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Calcium and Egg Activation in *Drosophila*

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Summary

In many animals, a rise in intracellular calcium levels is the trigger for egg activation, the process by which an arrested mature oocyte transitions to prepare for embryogenesis. In nearly all animals studied to date, this calcium rise, and thus egg activation, is triggered by the fertilizing sperm. However in the insects that have been examined, fertilization is not necessary to activate their oocytes. Rather, these insects' eggs activate as they transit through the female's reproductive tract, regardless of male contribution. Recent studies in *Drosophila* have shown that egg activation nevertheless requires calcium and that the downstream events and molecules of egg activation are also conserved, despite the difference in initial trigger. Genetic studies have uncovered essential roles for the calcium-dependent enzyme calcineurin and its regulator calcipressin, and have hinted at roles for calmodulin, in *Drosophila* egg activation. Physiological and in vitro studies have led to a model in which mechanical forces that impact the *Drosophila* oocyte as it moves through the reproductive tract triggers the influx of calcium from the external environment, thereby initiating egg activation. Future research will aim to test this model, as well as to determine the spatiotemporal dynamics of cytoplasmic calcium flux and mode of signal propagation in this unique system.

Keywords

Drosophila; oocyte; embryo; egg activation; calcium; mechanosensitive; calcineurin

Through the process of egg activation, a single resting cell—the mature oocyte—suddenly is triggered to undergo several major changes that give it the capacity, once fertilized, to give rise to every type of tissue in the adult. Understanding the intricacies of egg activation is fruitful not only for understanding how cells can transition between differentiated and totipotent states, but also has relevance to the development of assisted reproductive technologies.

In many organisms, mature oocytes remain stalled in a species-specific stage of meiosis. Release from this arrest point occurs during egg activation. This process initiates a number

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of changes at the molecular and cellular levels in the oocyte that prepare it to undergo embryogenesis after fertilization. The molecular changes include modification of vitelline membrane structure, alterations in RNA and protein pools through translation or degradation of stored maternal RNAs, and post-translational modifications made to existing proteins. These molecular events drive major changes at the cell level, including release from meiotic arrest, pronuclear fusion, and finally progression to embryonic mitoses.

In the vertebrate and invertebrate organisms studied, egg activation requires an increase in intracellular calcium levels within the oocyte (reviewed in [1-4]). In most cases, the calcium increase is triggered through the fertilizing sperm, commonly through activation of an intracellular calcium signaling pathway. For example, in mice the sperm introduces a specific isoform of phospholipase C, which activates the inositol triphosphate pathway in the fertilized egg, ultimately causing release of calcium stores from the endoplasmic reticulum (ER) [5].

However, the specific trigger for egg activation is different in at least some insects, including fruit flies and hymenoptera such as ants and bees (whose unfertilized eggs develop into males) [6, 7]. Egg activation in these insects does not require fertilization [8]. Instead, a mature oocyte begins to activate as it travels through the female reproductive tract. Activation is triggered by passage through the ovipositor in wasps [9], or upon the oocyte's release from the ovary into the oviduct in *Drosophila* [10]. Thus, insects afford a different view of egg activation: without the fertilizing sperm, their egg activation events are driven solely by maternal components within the oocyte. Experimental results derived from egg activation studies in *Drosophila* oocytes may therefore be of particular interest to researchers or physicians investigating the determinants of oocyte quality for application in assisted reproduction.

How does travel through the reproductive tract promote the egg activation program in *Drosophila*? The obvious candidate for an intermediary is calcium, but only very recently—six years ago—was evidence first obtained that indicated a requirement of calcium for *Drosophila* egg activation [11, 12]. Since then, some important discoveries have been made concerning the source of the calcium, the relationship of calcium to the trigger of the release from the ovary into the oviduct, and the components of the subsequent signaling pathway, but these are still very early days for studies of *Drosophila* egg activation. Our goals in this article are to summarize what has been reported, highlight questions that are not yet answered, and provide a framework for future investigation of this important area.

Genetic analysis indicates a requirement for calcium

Calcium signals in many cell types are commonly transmitted through the calcium binding protein calmodulin (CaM), which may then interact with other proteins to activate downstream events. Studies in vertebrates had previously pointed to roles for CaM-mediated pathways in egg activation. In mouse, for example, activity of the calmodulin-dependent kinase CaMKII increases with the multiple calcium spikes in the oocyte, and each wave of CaMKII activity progressively promotes further landmarks of egg activation [13]. CaMKII and the calcium-dependent phosphatase calcineurin also play a role in activation of *Xenopus* oocytes, where much of the signaling network downstream of the calcium rise has been identified. In resting *Xenopus* oocytes, meiosis is held in an arrested state by inhibition of the anaphase promoting complex, APC/C, whose action is required to initiate anaphase. The APC/C acts as an E3 ligase when bound to its coactivator CDC20 to signal the degradation of cell cycle regulators at the spindle assembly checkpoint, thus promoting progression from metaphase to anaphase (reviewed in [14]). In the resting cell, anaphase progression is blocked by inhibitory phosphorylation of CDC20 and by active Emi2, a spindle checkpoint

regulator [15]. Upon fertilization (and thus egg activation), the resulting rise in cytosolic Ca^{2+} activates (1) CaMKII, which places an inhibitory phosphorylation on Emi2; and (2) the calcium-dependent phosphatase calcineurin, which removes CDC20 phosphorylation. These post-fertilization events release inhibition of APC/C and allow progression through anaphase [15-17].

The first indication that calcium may play an important role in *Drosophila* egg activation came from genetic analysis: the gene for the calcineurin regulator *sarah* (Sra, calcipressin) was identified in a screen for mutations affecting early post-fertilization embryonic development. Embryos from *sra* mutant mothers arrest normally at metaphase I, but upon meiotic resumption at egg activation they are unable to proceed past anaphase I [11, 12]. Calcipressins typically regulate the activity of the Ca^{2+} /CaM-dependent phosphatase calcineurin (CaN) through direct binding [18-20]. Recent evidence establishes that during egg activation in flies, the calcipressin Sra activates calcineurin. First, the phenotype of embryos derived from germline clones of a null allele of the CnB subunit gene of calcineurin is remarkably similar to that of *sra* mutants: meiosis arrests properly at metaphase I but is unable to continue past anaphase I upon release from meiotic arrest [21]. Second, a recent study confirms that Sra associates with calcineurin *in vivo* and further shows that phosphorylation of Sra at Ser215 is necessary for calcineurin activity [22]. This residue is phosphorylated only in activated eggs and not in mature oocytes, and this phosphorylation event is dependent upon activity of glycogen synthase kinase (GSK-3, *shaggy*) [22]; however, it is not known how GSK-3 activity is turned on specifically at egg activation. Taken together, these results indicate the following scenario: upon egg activation, Sra becomes phosphorylated at a specific residue, which allows Sra to activate calcineurin.

Interestingly, Sra is bound to calcineurin both in ovaries, where calcineurin is not active, and in early embryos, where calcineurin is active [22]. In other organisms, calcipressin has been shown to bind groups of different targets for activating or repressive regulation depending on its phosphorylation status, and this association can be either repressive or activating [22,12]. The same may be true in *Drosophila*, where the association of Sra and calcineurin in the ovary may be inhibitory until Sra's phosphorylation at egg activation.

Roles for CaM and CaMKII in *Drosophila* egg activation have not yet been directly determined through genetics. We suspect that both proteins are important for signal transduction upon Ca^{2+} influx during egg activation. For example, the results just described indirectly point to at least one role for CaM through the calmodulin-dependent phosphatase calcineurin. In fact, CaM was identified in calcineurin-containing immunoprecipitates of Sra [22], further suggesting that CaM is required to convey the calcium signal to effector proteins. We discuss here the attempts to define genetically and directly the functions of CaM and CaMKII in *Drosophila* egg activation.

One challenge for such genetic analysis is that global knockout of either CaM or CaMKII is lethal to the animal, so, tissue-specific knockdowns must be tested. CaM-deficient germline clones were generated to study CaM's role in oogenesis and embryogenesis, but surprisingly, the germlines of these flies were not depleted of CaM protein. Though the germline cells were indeed *CaM*-null, it appeared that CaM protein was able to enter the germline cells from the surrounding somatic cells [23]. Thus, it was not possible to determine definitively whether CaM plays a role at egg activation. However, Andruss and colleagues observed that some mature oocytes absorbed less somatic CaM than others (anywhere between 28% and 76% that of heterozygous controls), and those that received relatively little somatic CaM show severe defects upon embryogenesis, though oogenesis occurs relatively normally [23]. These latter results support the idea that CaM plays a role in

the egg-to-embryo transition, although further experiments are needed to fully prove this hypothesis.

It has not yet been possible to test for a role for CaMKII in *Drosophila* egg activation because the location of the CaMKII gene, on *Drosophila*'s fourth chromosome, has precluded the generation of germline clones. However, a method to generate mitotic clones for fourth chromosome genes in the soma has just been reported [24]. Future tweaks to this system may make it possible to induce loss of CaMKII expression in the germline while maintaining normal expression in the soma.

Because generating germline clones deficient for CaM or CaMKII has not yet been possible, attempts have been made to knock down the amount or activity of these proteins using other means. Interfering RNA (Harvard Medical School, TRiP) or inhibitory peptides targeted against CaM [25] or CaMKII [26] can be expressed in the germline. Preliminary experiments of this type have not produced an effect on egg activation or fertility (A. Krauchunas, K. Kumar, Y. Lai, K. Sackton, C. Sartain, and M. Wolfner, unpublished data), but it is possible that the abundant maternal stores of CaM or CaMKII in oocytes cannot be sufficiently inhibited by these methods. Thus, at present we cannot evaluate whether CaM and CaMKII are essential for egg activation. We expect that new genetic techniques for germline analysis will allow us to study these proteins' roles in the future.

Exogenous calcium is needed for *Drosophila* egg activation

Early observations of *Drosophila* oocytes noted that laid eggs were already activated, even if they had not been fertilized [6, 8]. Oocytes in the ovaries were never activated, indicating that activation had to occur sometime between the release of eggs from the ovary and their being laid by the female. Subsequent detailed physiological studies indeed indicated that oocytes become activated as they pass into the oviduct [10]. Figure 1 demonstrates that transport through the oviduct promotes activation: among oocytes that had just begun to exit the ovary, only a small percentage had undergone activation. However, the percentage of activated oocytes increased progressively at positions further along the oviduct.

The specific egg activation trigger that occurs in the *Drosophila* oviduct is still unknown, though the available evidence points toward a mechanical stimulus independent of sperm [10, 27]. Mature oocytes in the ovary have a limp, deflated appearance; however, activated, laid eggs appear turgid and taut. Examination of the oviduct before and after egg laying indicates that fluid (possibly with any ions it contains) may be transferred from the oviduct epithelium to the egg during ovulation: the walls of the oviduct are swollen with fluid in female flies that have not yet ovulated an egg; after ovulation, the oviduct walls appear to have lost fluid (measured by the decrease in matrix space between the intima and the oviduct epithelium) and the laid egg to have gained fluid [6]. Therefore, one model for egg activation in *Drosophila* posits that this passage of fluid from the walls of the oviduct into the oocyte is sufficient to activate the egg [10]. Given the fact that females produce many eggs, this hypothesis would require a continual, net production of new fluid to flow into each subsequent oocyte. This transferred oviductal fluid could contain ions such as calcium that potentially could participate in egg activation. While *Drosophila* ovaries express RNAs for aquaporin water channels and, at low levels, for some other transporters such as the orthologs of NBC, NKCC1, and some potassium channels [28, 29], involvement of these molecules in egg activation has not been tested. Moreover, expression of aquaporins and ion transporters in the oviductal epithelium has not been examined.

A second model suggests that exit from the ovary and passage through the oviduct exerts pressure on the oocyte, activating mechanosensitive ion channels that ultimately trigger egg activation [10, 27]. In accordance with this model, simply pulling on the dorsal appendages

attached to the eggshell of a dissected *Drosophila* oocyte can induce egg activation [30], and external pressure on the oocyte can accelerate its activation in a buffer that contains calcium [27] (see below). These two models (fluid transfer, and mechanosensitive triggers) are not mutually exclusive. One can imagine, for example, that swelling of the egg due to hydration from oviductal fluid would cause mechanical stress in the oocyte membrane, which could activate ion channels and initiate egg activation.

It has been possible to analyze the trigger for egg activation by using an *in vitro* system. Oocytes dissected from the ovary are incubated in an “activation buffer” that is hypotonic to the egg and contains the appropriate ionic makeup (see below) [6, 31]. This method of hypoosmotic egg activation causes the oocyte to take up fluid and to become turgid, perhaps mimicking the hydration that occurs in the oviduct. These *in vitro* activated oocytes show all the signs of normal *in vivo* egg activation, including normal progression through meiotic stages [31], translation of stored mRNAs [27], and vitelline membrane crosslinking [31].

This latter feature of egg activation causes the eggshell to become impermeable to small molecules, providing a phenotype that allows investigators to test whether or not an oocyte has activated: oocytes that have undergone egg activation are impermeable to bleach, and will survive upon incubation in a bleach solution. In contrast, oocytes that have not activated will lyse during incubation with bleach. Using this assay, supplemented with assays of meiotic progression and translational initiation, Horner and Wolfner tested the hypothesis that mechanical stress plays a role in activation of *Drosophila* oocytes [27]. They found that oocytes that are treated with hydrostatic pressure during the *in vitro* activation procedure tend to activate at a faster rate than controls. This result suggests that mechanical stimuli encountered in the oviduct likely contribute to egg activation.

Calcium is a critical component in this assay. The presence of calcium in the activation buffer is essential for hypoosmotic activation of *Drosophila* oocytes *in vitro*: oocytes dissected from the ovary cannot activate if incubated in calcium-depleted activation buffer [27]. Furthermore, *in vitro* activation of dissected *Drosophila* oocytes can be inhibited by treatment with gadolinium, which blocks some ion channels including mechanosensitive stretch-activated channels. The effects of gadolinium and the buffer constitution suggest that calcium ions enter the oocyte from the external environment to trigger activation [27]. Taken together, the available data are most consistent with a model in which calcium from the oviductal fluid enters the oocyte from the outside due to a mechanical stimulus experienced by the oocyte as it passes through the oviduct, and this influx of calcium ultimately triggers egg activation.

This model requires ion channels to mediate this calcium influx. The identity of these channels is not yet known, although promising candidates exist, particularly among the mechanosensitive channels. These could, in theory, be activated through tension across the egg surface during egg swelling, through pressure exerted on the egg during ovulation, or through hypoosmolarity. Several members of the transient receptor potential (TRP) family of calcium channels (reviewed in [32]) are activated by mechanical stress, including *nompC* (which is activated by cytoskeletal tension [33]), *nanchung*, and *inactive* (both activated by hypoosmolarity [34, 35]). Although transcripts encoding these particular TRP channels are not detectable by RT-PCR in the adult ovary [27], and thus cannot be the sensors for the egg activation trigger, a fourth TRP protein that may be responsive to mechanical stimuli, TRPA encoded by the *painless* gene, is expressed in the ovary [27], but it has not yet been tested for a role in egg activation. In addition, mechanosensitive channels of a different class, the DEG/ENaC family are expressed in ovaries and thus are candidates for involvement in egg activation. Only one member of this family, *ripped pocket* (*rpk*), had been tested, and its mutant exhibited normal fertility [27]. However, that mutant is not null so it is not possible

to conclusively rule out a role for *rpk* in egg activation. Additional mechanosensitive channels of the TRP and DEG/ENaC families are expressed in the ovary [28, 29] (Table 1). An important direction for future study is to assess the roles of these ovary-expressed channels in egg activation.

Attempts to image calcium within the *Drosophila* oocyte

In many organisms, including sea urchins and mice [36, 37], time-lapse videos of egg activation have revealed waves or multiple oscillations of the calcium rise at egg activation, often initiating from the site of sperm entry. It would similarly be intriguing to determine the dynamics of calcium levels in activating *Drosophila* oocytes, but this goal has been elusive.

Determining the calcium dynamics in *Drosophila* oocytes requires the introduction of appropriate calcium sensors into these large cells. Injection directly into the cytoplasm of the mature oocyte, traditionally used for many of the organisms in which calcium waves have been detected during activation, is problematic for *Drosophila*, given the mechanosensory trigger for egg activation. To overcome this issue, *Drosophila* genetics permits the expression of calcium sensors encoded by transgenes. Such sensors often utilize some iteration of a fluorescent molecule bound to calmodulin [38, 39], which contains four Ca^{2+} binding sites. In these sensors, the fluorophore weakly fluoresces under low calcium conditions; upon calcium binding, calmodulin undergoes a conformational change that also adjusts the structure of the linked fluorescent protein to enhance fluorescence. Transgenic *Drosophila* strains are available in which such constructs, commonly the GCaMP family or cameleon constructs, have been used with great success in the soma of *Drosophila* [38, 39]. Modifications will need to be made for their use in egg activation, however. The currently-available sensors are regulated by the traditional GAL4-UAS expression system, which does not allow high expression in the female germline [40], so new versions that are amenable to high expression in oocytes are needed. Moreover, the *Drosophila* oocyte yolk and vitelline membrane exhibit high levels of green autofluorescence, requiring sensors that emit in a different color range.

An alternative to genetically encoded sensors are calcium indicators that freely diffuse into the cell. An oocyte can be maintained in an unactivated state for some time in a hypertonic solution [31], so under these conditions a small molecule sensor could, in theory, be loaded into the oocyte without causing the egg to activate. There are a number of commercially available acetoxymethyl (AM)-ester forms of fluorescent calcium sensors in a broad range of colors that may be used in this way. AM-ester conjugates have a neutral charge and thus can diffuse across cell membranes; once inside, the cell's own esterases cleave the compound, resulting in a charged, mature form of the sensor that is unable to exit the cell. Such diffusible small molecule sensors could possibly be used to image calcium dynamics in *Drosophila* oocytes during activation.

Once a reliable method is available to examine calcium flux in activating oocytes, several important questions can be addressed, including: From which point in the oocyte does the calcium flux initiate? Is there a uniform rise in Ca^{2+} or a directional wave across the cytoplasm? Are there multiple oscillations or only one? Does the calcium flux occur only in the cytoplasm immediately under the vitelline membrane, or does it penetrate the center yolk as well?

The answers to these questions would help choose between several interesting scenarios concerning the nature of the trigger for egg activation. (1) If the *Drosophila* oocyte opens stretch-activated calcium channels as a result of fluid uptake in the oviduct, which likely exerts pressure across all parts of the egg membrane uniformly, we would expect an isometric rise in cytoplasmic calcium levels, instead of a wave initiating from a single point

(as with systems dependent upon sperm entry). (2) Since the posterior pole of the oocyte enters the oviduct first, and thus pressure is first exerted posteriorly, a calcium rise may initiate from the posterior and propagate anteriorly *in vivo*. (3) The calcium rise may initiate from the micropyle (the small opening through which the sperm enters) on the anterior end of the oocyte, which is analogous to the point of sperm entry in oocytes of other organisms, even though the *Drosophila* egg activation does not require fertilization. In this case, a calcium wave would propagate from the anterior to the posterior.

We need to define not only the sites at which calcium is introduced into the egg from the outside, but also the mechanisms by which the calcium signal is transmitted through the egg. Intracellular calcium release events are almost certainly required to propagate the signal across the large (0.5-1mm) oocyte. Knowledge from other systems will be instructive. In mice and other vertebrate species, several different types of calcium channels work together to propagate the influx that occurs upon egg activation [2]. While the first calcium transient results from Ca^{2+} release from the ER following sperm-induced phosphoinositide signaling, further oscillations depend upon a number of different calcium channels and ion pumps. When Ca^{2+} levels are depleted from ER stores, sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA) pumps on the ER membrane must actively transport Ca^{2+} from the cytosol back into the ER. Furthermore, plasma membrane Ca^{2+} -ATPase (PMCA) pumps on the cell surface move Ca^{2+} out of the cell to restore normal cytosolic Ca^{2+} levels. Thus, for each oscillation, the intracellular Ca^{2+} levels are elevated for several minutes. In organisms whose oocytes undergo multiple waves at fertilization, oscillations following the initial sperm-induced transient rely not only upon Ca^{2+} from the ER, but also Ca^{2+} influx from the external environment [41]. The necessity of these ATPases has not yet been tested in *Drosophila* egg activation, although both PMCA and SERCA are both highly expressed in the *Drosophila* female germline [28, 29]. The IP_3 receptor is expressed in the *Drosophila* ovary [28, 29], suggesting that *Drosophila* oocytes might have the capacity to undergo single or multiple calcium oscillations or calcium-induced calcium release through IP_3R , even though their initial calcium rise derives from external sources rather from the ER. Further studies are required to definitively determine whether IP_3R plays an essential role in *Drosophila* egg activation. The ryanodine receptor, which has been implicated in calcium oscillations in other animals, is not expressed in *Drosophila* ovaries [28, 29]. Moreover, germline clones of a ryanodine receptor mutant do not impair female fertility [42], further indicating that this molecule is not involved in any changes in calcium levels during egg activation in *Drosophila*. On the other hand, not all organisms' oocytes undergo multiple oscillations: in the large eggs of frogs and fish, for example, only a single large calcium transient is produced [43]. The same may also be true for *Drosophila*, whose oocytes are also considerably larger than those of mammals. Once live imaging experiments have been optimized for *Drosophila* oocytes, questions pertaining to the number, speed, and origin of activating calcium transients will likely be answered.

Effectors downstream of the calcium signal are conserved

Though the initial trigger for egg activation in *Drosophila* differs from that of other species, many other components of signal propagation and downstream effectors of the signal may be conserved once calcium has entered the egg. As stated previously, the phosphatase calcineurin and the kinase CaMKII are both required for egg activation in *Xenopus*. New evidence suggests that phosphatases and kinases, potentially including calcineurin and CaMKII, may be needed simultaneously at egg activation in *Drosophila*. A recent study demonstrated that approximately 30% of the *Drosophila* oocyte proteome undergoes phosphorylation changes (dephosphorylation more commonly than phosphorylation) within 30 minutes of egg activation, and identified over 300 proteins that undergo changes in phosphorylation state (phosphorylation and/or dephosphorylation) during this transition

[44]. These findings suggest that kinases and phosphatases acting at egg activation might act on separate sets of proteins through phosphorylation changes that either activate or inhibit targets. In addition to calcineurin and, presumably, CaMKII, activity of other phosphomodulating proteins is regulated at egg activation. These include GSK-3, which phosphorylates Sra in order to activate calcineurin [22], and MAPK, which is active in mature oocytes but whose activity is attenuated at egg activation [45]. The action of multiple kinases and phosphatases at this time, many of which likely act upon multiple targets, can result in the coordinated yet widespread changes that are required for the resting oocyte to become capable of undergoing embryogenesis.

In addition to the proteins directly affected by calcium, important factors that act further downstream of the initial calcium signal to carry out the events of egg activation are conserved between *Drosophila* and other organisms. For example, GLD-2-family poly(A) polymerases facilitate poly(A)-tail extension of stored transcripts in flies, worms, and frogs [46-49]; mice also express a GLD-2 homolog in mature oocytes [50]. The function of CDC20 proteins is also conserved: the *Drosophila* CDC20 homolog *cortex* is required for meiotic resumption, as in other organisms [51]. Furthermore, 83% of the *Drosophila* proteins that undergo phosphomodulation events at egg activation are conserved across species [44]. These examples suggest that many factors involved in *Drosophila* egg activation are analogous to those in other species, including mammals.

Conclusions

Work to date shows that *Drosophila* egg activation has unique and fascinating properties. Activation is not initiated by fertilization, but instead requires movement of the egg through the oviduct (Figure 2). Calcium is an essential requirement, but it enters the egg from the outside environment rather than being released from internal stores. These basic findings raise a variety of unanswered questions. There is a very likely mechanosensory trigger, but which part of the egg detects the stimulus, how the stimulus is detected, and how it brings about a rise in calcium are all unknown. The required calcium channels hypothesized to carry calcium across the oocyte's plasma membrane are yet to be indentified. We also have no knowledge of how the influx is propagated, or whether there is, subsequently, a single wave or multiple oscillations in intracellular calcium concentrations.

Despite these outstanding questions, *Drosophila* is rising to the forefront as a valuable and unique model system in which to study egg activation. Because *Drosophila* does not require sperm for activation to occur, we can separate the events of activation from those of fertilization, allowing for a more precise view of egg activation that is also pertinent to other species where the two are inseparable. Additionally, though the specific trigger of calcium influx differs from other organisms, the available evidence indicates that the resulting signaling events are similar to those in other species. Thus, given the availability of genetic tools and ease of manipulation of the organism, along with the similar requirement for calcium and relevance to other organisms, *Drosophila* is a useful and viable model for egg activation.

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References

- [1]. Whitaker M. Calcium signalling in early embryos. *Philos Trans R Soc Lond B Biol Sci.* 2008; 363:1401–1418. [PubMed: 18263556]

- [2]. Malcuit C, Kurokawa M, Fissore RA. Calcium oscillations and mammalian egg activation. *J Cell Physiol.* 2006; 206:565–573. [PubMed: 16155907]
- [3]. Miyazaki S, Ito M. Calcium signals for egg activation in mammals. *J Pharmacol Sci.* 2006; 100:545–552. [PubMed: 16799264]
- [4]. Townley IK, Roux MM, Foltz KR. Signal transduction at fertilization: the Ca²⁺ release pathway in echinoderms and other invertebrate deuterostomes. *Semin Cell Dev Biol.* 2006; 17:293–302. [PubMed: 16545968]
- [5]. Rice A, Parrington J, Jones KT, Swann K. Mammalian sperm contain a Ca(2+)-sensitive phospholipase C activity that can generate InsP(3) from PIP(2) associated with intracellular organelles. *Dev Biol.* 2000; 228:125–135. [PubMed: 11087632]
- [6]. Mahowald AP, Goralski TJ, Caulton JH. In vitro activation of *Drosophila* eggs. *Dev Biol.* 1983; 98:437–445. [PubMed: 6409691]
- [7]. King PE, Rafai J. A possible mechanism for initiating the parthenogenetic development of eggs in a parasitoid Hymenopteran, *Nasonia vitripennis*. *The Entomologist.* 1970; 106:118–120.
- [8]. Doane WW. Completion of meiosis in uninseminated eggs of *Drosophila melanogaster*. *Science.* 1960; 132:677–678. [PubMed: 13817039]
- [9]. Went DF, Krause G. Egg activation in *Pimpla turionellae* (Hym.). *Naturwissenschaften.* 1974; 61:407–408. [PubMed: 4474605]
- [10]. Heifetz Y, Yu J, Wolfner MF. Ovulation triggers activation of *Drosophila* oocytes. *Dev Biol.* 2001; 234:416–424. [PubMed: 11397010]
- [11]. Horner VL, Czank A, Jang JK, Singh N, Williams BC, Puro J, Kubli E, Hanes SD, McKim KS, Wolfner MF, Goldberg ML. The *Drosophila* calcipressin sara is required for several aspects of egg activation. *Curr Biol.* 2006; 16:1441–1446. [PubMed: 16860744]
- [12]. Takeo S, Tsuda M, Akahori S, Matsuo T, Aigaki T. The calcineurin regulator sra plays an essential role in female meiosis in *Drosophila*. *Curr Biol.* 2006; 16:1435–1440. [PubMed: 16860743]
- [13]. Markoulaki S, Matson S, Abbott AL, Ducibella T. Oscillatory CaMKII activity in mouse egg activation. *Dev Biol.* 2003; 258:464–474. [PubMed: 12798302]
- [14]. Peters J-M. The anaphase promoting complex/cyclosome: a machine designed to destroy. *Nat Rev Mol Cell Biol.* 2006; 116:221–234.
- [15]. Hansen DV, Tung JJ, Jackson PK. CaMKII and polo-like kinase 1 sequentially phosphorylate the cytostatic factor Emi2/XErp1 to trigger its destruction and meiotic exit. *Proc Natl Acad Sci U S A.* 2006; 103:608–613. [PubMed: 16407128]
- [16]. Mochida S, Hunt T. Calcineurin is required to release *Xenopus* egg extracts from meiotic M phase. *Nature.* 2007; 449:336–340. [PubMed: 17882219]
- [17]. Nishiyama T, Yoshizaki N, Kishimoto T, Ohsumi K. Transient activation of calcineurin is essential to initiate embryonic development in *Xenopus laevis*. *Nature.* 2007; 449:341–345. [PubMed: 17882220]
- [18]. Fuentes JJ, Genesca L, Kingsbury TJ, Cunningham KW, Perez-Riba M, Estivill X, de la Luna S. DSCR1, overexpressed in Down syndrome, is an inhibitor of calcineurin-mediated signaling pathways. *Hum Mol Genet.* 2000; 9:1681–1690. [PubMed: 10861295]
- [19]. Grolach J, Fox DS, Cutler NS, Cox GM, Perfect JR, Heitman J. Identification and characterization of a highly conserved calcineurin binding protein, CBP1/calciressin, in *Cryptococcus neoformans*. *EMBO J.* 2000; 19:3618–3629. [PubMed: 10899116]
- [20]. Kingsbury TJ, Cunningham KW. A conserved family of calcineurin regulators. *Genes Dev.* 2000; 14:1595–1604. [PubMed: 10887154]
- [21]. Takeo S, Hawley RS, Aigaki T. Calcineurin and its regulation by Sra/RCAN is required for completion of meiosis in *Drosophila*. *Dev Biol.* 2010; 344:957–967. [PubMed: 20561515]
- [22]. Takeo S, Swanson SK, Nandanan K, Nakai Y, Aigaki T, Washburn MP, Florens L, Hawley RS. Shaggy/glycogen synthase kinase 3beta and phosphorylation of Sarah/regulator of calcineurin are essential for completion of *Drosophila* female meiosis. *Proc Natl Acad Sci U S A.* 2012; 109:6382–6389. [PubMed: 22421435]
- [23]. Andruss BF, Bolduc C, Beckingham K. Movement of calmodulin between cells in the ovary and embryo of *Drosophila*. *Genesis.* 2004; 38:93–103. [PubMed: 14994272]

- [24]. Sousa-Neves R, Schinaman JM. A novel genetic tool for clonal analysis of fourth chromosome mutations. *Fly* (Austin). 2012; 6:49–56. [PubMed: 22198523]
- [25]. VanBerkum MF, Goodman CS. Targeted disruption of Ca(2+)-calmodulin signaling in *Drosophila* growth cones leads to stalls in axon extension and errors in axon guidance. *Neuron*. 1995; 14:43–56. [PubMed: 7826640]
- [26]. Griffith LC, Verselis LM, Aitken KM, Kyriacou CP, Danho W, Greenspan RJ. Inhibition of calcium/calmodulin-dependent protein kinase in *Drosophila* disrupts behavioral plasticity. *Neuron*. 1993; 10:501–509. [PubMed: 8384859]
- [27]. Horner VL, Wolfner MF. Mechanical stimulation by osmotic and hydrostatic pressure activates *Drosophila* oocytes in vitro in a calcium-dependent manner. *Dev Biol*. 2008; 316:100–109. [PubMed: 18304524]
- [28]. Chintapalli VR, Wang J, Dow JA. Using FlyAtlas to identify better *Drosophila melanogaster* models of human disease. *Nat Genet*. 2007; 39:715–720. [PubMed: 17534367]
- [29]. McQuilton P, St Pierre SE, Thurmond J. FlyBase 101--the basics of navigating FlyBase. *Nucleic Acids Res*. 2012; 40:D706–714. [PubMed: 22127867]
- [30]. Endow SA, Komma DJ. Spindle dynamics during meiosis in *Drosophila* oocytes. *J Cell Biol*. 1997; 137:1321–1336. [PubMed: 9182665]
- [31]. Page AW, Orr-Weaver TL. Activation of the meiotic divisions in *Drosophila* oocytes. *Dev Biol*. 1997; 183:195–207. [PubMed: 9126294]
- [32]. Montell C. *Drosophila* TRP channels. *Pflugers Arch*. 2005; 451:19–28. [PubMed: 15952038]
- [33]. Walker RG, Willingham AT, Zuker CS. A *Drosophila* mechanosensory transduction channel. *Science*. 2000; 287:2229–2234. [PubMed: 10744543]
- [34]. Gong Z, Son W, Chung YD, Kim J, Shin DW, McClung CA, Lee Y, Lee HW, Chang DJ, Kaang BK, Cho H, Oh U, Hirsh J, Kernan MJ, Kim C. Two interdependent TRPV channel subunits, inactive and Nanchung, mediate hearing in *Drosophila*. *J Neurosci*. 2004; 24:9059–9066. [PubMed: 15483124]
- [35]. Kim J, Chung YD, Park DY, Choi S, Shin DW, Soh H, Lee HW, Son W, Yim J, Park CS, Kernan MJ, Kim C. A TRPV family ion channel required for hearing in *Drosophila*. *Nature*. 2003; 424:81–84. [PubMed: 12819662]
- [36]. Stricker SA, Centonze VE, Paddock SW, Schatten G. Confocal microscopy of fertilization-induced calcium dynamics in sea urchin eggs. *Dev Biol*. 1992; 149:370–380. [PubMed: 1730391]
- [37]. Deguchi R, Shirakawa H, Oda S, Mohri T, Miyazaki S. Spatiotemporal analysis of Ca(2+) waves in relation to the sperm entry site and animal-vegetal axis during Ca(2+) oscillations in fertilized mouse eggs. *Dev Biol*. 2000; 218:299–313. [PubMed: 10656771]
- [38]. Tian L, Hires SA, Mao T, Huber D, Chiappe ME, Chalasani SH, Petreanu L, Akerboom J, McKinney SA, Schreiter ER, Bargmann CI, Jayaraman V, Svoboda K, Looger LL. Imaging neural activity in worms, flies and mice with improved GCaMP calcium indicators. *Nat Methods*. 2009; 6:875–881. [PubMed: 19898485]
- [39]. Strutz A, Völler T, Riemensperger T, Fiala A, Sachse S. 3. Calcium imaging of neural activity in the olfactory system of *Drosophila*. *Neuromethods*. 2012; 72:43–70.
- [40]. Rorth P. Gal4 in the *Drosophila* female germline. *Mech Dev*. 1998; 78:113–118. [PubMed: 9858703]
- [41]. Miao YL, Stein P, Jefferson WN, Padilla-Banks E, Williams CJ. Calcium influx-mediated signaling is required for complete mouse egg activation. *Proc Natl Acad Sci U S A*. 2012; 109:4169–4174. [PubMed: 22371584]
- [42]. Sullivan KMC, Scott K, Zuker CS, Rubin GM. The ryanodine receptor is essential for larval development in *Drosophila melanogaster*. *Proc Natl Acad Sci U S A*. 2000; 97:5942–5947. [PubMed: 10811919]
- [43]. Stricker SA. Comparative biology of calcium signaling during fertilization and egg activation in animals. *Dev Biol*. 1999; 211:157–176. [PubMed: 10395780]
- [44]. Krauchunas AR, Horner VL, Wolfner MF. Protein phosphorylation changes reveal new candidates in the regulation of egg activation and early embryogenesis in *D. melanogaster*. *Dev Biol*. 2012; 370:125–134. [PubMed: 22884528]

- [45]. Sackton KL, Buehner NA, Wolfner MF. Modulation of MAPK activities during egg activation in *Drosophila*. *Fly* (Austin). 2007; 1:222–227. [PubMed: 18820472]
- [46]. Cui J, Sackton KL, Horner VL, Kumar KE, Wolfner MF. Wispy, the *Drosophila* homolog of GLD-2, is required during oogenesis and egg activation. *Genetics*. 2008; 178:2017–2029. [PubMed: 18430932]
- [47]. Benoit P, Papin C, Kwak JE, Wickens M, Simonelig M. PAP- and GLD-2-type poly(A) polymerases are required sequentially in cytoplasmic polyadenylation and oogenesis in *Drosophila*. *Development*. 2008; 135:1969–1979. [PubMed: 18434412]
- [48]. Wang L, Eckmann CR, Kadyk LC, Wickens M, Kimble J. A regulatory cytoplasmic poly(A) polymerase in *Caenorhabditis elegans*. *Nature*. 2002; 419:312–316. [PubMed: 12239571]
- [49]. Barnard DC, Ryan K, Manley JL, Richter JD. Symplekin and xGLD-2 are required for CPEB-mediated cytoplasmic polyadenylation. *Cell*. 2004; 119:641–651. [PubMed: 15550246]
- [50]. Nakanishi T, Kubota H, Ishibashi N, Kumagai S, Watanabe H, Yamashita M, Kashiwabara S, Miyado K, Baba T. Possible role of mouse poly(A) polymerase mGLD-2 during oocyte maturation. *Dev Biol*. 2006; 289:115–126. [PubMed: 16325797]
- [51]. Chu T, Henrion G, Haegeli V, Strickland S. Cortex, a *Drosophila* gene required to complete oocyte meiosis, is a member of the Cdc20/fizzy protein family. *Genesis*. 2001; 29:141–152. [PubMed: 11252055]

Figure 1. Ovulation triggers egg activation in *Drosophila*

Heifetz and colleagues [10] demonstrated that oocytes dissected from different positions within the oviduct (A) show differences in extent of activation (B) as indicated by their egg permeabilization index, a measure of bleach resistance. Oocytes that have progressed further along the oviduct (with stage IV being the most advanced) are more likely to have activated than those that are just exiting the ovary (stage 1 (oocytes within the ovary are never activated)). Other metrics, such as meiosis resumption confirm that oocytes in transit through the oviducts are activating [10]. Figure reprinted from Heifetz et al. [10], with permission.

Figure 2. Model of egg activation in *Drosophila*

The current model for egg activation posits that a mechanical tension force (F_T) on the oocyte during its transit through the oviduct activates mechanosensitive Ca^{2+} channels. Ca^{2+} enters the cytoplasm where it binds and activates calmodulin (CaM; (transition from inactive CaM_i to active CaM_a), which can then act on downstream effectors like CaMKII and calcineurin (CaN) (dashed lines indicate that evidence has not yet been provided; subscripts “i” and “a” indicate inactive and active forms, respectively). Separately, calcipressin (Sra) is phosphorylated by GSK-3 upon egg activation, which allows Sra to positively regulate CaN. These phospho-modulatory proteins, as well as as-yet unidentified kinases and phosphatases, are proposed to modulated the phosphorylation state, and thus activity, of over 300 proteins [44], leading to the cellular changes of egg activation.

Table 1

Expression of mechanosensitive channels in the adult ovary [28, 29].

	Expressed (high, moderate, low)	Not expressed
TRP	<i>trpm, trpml, pain</i>	<i>trp, trpy, trpl, nompc, nan, iav, trpa1, pkd2, trpml</i>
DEG/ENaC	<i>rpka, ppk28</i>	<i>ppk, ppk4, ppk6, ppk7, ppk10, ppk11, ppk12, ppk13, ppk14, ppk16, ppk19, ppk20, ppk21, ppk23, ppk28</i>