RESEARCH ARTICLE

A single and rapid calcium wave at egg activation in Drosophila

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ABSTRACT

Activation is an essential process that accompanies fertilisation in all animals and heralds major cellular changes, most notably, resumption of the cell cycle. While activation involves wave-like oscillations in intracellular Ca²⁺ concentration in mammals, ascidians and polychaete worms and a single Ca²⁺ peak in fish and frogs, in insects, such as Drosophila, to date, it has not been shown what changes in intracellular Ca²⁺ levels occur. Here, we utilise ratiometric imaging of Ca²⁺ indicator dyes and genetically encoded Ca²⁺ indicator proteins to identify and characterise a single, rapid, transient wave of Ca²⁺ in the Drosophila egg at activation. Using genetic tools, physical manipulation and pharmacological treatments we demonstrate that the propagation of the Ca²⁺ wave requires an intact actin cytoskeleton and an increase in intracellular Ca²⁺ can be uncoupled from egg swelling, but not from progression of the cell cycle. We further show that mechanical pressure alone is not sufficient to initiate a Ca²⁺ wave. We also find that processing bodies, sites of mRNA decay and translational regulation, become dispersed following the Ca²⁺ transient. Based on this data we propose the following model for egg activation in Drosophila: exposure to lateral oviduct fluid initiates an increase in intracellular Ca²⁺ at the egg posterior via osmotic swelling, possibly through mechano-sensitive Ca²⁺ channels; a single Ca²⁺ wave then propagates in an actin dependent manner; this Ca²⁺ wave co-ordinates key developmental events including resumption of the cell cycle and initiation of translation of mRNAs such as bicoid.

KEY WORDS: Egg activation, Calcium imaging, Drosophila, Calcium transient

INTRODUCTION

The eggs of different species of animals are arrested at distinct stages of meiosis and egg activation is required in all species to transform the egg to a cell capable of initiating embryogenesis following fertilisation. Conserved changes in the egg at activation include: adjusting the composition of the outer membranes and egg shell; cortical granule exocytosis, also known as cortical reaction; the resumption of meiosis via inactivation of MPF and activation of APC, mRNA translation activation or degradation, as well as cytoskeletal rearrangements (Horner and Wolfiner, 2008; Houston, 2012; Krauchunas and Wolfiner, 2013). In most animals studied to date it has been shown that changes in intracellular Ca²⁺ concentrations play a major role in setting these events in motion (Krauchunas and Wolfiner, 2013). Ca²⁺ is a well-known second messenger involved in many signal transduction cascades in cells and tissues co-ordinating muscle contraction, transport processes, cell division and growth and enzyme activities (Berridge, 2005; Clapham, 2007). Intracellular Ca²⁺ concentrations are kept highly regulated at approximately 100 nM as prolonged exposure to high levels of Ca²⁺ is toxic to the cell. As a result, there is typically a large difference in the Ca²⁺ concentration between the internal and external cellular environments and signalling events are transient and/or restricted spatially. This is maintained by several ATP-dependent pumps and storage of Ca²⁺ in intracellular organelles, such as the endoplasmic reticulum (ER) or mitochondria (Berridge, 2005; Clapham, 2007).

The specific spatiotemporal dynamics of the transient increases in cytoplasmic Ca²⁺ have been characterised across many species during egg activation and reveal important differences between species (Stricker, 1999). In Drosophila, despite genetic evidence that Ca²⁺ signalling is required at egg activation (Horner et al., 2006), to date, a Ca²⁺ transient has not been observed. Unlike many animals, Drosophila do not require fertilisation to trigger activation (Doane, 1960). In vivo experiments in Drosophila have shown that activation occurs during the transition from the ovary to the oviduct prior to fertilisation in the uterus (Heifetz et al., 2001). Egg activation relies entirely upon maternal factors that, without the addition of the sperm, can trigger the Drosophila egg to complete meiosis (Doane, 1960), modify the vitelline membrane (Heifetz et al., 2001) and initiate translation or degradation of some mRNAs (Macdonald and Struhl, 1986; Tadros and Lipshitz, 2005).

With egg activation taking place independently of fertilisation in Drosophila, both mechanical stimulation during the passage of the egg through the female reproductive tract and osmotic pressure from the fluid composition in the oviduct have been proposed as a trigger of activation (Went and Krause, 1973; Heifetz et al., 2001; Horner and Wolfiner, 2008). Ex vivo experiments on Drosophila eggs show that physical pulling on the dorsal appendages can cause the resumption of meiosis (Endow and Komma, 1997), placing them into a hypotonic buffer causes them to swell and activate (Mahowald et al., 1983; Page and Orr-Weaver, 1997) and more recently, osmotic and hydrostatic pressure can activate the egg (Horner and Wolfiner, 2008). Before activation, mature eggs appear shrivelled and dehydrated, whereas following activation in the female or ex vivo with activation buffer, eggs are turgid and enlarged (Mahowald...
et al., 1983). Furthermore, evidence shows that the oviduct matrix is more hydrated in virgin females that have undergone ovulation, compared to that in females in the process of ovulating (Mahowald et al., 1983), raising the possibility that fluid is transferred to the egg from the oviduct matrix during ovulation.

While the mechanism of triggering egg activation is unclear, the importance of Ca$^{2+}$ signalling is well established. Mutations in the *Drosophila* calcipressin *sarah* (*sra*), part of a pathway with the Ca$^{2+}$-dependent phosphatase calcineurin, result in female eggs, and subsequently to test how these changes are co-ordinated, we utilised micro-injected Ca$^{2+}$-decting dye and a genetically encoded Ca$^{2+}$ indicator. We identified a single, rapid Ca$^{2+}$ wave passing through the mature oocyte. Genetic, pharmacological and physical manipulation reveals that the Ca$^{2+}$ wave is not initiated by pressure on the egg alone but is dependent on both a functional actin cytoskeleton and the calcineurin signalling pathway. Our data supports a model where the fluid in the lateral oviduct, which rehydrates the egg, triggers Ca$^{2+}$ wave initiation at the posterior through mechanosensitive channels. Finally, we show that processing (P) bodies are dispersed following the Ca$^{2+}$ wave and propose that this is required to activate the translation of stored mRNAs in the egg as a response to activation.

### MATERIALS AND METHODS

#### Fly strains

Stocks were raised on standard cornmeal–agar medium at 21 or 25°C: wild-type, OregonR; UAS myristoylated (myr)-GCaMP5 (Melom and Littleton, 2013); GAL4::VP16-nosUTR (Van Doren et al., 1998); *sarah*108; *sarah*428 (Horner et al., 2006); Me31B:GFP (Buszczak et al., 2007). For all experiments, mated females were fattened on yeast for 24–48 hours at 25°C.

#### Preparation of egg chambers for imaging

For live imaging, oocytes were prepared as described previously (Weil et al., 2012). Ovaries from well-fed females were dissected and individual egg chambers separated from ovarioles using a dissecting probe in series95 halocarbon oil (KMZ chemicals) on 22 × 40 cover slips, #1.5 (Menzel-Gläser). Buffer was then added by glass pipette on top of the sample. Precise start times are not always clear due to variability in oil displacement sample to sample. Furthermore, the propagation of the applied medium was not always consistent. As far as possible these variables was controlled for by selecting the starting time point based on bright-field images.

#### Embryo collection

As described previously (Parton et al., 2010), embryos were collected at 25°C between 0–4 hours on yeasted apple juice agar plates then dechorionated for 2 minutes in 50% bleach. For imaging, embryos were adhered with heptane glue to a #1.5 coverslip then covered with series700 halocarbon oil (KMZ chemicals).

#### Microinjection

Preparation for microinjection was carried out as described previously (Weil et al., 2012). Stage 14 egg chambers were prepared as above. A 2×10 mm piece of glass was placed on the coverslip to unblock the needle if required. A Femtotips II microinjection needle (Eppendorf) and a gas pressure injection system (Tritech Research) were used to inject the egg chambers. Dye aqueous stock solutions were made at 1 mM Calcium Green-1 Dextran, Ms 10,000 (Invitrogen) and 1 mM Texas Red Dextran, Mr 10,000 (Invitrogen). For ratiometric calcium imaging, dye stocks were mixed 1:1 then briefly centrifuged at high speed prior to loading into the injection needle and injection. After injection, 45 minutes was left for recovery and dye diffusion before addition of activation buffer. Calcium Green-1 Kd for Ca$^{2+}$=190 mM. For artificial elevation of cytoplasmic Ca$^{2+}$ levels, 20–100 pl of 10 mM CaCl$_2$ was injected.

#### Solutions, pharmacological treatments and ex vivo activation

To activate the oocytes ex vivo, a standard hypotonic activation buffer, first developed by Mahowald and colleagues (Mahowald et al., 1983) and also described by Page and Orr-Weaver (Page and Orr-Weaver, 1997), was used, composed of: 3.3 mM NaH$_2$PO$_4$, 16.6 mM KH$_2$PO$_4$, 10 mM NaCl, 50 mM KCl, 5% polyethylene glycol 8000, 2 mM CaCl$_2$, brought to pH 6.4 with a 1:5 ratio of NaOH:KOH. Other solutions used include Schneider’s Insect medium (Sigma) for control experiments, 10 mM CaCl$_2$ and activation buffer with 10 μg/ml final concentration cytochalasin-D (Sigma).

### RESULTS

#### A single transient Ca$^{2+}$ wave accompanies egg activation in *Drosophila*

Although mutations in the Ca$^{2+}$ signalling pathway have been shown to disrupt egg activation (Horner et al., 2006), to date, Ca$^{2+}$ signalling events have not been recorded during *Drosophila* egg activation. To investigate this we monitored the change in intracellular Ca$^{2+}$ by ratiometric imaging on ex vivo isolated mature oocytes (stage 14). Prior to addition of activation buffer, both Calcium Green-1 Dextran (10 kDa) and Texas Red Dextran (10 kDa) were micro-injected in equal concentrations and allowed to evenly diffuse through the cytoplasm (45 minutes) to provide a baseline measure of intracellular Ca$^{2+}$ concentration. Upon activation, we observed a consistent, rapidly propagating increase in intracellular Ca$^{2+}$, followed by a slower “swelling” phase, decrease (Fig. 1A,B, n=8). These events were concomitant with the physical “swelling” response normally associated with activation (data not shown). To confirm that the change in Ca$^{2+}$ was due to activation rather than simply mechanical perturbation, Schneider’s Insect medium was applied to the post-injected egg chambers (Fig. 1C, n=8). In these cases there was no Ca$^{2+}$ wave or swelling, however, there was a small local Ca$^{2+}$ elevation associated with the injection site, which did not propagate. We verified that the ratio imaging method was responding to Ca$^{2+}$ by artificially elevating the Ca$^{2+}$ concentration locally through microinjection of CaCl$_2$ (data not shown).

The Ca$^{2+}$ transient rapidly propagates as a wave from the cortical posterior

While injected dyes allowed us to initially observe and make preliminary characterisation of the calcium response we found it difficult to thoroughly analyse speed and magnitude. To
overcome this and in order to further characterise the Ca\textsuperscript{2+} transient during activation by standard hypotonic activation buffer, we used the genetically encoded Ca\textsuperscript{2+} indicator Ca\textsuperscript{2+}-sensitive GFP (GCaMP) (Nakai et al., 2001) under the control of a germ line specific GAL4 driver. We found that using the myristoylated variant of the indicator (Melom and Littleton, 2013), targeting it to the inner leaflet of the plasma membrane, resulted in a dramatic increase in the signal-to-noise ratio between the GFP and auto-fluorescent yolk granules in the cytoplasm of the egg. This approach showed a similar transient Ca\textsuperscript{2+} increase to that detected with injected dyes (Fig. 1B, Fig. 2A; supplementary material Movies 1–3). Using UAS-\textit{myrGCaMP5}, we could clearly see that the Ca\textsuperscript{2+} transient propagated as a wave (Fig. 2A, arrowheads) and subsequently returned to basal levels (Fig 2A). Initiation of the wave following the addition of activation buffer was observed on average at 19\textsuperscript{15}0\textsuperscript{17.5}0 (n=26, SEM) and the completion of the wave resulting in the whole oocyte showed an increase in intracellular calcium after 3\textsuperscript{97}35\textsuperscript{06}41.10 (n=16, SEM). Recovery begins 4\textsuperscript{49}06\textsuperscript{06}50.60 (n=10, SEM) to complete. The majority of waves initiated from the posterior pole (69\%) versus the anterior (18\%) or lateral cortex (13\%) (n=29). Even with prolonged observations, we did not detect any further transients or oscillating waves after ex-vivo activation (Fig. 2A, t=70") or in the early embryo (0–2 hours) (data not shown).

Expression of the UAS-\textit{myrGCaMP5} Ca\textsuperscript{2+} reporter did not interfere with the expected morphological changes in the oocyte associated with activation, including swelling, rounding at the poles and increased rigidity (supplementary material Movie 1), which were similar to control experiments where the reporter was
Mechanical stimulation alone does not support the propagating Ca\textsuperscript{2+} wave

It has long been postulated that both mechanical stimulation and external cues contribute to Drosophila egg activation during deposition (Heifetz et al., 2001; Horner and Wolfrner, 2008; Krauchunas and Wolfrner, 2013). Various methods have previously been used to study this question, for example, physical pulling on the dorsal appendages of mature oocytes has been shown to cause the resumption of meiosis (Endow and Komma, 1997) and placing mature oocytes into a hypotonic chamber has been shown to cause the resumption of meiosis (Endow and Komma, 1997) and placing mature oocytes into a hypotonic chamber is clearly capable of multiple transients, activation involves only a single propagating wave.

External Ca\textsuperscript{2+} alone does not trigger a propagating Ca\textsuperscript{2+} wave

In order to better understand the basis of the Ca\textsuperscript{2+} wave during activation by standard hypotonic activation buffer, we tested if the application of solutions with different properties had an effect on intracellular Ca\textsuperscript{2+} in the egg. We already showed that Schneider’s Insect medium (5.4 mM CaCl\textsubscript{2}, 2.7× more CaCl\textsubscript{2} than in activation buffer) did not trigger a Ca\textsuperscript{2+} transient or activation. However, addition of 10 mM CaCl\textsubscript{2}, which is five times more concentrated than the CaCl\textsubscript{2} in activation buffer, caused a rapid increase in intracellular Ca\textsuperscript{2+} from all regions of the cortex, accelerated swelling and lysis within minutes (Fig. 3A). Next, we demonstrated that altering the membrane potential, through the application of a high potassium medium (Ataman et al., 2008), caused no swelling or intracellular Ca\textsuperscript{2+} change (data not shown). Finally, we showed that hypo-osmotic shock by addition of dH\textsubscript{2}O promotes accelerated swelling as well as an increase in Ca\textsuperscript{2+} in the egg (Fig. 3B). Interestingly, with the addition of water we often observe a recovery of intracellular Ca\textsuperscript{2+} to normal levels and occasionally a second increase in Ca\textsuperscript{2+} (Fig. 3B). Together, this data suggests that mechanical pressure from hypo-osmotically induced swelling is a likely mechanism for initiation of the Ca\textsuperscript{2+} wave, possibly initially triggered by entry of external Ca\textsuperscript{2+}. The data also shows that while the egg chamber is clearly capable of multiple transients, activation involves only a single propagating wave.

Fig. 2. A rapidly propagating Ca\textsuperscript{2+} response at egg activation detected by transgenic indicator. (A) Time series of a mature oocyte expressing UAS-myrGCaMP5 following the addition of activation buffer, (t=0) shows an expected baseline of fluorescence (note: dorsal appendages to the left show typical autofluorescence). After the addition of activation buffer, the mature oocyte swells and a wave of increases Ca\textsuperscript{2+}-concentration initiates from the posterior pole (t=16.05°, arrowhead). The posterior wave propagates across the mature oocyte (t=33.2°, arrowhead). Following a brief period when the whole cell has an increase in Ca\textsuperscript{2+} (t=8.2°), a slower recovery commences (t=16.05°) and leaves the cell a similar Ca\textsuperscript{2+} concentration as prior to addition of activation buffer (t=19.25°) and no oscillations are detected. (B) Time series of a mature oocyte not expressing UAS-myrGCamp-5 (without the tub-GAL4VP16 driver) or (C) wild-type following the addition of activation buffer. Corresponding bright-field images show the mature oocytes swelling and fluorescent images show no change in fluorescence, as expected (n=7). (D) Mature oocytes expressing tub-GAL4VP16 and UAS-myrGCamp-5 following the addition of Schneider’s Insect media does not show the mature oocyte swelling and an increase in Ca\textsuperscript{2+} is not detected (n=7). Scale bars A–D=100 μm. Max projection A=41 μm, B=40.3 μm, C=43.7 μm, D=33.2 μm.

Fig. 3. Osmotically induced swelling of the mature oocyte results in an increase in Ca\textsuperscript{2+} and lysis. (A,B) Time series of a mature oocyte expressing UAS-myrGCaMP5 following the addition of 10 mM CaCl\textsubscript{2} (A) or water (B). (A) Addition of 10 mM CaCl\textsubscript{2} results in a rapid cortical increase in intracellular Ca\textsuperscript{2+}, accelerated swelling and lysis (n=8). (B) Addition of water causes a rapid increase in intracellular Ca\textsuperscript{2+} around the circumference of the cell (n=10). The cell recovers to pre-activation levels before showing second increase in intracellular Ca\textsuperscript{2+} before lysis. Corresponding bright-field images show a continued swelling of the mature oocyte until lysis. Scale bars A,B=100 μm. Max projection A=27.7 μm, B=41 μm.
Other factors must be required to couple the two events. Swelling alone is not sufficient to initiate and drive the Ca²⁺ wave. Previous work has shown that the actin cytoskeleton is re-organised at activation (Weil et al., 2008). We tested if disruption of the actin cytoskeleton via pharmacological disruption could also block the Ca²⁺ wave. In the presence of activation buffer with cytochalasin-D, the wild-type egg swells, but the wave appears to stutter and retract prematurely, never encompassing the whole oocyte as observed in standard conditions (Fig. 6C; supplementary material Movie 4). These results demonstrate a role for the cytoskeleton in coupling swelling and propagation of the Ca²⁺ wave.

### Ca²⁺ transients occur in oocytes passing through the ex vivo oviduct

Amongst the earliest changes associated with activation occur upon entrance of the oocyte into the lateral oviduct, for example an increase in the cross-linking of the vitelline membrane (Heifetz et al., 2001). In order to test whether Ca²⁺ signalling is associated with these early physiological events, we dissected the full reproductive machinery of the female and observed mature oocytes that were at the entrance of the lateral oviduct (Fig. 5C–E). Despite extensive imaging challenges, we were able to detect an increase in Ca²⁺ at the posterior of some oocytes as they entered the lateral oviduct (Fig. 3D). These results confirm the Ca²⁺ transient as part of the normal process of egg activation in *Drosophila*.

### Requirements for initiation and propagation of the Ca²⁺ transient

To explore the relationship between the Ca²⁺ wave and other aspects of egg activation, we tested mature oocytes mutant for the *Drosophila* calcipressin, sra, which has previously been shown to block release of the cell cycle (Horner et al., 2006; Takeo et al., 2006). We find that in a sra mutant background *ex vivo* activated mature oocytes swell normally but no Ca²⁺ wave is detected in 13 of 14 samples (Fig. 6A). The fact that swelling and the initiation and propagation of the Ca²⁺ wave can be uncoupled in this way shows that swelling is not dependent upon the Ca²⁺ wave and furthermore, swelling alone is not sufficient to initiate and drive the Ca²⁺ wave. Other factors must be required to couple the two events.

To test if a localised increase in Ca²⁺ alone could initiate and enable propagation of a wave, we injected CaCl₂ directly. We show that both a low or high volume CaCl₂ displays a proportional rise in the intracellular concentration of Ca²⁺ detected (Fig. 6B). We also show that despite this local increase, the cell recovers back to pre-injected levels and that the local increase in Ca²⁺ does not cause a wave to propagate in the mature oocyte.

Previous work has shown that the actin cytoskeleton is reorganised at activation (Weil et al., 2008). We tested if disruption of the actin cytoskeleton via pharmacological disruption could also block the Ca²⁺ wave. In the presence of activation buffer with cytochalasin-D, the wild-type egg swells, but the wave appears to stutter and retract prematurely, never encompassing the whole oocyte as observed in standard conditions (Fig. 6C; supplementary material Movie 4). These results demonstrate a role for the cytoskeleton in coupling swelling and propagation of the Ca²⁺ wave.

### The Ca²⁺ transient co-ordinates downstream events of egg activation

Activation in *Drosophila* heralds a cascade of events, including translational initiation of several mRNA’s: *bcd*, *nanos*, *hunchback*, *caudal*, *Toll*, *torso*, *smaug*, and *string* (Tadros and Lipshitz, 2005). Previous work has established the presence of cytoplasmic regions in the oocyte termed P bodies, where mRNA translation is not supported and which, therefore, act in repressing the translation of stored mRNA’s such as *bcd* (Weil et al., 2012). Activation has previously been shown to dissipate P bodies at the anterior pole of the oocyte, releasing *bcd* mRNA for translation (Weil et al., 2012). To test the involvement of the Ca²⁺ transient...
in regulation of these P bodies and consequently in regulating translation regulation, we examined the common P body marker, Me31B, at activation (Fig. 7A–C). We show that Me31B particles present in the mature oocyte, disperse following ex vivo activation (n=11). We observe a similar dispersed pattern of Me31B in the early embryo when compared to the large foci in stage 14 oocyte (Fig. 7D,E).

DISCUSSION

In this study we visualised a rapid wave of intracellular Ca\(^{2+}\) in the Drosophila oocyte at egg activation by two independent methods. We were able to demonstrate a requirement for the calcipressin encoded by the sra gene as well as as cin for the initiation and propagation of the wave. We further show that mechanical pressure alone was insufficient to initiate and propagate a Ca\(^{2+}\) wave. Our data supports a model where at ovulation: (1) a stage 14 oocyte enters the lateral oviduct; (2) fluid in the oviduct initiates a change in intracellular Ca\(^{2+}\) at the posterior pole; (3) osmotic swelling and likely IP3 mediated release of intracellular Ca\(^{2+}\); (4) Ca\(^{2+}\) release by IP3 is in part controlled by a functional actin cytoskeleton; (5) a wave of Ca\(^{2+}\) propagates through the cell by Ca\(^{2+}\)-induced Ca\(^{2+}\) release; (6) Ca\(^{2+}\) is reabsorbed into internal stores; (7) resumption of the cell cycle and translation of maternal mRNAs.

In most animals, Ca\(^{2+}\) release, in the form of a wave or oscillation, at egg activation is initiated by the sperm (Stricker, 1999). This results in cortical granule exocytosis to block polyspermy and the start of development though initiation of the cell’s metabolism. Here we report a single rapid wave of Ca\(^{2+}\) in the Drosophila egg. This wave is unique with respect to other model systems insofar as it occurs prior to fertilisation but does appear to have similar properties to the waves observed in jellyfish and frogs. While gamete membrane fusion or binding of the gamete to receptors prior to fusion are common mechanisms to initiate activation, Drosophila must receive a different signal to set events into motion. One possibility is that a signal in the oviduct fluid bind to a receptor in the oocyte and triggers the tyrosine kinase, phospholipase C, phosphatidylinositol (4,5)-bisphosphate (PIP2), inositol 1,4,5-trisphosphate (IP3) cascade as in other systems (Berridge, 2005; Clapham, 2007), however this is not consistent with our ex vivo experiments.

While we cannot completely rule out a role for physical pressure or additional contribution from a specific factor for activation, our data supports a model where an increase in Ca\(^{2+}\) due to osmotic swelling enables initiation of activation. While direct injection of CaCl\(_2\) does not result in the propagating increase in calcium, we do observe that Schneider’s media which

Fig. 6. Ca\(^{2+}\) wave is compromised in sra mutant and by actin disruption. (A) sarah mutant mature oocyte expressing UAS-myrGCamp-5. Addition of activation buffer (t=0') does not show a change in intracellular Ca\(^{2+}\), while swelling and showing physical changes associated with egg activation (n=13/14). (B) Mature oocyte microinjected with Calcium Green-1 and Texas Red Dextran and left for dyes to diffuse for 45 minutes. A low volume injection of 10 mM CaCl\(_2\) (t=10', white arrowhead) shows a slight local increase in the Ca\(^{2+}\) concentration. Higher volume injection of 10 mM CaCl\(_2\) (t=175', white arrowhead) shows a clear local rise in the intracellular concentration of Ca\(^{2+}\) detected. Continued observation shows that the local increase in Ca\(^{2+}\) does not cause a wave to propagate in the mature oocyte. (C) Wild-type mature oocyte expressing UAS-myrGCamp-5 cultured in activation buffer with 10 µg/ml cytochalasin-D (n=21/24 show a complete loss or compromised wave). Intracellular Ca\(^{2+}\) increases from the posterior pole as in wild-type (t=2', white arrowhead). This posterior wave fails to move across the entire cell (t=3'32', white arrowhead), retracting to the posterior prematurely. A similarly compromised anterior wave fails to propagate fully (t=7'04", white arrowhead). Corresponding bright-field images show that swelling and separation of dorsal appendages occur as expected with egg activation. Scale bars A–C=100 µm. Max projection A=40 µm, C=41.5 µm.

Fig. 7. P bodies disperse at egg activation. (A–E) Mature oocytes and early embryo expressing Me31B::GFP labelling P bodies. (A) Time series of ex vivo mature oocyte following the addition of activation buffer (t=0') show Me31B foci dispersing. (B,C) Higher magnification 63x 1.4 NA lens before (B) and after (C) the addition of activation buffer. Large Me31B particles fall apart following the addition of activation buffer and swelling, consistent with observations of stage 14 oocyte (D) and early embryo (E). Scale bars A,B=100 µm, C–E=40 µm. Max projection A=40 µm, B–E single frame.
enters the mature oocyte through the injection site can generate a sequential increase in intracellular Ca\(^{2+}\). This increase is not comparable in speed, time or distance covered to the normal wave we observe at activation. We observe initiation of the Ca\(^{2+}\) wave in \textit{ex vivo} samples primarily from the posterior suggesting that there is something unique about this region of the cell. This could be due to a posterior enrichment of ER or Ca\(^{2+}\) channels. The former is unlikely as labelling for ER does not reveal any local enrichment (data not shown).

In sea urchin eggs, microinjection of purified IP3 is sufficient to cause cortical granule exocytosis (Busa et al., 1985; Whitaker and Irvine, 1984) and blocking IP3 prevents a Ca\(^{2+}\) release (Berridge, 2005; Clapham, 2007). In \textit{Drosophila}, IP3 receptor germline clones fail to produce viable embryos and ovary extracts detect expression of the IP3 receptor (Acharaya et al., 1997; Chintapalli et al., 2007; McQuilton et al., 2012). Our finding that disruption of the actin cytoskeleton results in a compromised Ca\(^{2+}\) wave hints at signalling through an actin-IP3 receptor interaction. We know from previous work that the F-actin cytoskeleton is rearranged at activation (Weil et al., 2008). Tissue culture, brain and pancreatic cells from mammals also show that actin physically interacts with the IP3 receptors (Fujimoto et al., 1995; Joseph and Samanta, 1993; Turvey et al., 2005). In starfish, disruption of actin resulted in a decrease in intracellular Ca\(^{2+}\) at egg activation (Kyozuka et al., 2008). Following what we hypothesise to be an actin dependent IP3 pathway release of Ca\(^{2+}\), it is likely that the Ca\(^{2+}\) would be absorbed into internal stores resulting in base levels of internal Ca\(^{2+}\) observed. An alternative is that the Ca\(^{2+}\) change could be mediated through the transient receptor potential and DEG/ENaC channel families which have been shown to be expressed in \textit{Drosophila} adult ovary (Chintapalli et al., 2007; Horner and Wolflner, 2008; McQuilton et al., 2012). It is clear that further experimental work would be required to decipher which mechanisms are operating. Whatever the model, it is likely that the Ca\(^{2+}\) transient observed is important in regulating the subsequent events downstream of activation.

Now that we know that a calcium signalling event accompanies activation in \textit{Drosophila}, this begs the question, which downstream events are triggered by the transient Ca\(^{2+}\) wave and how the events are activated. For example, the post-transcriptional regulation of maternal transcripts is critical to the subsequent development of the \textit{Drosophila} egg post-activation (Tadros and Lipshitz, 2005; Tadros et al., 2007). The anterior determinant \textit{bcd} mRNA shows Poly(A) elongation at activation in a \textit{sra} dependent manner (Horner et al., 2006). Visualisation at activation has previously shown that \textit{bcd} mRNA is released from anchoring in large aggregates at anterior pole and is no longer observed in P bodies (Weil et al., 2008; Weil et al., 2012). The loss of P bodies around the entire egg at activation could be a global mechanism for post-transcriptional regulation at egg activation. It remains unclear how Ca\(^{2+}\) mediates the observed changes in mRNA and P bodies. While Ca\(^{2+}\) signalling typically requires transaction proteins, such as calmodulin, another possibility is that Ca\(^{2+}\) mediates change through direct binding to its target. In this model, which is not restricted to \textit{Drosophila} or egg activation, calcium bound to mRNA or P body components would thus alter their conformation, charge or association. Further examination of mRNA untranslated regions and protein dissociation in different calcium concentrations are required to test this hypothesis. \textit{Drosophila}, with its advantages for live cell imaging and the availability of genetic tools will be an ideal system to approach these questions and determine whether similar mechanisms operate in other systems.

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**Competing interests**

The authors declare no competing or financial interests.

**Author contributions**


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