsuperscript{2+} concentration developed from the cortex to the cytoplasm, the highest level of Ca\textsuperscript{2+} being restricted to the cortical areas (Fig. 5A; and confocal microscopy observation, data not shown). However, it is important to emphasize that the hormone-induced Ca\textsuperscript{2+} signal arose entirely from the internal source, as it also occurred in oocytes bathed in calcium-free seawater (data not shown). In agreement with the experiments on the Ca\textsuperscript{2+}-related second messengers, the peak of Ca\textsuperscript{2+} release in the cofilin-injected oocytes was delayed by about 10 s in comparison with the control oocytes. Interestingly, when the Ca\textsuperscript{2+} signal subsided after 6 min, the basal level of cytoplasmic Ca\textsuperscript{2+} in the cofilin-injected cells was consistently lower than that of the controls (Fig. 5B). This result is in agreement with our observation that cofilin injection itself lowers the basal level of the Ca\textsuperscript{2+} in the cytoplasm of starfish oocyte (data not shown).
Cofilin alters the sperm-induced Ca²⁺ response during fertilization

Ca²⁺ signalling during fertilization of starfish oocytes is characterized by an instant Ca²⁺ elevation that initiates just beneath the plasma membrane (cortical flash) and by the subsequent propagation of the Ca²⁺ wave to the entire cytoplasm [7–9]. In the absence of injected cofilin, the sharp cortical flash initiated after the sperm had contacted the oocyte (Fig. 6A). The cortical Ca²⁺ flash then disappeared very rapidly. Then, a wave of Ca²⁺ ensued from the sperm-oocyte interaction site and propagated to the opposite side of the oocyte (Fig. 6A).

In the presence of injected cofilin, the cortical flash was either abolished (12 cases out of 40) or significantly reduced (28 cases out of 40) in the fertilized oocytes (Fig. 6A). Despite the impairment of the cortical flash, cofilin substantially enhanced the propagation phase of the Ca²⁺ wave. When the increased Ca²⁺ was quantified over the entire cytoplasm, the peak of the signal in cofilin-preinjected oocytes was 32% higher than in the control (Fig. 6B). After the Ca²⁺ signal had subsided (>7 min), the basal level of cytoplasmic Ca²⁺ in the cofilin-injected oocytes was consistently lower than that of the control (Fig. 6B).

Discussion

Actin-binding protein cofilin/ADF not only stimulates treadmilling of F-actin, but also severs or twists actin filaments in vitro [22,23]. In vivo, the function of cofilin has been mainly linked to cell motility, migration, and cytokinesis [24–26]. In the present work, we have reported the first evidence that cofilin can also modulate the intracellular Ca²⁺ signalling.

It has been known that the increase of Ca²⁺ is mainly due to the release from the intracellular stores through InsP₃- and cADPr/ryanodine-sensitive channels (reviewed in [27]), but we have recently demonstrated that the novel second messenger NAADP mediates Ca²⁺ influx through plasma membrane calcium channels [20].

The results of the present study indicate that cofilin modulates the kinetics of cellular Ca²⁺ increase by all three major Ca²⁺-releasing second messengers, i.e., InsP₃, cADPr, and NAADP. In addition, injected cofilin also altered the spatio-temporal pattern of physiologically occurring Ca²⁺ signals during oocyte maturation and fertilization. Based on these results, several possibilities can be considered on the role of cofilin in Ca²⁺ signalling. Cofilin might directly modulate the activity of Ca²⁺-releasing channels. Or, as it seems more likely, cofilin could fine-tune their activity indirectly by remodelling the cytoskeleton of the microenvironment. In line with this possibility, recent studies have demonstrated that the Ca²⁺ influx across the plasma membrane is regulated by cofilin [28,29]. Alternatively, cofilin might control Ca²⁺ signalling by still unknown mechanism directly involving the cytoskeleton. There have been reports suggesting that F-actin can store and release Ca²⁺ in vitro, as an actin-binding protein, profilin, could release Ca²⁺ by promoting treadmilling of actin filaments in vitro [30–32]. We have observed that the microinjection of gelsolin, another actin-binding protein sharing similar structural and functional properties to cofilin, also enhances Ca²⁺ release from the InsP₃-sensitive stores. In contrast, injection of other proteins did not produce such result (data not shown).

Although cofilin/ADF is yet to be cloned from starfish, a protein homologous to mammalian cofilin was purified from starfish oocytes (Asterias amurensis) [33,34]. Using the antibody against this protein, we have detected the expected 17 kDa band in the oocytes of Mediterranean starfish A. auranciacus (Fig. 7). Hence, the regulation of Ca²⁺ signalling by cofilin featured in this study is likely to be physiologically relevant to starfish oocytes. Future studies will be necessary to understand the general physiological significance and the molecular basis of our observations.

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References


Fig. 7. A cofilin/ADF protein is expressed in starfish oocytes. Total proteins (50 µg) extracted from immature (lane 1) and 1-MA-matured (lane 2) oocytes were resolved on 15% SDS–PAGE and subjected to Western blot analysis as described in the Materials and methods section. Cofilin is present in both immature and mature starfish oocytes used in this study.


