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Report

EGG-3 Regulates Cell-Surface and Cortex Rearrangements during Egg Activation in *Caenorhabditis elegans*

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Summary

Fertilization triggers egg activation and converts the egg into a developing embryo. The events of this egg-to-embryo transition typically include the resumption of meiosis, the reorganization of the cortical actin cytoskeleton, and the remodeling of the oocyte surface [1–3]. The factors that regulate sperm-dependent egg-activation events are not well understood. *Caenorhabditis elegans* EGG-3, a member of the protein tyrosine phosphatase-like (PTPL) family [4], is essential for regulating cell-surface and cortex rearrangements during egg activation in response to sperm entry. Although fertilization occurred normally in *egg-3* mutants, the polarized dispersal of F-actin is altered, a chitin eggshell is not formed, and no polar bodies are produced. EGG-3 is associated with the oocyte plasma membrane in a pattern that is similar to CHS-1 and MBK-2. CHS-1 is required for eggshell deposition [5–7], whereas MBK-2 is required for the degradation of maternal proteins during the egg-to-embryo transition [8–12]. The localization of CHS-1 and EGG-3 are interdependent and both genes were required for the proper localization of MBK-2 in oocytes. Therefore, EGG-3 plays a central role in egg activation by influencing polarized F-actin dynamics and the localization or activity of molecules that are directly involved in executing the egg-to-embryo transition.

Results and Discussion

The *egg-3* gene (F44F4.2) was identified as having a role in *C. elegans* fertility through a large-scale RNAi analysis by a nonredundant cDNA library [13]. RNAi of *egg-3* resulted in sterile hermaphrodites that produced unshelled oocytes similar to *egg-1* and *egg-2* mutants [14] (see Supplemental Experimental Procedures available with this article online). The *egg-3* gene has no clear homolog outside nematodes but encodes a member of the protein tyrosine phosphatase-like (PTPL) family [4, 15]. This emerging family of molecules includes the *Arabidopsis PASTICCINO2* (PAS2) that is involved in cell growth and differentiation, as well as *C. elegans sdf-9* that is involved in the regulation of dauer larva formation and insulin-like signaling [15–19]. PTP domains contain the conserved motif (I/V)HCXAGXXR(S/T)G, with cysteine and arginine residues being essential for catalysis [20]. PTPL family proteins are characterized by a lack of these cysteine or arginine residues, but they can bind phosphorylated substrates [4, 15, 21]. For example, the PTPL family protein PAS2 has been shown to lack phosphatase activity but bind a phosphorylated cyclin-dependent kinase [17]. The catalytic arginine residue is missing in *egg-3* (Figure 1A). Therefore, EGG-3 may also lack phosphatase activity similar to other PTPL family proteins.

For further analysis of *egg-3* function, a deletion allele of *egg-3(tm1191)* was obtained from the National Bioresource Project for the Nematode (Japan). This strain carries a 430 base pair deletion that is predicted to result in a protein lacking part of the PTPL domain (Figure 1B). Brood size analysis showed that *egg-3(tm1191)* homozygous hermaphrodites and deficiency transheterozygous *egg-3(tm1191)/mnDf90* hermaphrodites produced no viable progeny identical to *egg-3(RNAi)* (Table S1). *egg-3(tm1191)* mutant sterility could be rescued by a transgene that included a wild-type copy of the *egg-3* gene (Table S1) or a GFP:EGG-3 fusion driven by a germline-specific promoter (see below). Together, these data established that *egg-3(RNAi)* and *egg-3(tm1191)* represent a strong loss of function.

Crossing wild-type males to *egg-3* mutant hermaphrodites (n = 15) did not rescue fertility. Male worms that were homozygous for *egg-3(tm1191)* were completely fertile and produced sperm with normal morphology (data not shown). This indicated that infertility defects were associated with oocyte development or function rather than sperm development or function. Gametogenesis, ovulation, fertilization, and the block to polyspermy were observed to occur normally in *egg-3* mutants [22] (Figures 1C and 1D; Table S2). However, several aspects of egg activation were abnormal in *egg-3* mutants. With transgenic animals carrying GFP fused to the filamentous actin-binding domain of *Drosophila melanogaster* moesin [23] (GFP-moe) (Figure 1E), it was observed that an "actin cap" forms in the presumptive posterior of the egg cortex approximately 2–4 min after the entry of the oocyte into the spermatheca (the site of fertilization) (N.V.V. and F.P., unpublished data). This actin cap formation is dependent on fertilization. Thereafter, the F-actin cap is dispersed to the posterior half of the embryos as they leave the spermatheca and enter the

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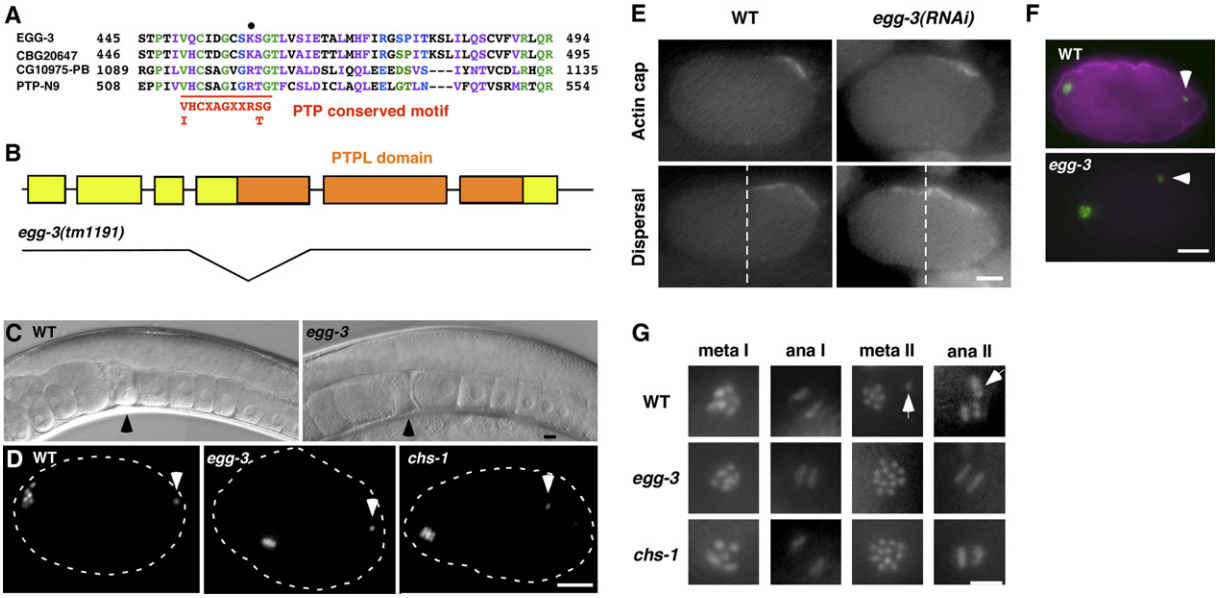

Figure 1. The PTPL Family Protein EGG-3 Is Required for Egg Activation

(A) Alignment of the phosphatase domain of EGG-3 and the ortholog in *C. briggsae* (CBG20647), phosphatases in *Drosophila melanogaster* (CG10975-PB), and in *Homo sapiens* (PTP-N9) with Clustal W software. Green, identical; purple, conserved; blue, semiconserved. Altered amino acid residues in the PTP motif are marked.

(B) Gene structure of *egg-3*. *egg-3(tm1191)* mutants deleted 430 bp, from the middle of intron 3 to the end of exon 4. cDNA yk1434 g11 included SL1 sequence upstream of exon 1.

(C) DIC images of wild-type and *egg-3(tm1191)* hermaphrodites. Arrowheads indicate spermatheca. Scale bar represents 10 μm.

(D) DAPI staining of newly fertilized embryos of wild-type, *egg-3(tm1191)*, and *chs-1(ok1120)*. Arrowheads indicate sperm DNA. Scale bar represents 10 μm.

(E) Polarized dispersal of F-actin is lost in *egg-3(RNAi)* worms. The dispersal of the actin cap crossed 50% egg length as denoted by dashed line in *egg-3(RNAi)* embryos. Wild-type control 15/15 embryos. *egg-3(RNAi)* 5/7 embryos. Scale bar represents 10 μm.

(F) Chitin staining of wild-type and *egg-3(tm1191)* embryos. Arrowheads indicate sperm DNA. Magenta, chitin; green, DAPI. Scale bar represents 10 μm.

(G) GFP-histone in wild-type, *egg-3(tm1191)*, and *chs-1(ok1120)* embryos during meiosis. meta I, metaphase I; ana I, anaphase I; meta II, metaphase II; ana II, anaphase II. Arrows indicate polar body. Scale bar represents 5 μm.

uterus (N.V.V. and F.P., unpublished data) (Figure 1E). In *egg-3(RNAi)* worms, normal cortical rearrangements occurred during meiotic maturation [22] and the actin cap was indistinguishable from wild-type. However, F-actin dispersed aberrantly to the anterior side of the cortex (Table S2; Figure 1E). These experiments show that *egg-3* is not required for the formation of the actin cap but is required for the polarized dispersal of the actin cytoskeleton in the cell cortex during the egg-to-embryo transition.

In many species, the egg surface changes dramatically after fertilization and during the egg-to-embryo transition. In *C. elegans*, a chitinous eggshell is secreted in response to sperm entry and covers the embryo to provide chemical impermeability and mechanical support for embryonic development [24]. Eggshell formation is thought to start in the spermatheca within the first 5 min after fertilization. In *C. elegans*, the eggshell consists of three layers [25]. Based on comparisons to parasitic nematodes [26], these three layers are thought to be an outer vitelline membrane, a middle chitin-containing layer, and an inner lipid-rich layer. We see that at least the chitin-containing layer is already formed at metaphase I in wild-type embryos (Figure 1F). However, *egg-3* mutant embryos lacked chitin layers at metaphase I (Figure 1F) and all later stages (endomitotic stages after meiosis; data not shown). Therefore, *egg-3* function is required for the formation of the eggshell chitin layer after fertilization.

Sperm entry also triggers the resumption of meiosis and polar body formation [27] (Figure 1G). We observed oocyte chromosomes during meiosis by using GFP-histone and GFP-tubulin in wild-type and *egg-3* mutants. In *egg-3* mutants, meiotic spindle translocation and rotation occurred normally [27] (Figure S1), and chromosomes segregated properly until anaphase I (Figure 1G). However, despite meiotic progression, no polar bodies were formed after the completion of meiosis I and II (Figure 1G and data not shown). Additionally, 12 individualized chromosomes were often seen at the beginning of meiosis II in *egg-3* mutants (Figure 1G). We also observed that CYB-1:GFP (cyclin B) was degraded during meiosis II in both wild-type [28, 29] and *egg-3* mutant oocytes (Figure S2). This further suggests that there is no significant meiotic delay and meiotic progression is not dependent on EGG-3 function. We conclude that EGG-3 is not required for meiotic progression but is required for polar body formation. These meiotic defects seen in *egg-3* mutants are similar to those seen in embryos depleted of F-actin by latrunculin A treatment and profilin *pfn-1(RNAi)* embryos [30].

To examine the subcellular localization of EGG-3, we created transgenic worm strains carrying integrated *gfp* or *mCherry:egg-3* fusions driven by the

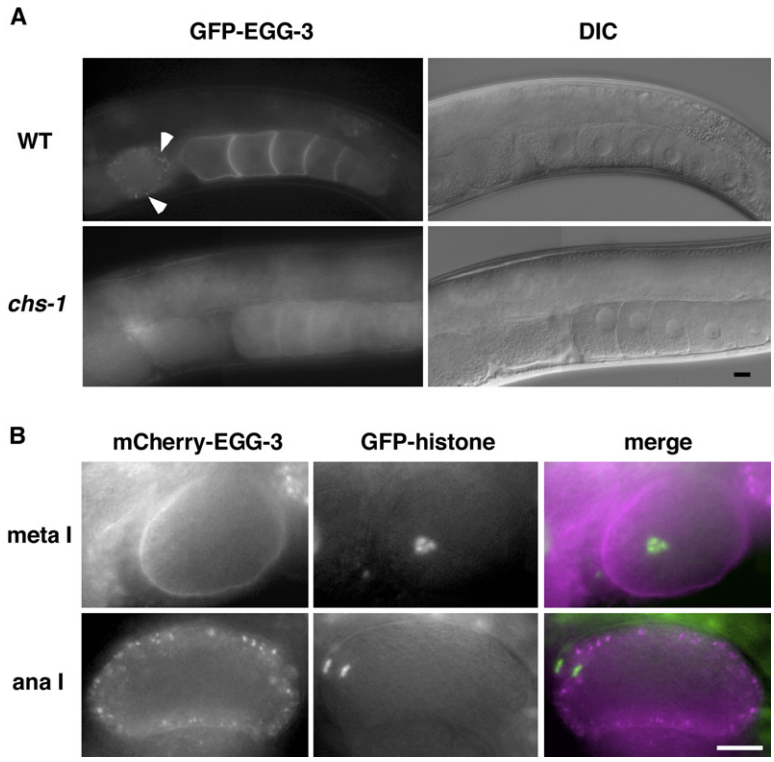

Figure 2. GFP:EGG-3 Is Associated with Oocyte Membranes and Its Localization Is Dependent on *chs-1*

(A) GFP:EGG-3 is associated with oocyte plasma membranes and formed foci after fertilization. The bulk of GFP:EGG-3 is cytoplasmic in *chs-1(ok1120)* mutants. Arrowheads indicate foci.

(B) mCherry:EGG-3 leaves membranes and forms foci from anaphase I. meta I, metaphase I; ana I, anaphase I; magenta, mCherry:EGG-3; green, GFP:histone.

Scale bars represent 10 μm.

germline-specific *pie-1* promoter [31]. GFP:EGG-3 was associated with the plasma membrane of oocytes and newly fertilized embryos (Figure 2A). Later, it moved to cytoplasmic foci and was subsequently degraded (Figure 2A). To determine the timing of this dynamic relocalization, we constructed worm strains expressing both GFP-histone and mCherry:EGG-3. In these animals, we were able to determine that mCherry:EGG-3 moved from a membrane-associated localization pattern to foci at anaphase I (Figure 2B). This redistribution did not depend on fertilization but did depend on meiotic progression. GFP:EGG-3 was localized normally and formed foci in *spe-9(hc88)* fertilization-defective mutants [32] (Figure S3A). However, GFP:EGG-3 stayed associated with the plasma membrane in *mat-1(RNAi)* worms where oocytes were arrested at metaphase I [33, 34] (Figure S3A).

Because *egg-3* mutant oocytes lacked an eggshell, we reasoned that we might be able to detect an interaction between EGG-3 and molecules required for eggshell formation. The *chs-1* gene encodes a chitin synthase that is predicted to have 15 transmembrane domains and catalyzes the polymerization of UDP-N-acetyl-glucosamine to produce chitin [5–7]. The *chs-1* gene is essential for eggshell formation and *chs-1* mutants produce oocyte phenotypes that are similar to *egg-3* mutants. In *chs-1* mutants, fertilization occurred but polar bodies and eggshell formation were lacking (Figures 1D and 1G) [7]. Furthermore, 12 individualized chromosomes were often seen in *chs-1* mutant oocytes (Figure 1G).

In order to examine CHS-1 localization, we created worms expressing GFP:CHS-1 with the *pie-1* promoter. GFP:CHS-1 was also associated with the oocyte surface and colocalized with mCherry:EGG-3 in foci in newly fertilized embryos (Figures 3A and 3B). Most GFP:EGG-3 was not associated with oocyte membranes and was cytoplasmic in *chs-1* mutants (Figure 2A). The converse was also true. The amount of GFP:CHS-1 on the oocyte membrane was decreased in *egg-3* mutants when compared to a wild-type background (Figure 3B), similar to *egg-3(RNAi)* worms (data not shown). These results indicate that EGG-3 and CHS-1 are membrane-associated proteins and that their normal localization patterns are interdependent.

The DYRK Kinase MBK-2 is required for the egg-to-embryo transition in *C. elegans* and marks maternal proteins for timely degradation [8–12]. The localization pattern of GFP:MBK-2 appears to be identical to GFP:EGG-3. We confirmed colocalization by constructing a strain of worms carrying mCherry:EGG-3 and GFP:MBK-2 (Figure 4B). Furthermore, GFP:MBK-2 was not associated with the oocyte plasma membrane in *egg-3* or *chs-1* mutants (Figure 4A), similar to *chs-1(RNAi)* worms [35] (data not shown). In contrast, loss of *mbk-2* function with RNAi did not alter the association of GFP:EGG-3 and GFP:CHS-1 with the oocyte plasma membrane (Figures S3A and S3B). We conclude that EGG-3 and CHS-1 are required for the proper localization of MBK-2.

We have shown that EGG-3 is clearly required for a number of important events of egg activation. These events include the proper polarized reorganization of the cortex actin cytoskeleton, eggshell formation, and polar body formation. The dynamic localization patterns of EGG-3 and CHS-1 were interdependent and MBK-2 required EGG-3 and CHS-1 for its proper localization in oocytes. A number of hypotheses have been proposed for the biochemical function of PTPL family proteins

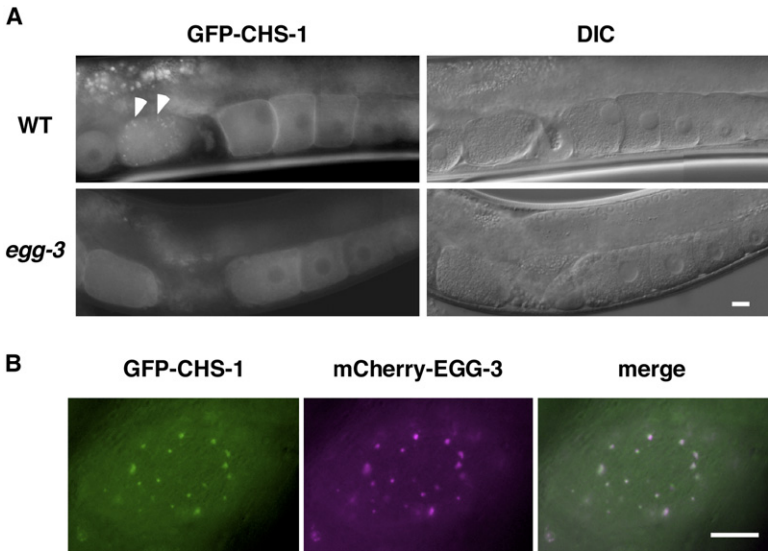

Figure 3. GFP:CHS-1 Is Associated with the Oocyte Plasma Membrane and Colocalized with mCherry-EGG-3

(A) GFP:CHS-1 (nnIs2) is associated with the oocyte plasma membrane and formed foci after fertilization. In *egg-3(tm1191)* mutants, GFP:CHS-1(nnIs2) associated with the plasma membrane was decreased. GFP:CHS-1(nnEx2) showed the same results by *egg-3* RNAi-by-soaking (data not shown). Arrowheads indicate foci.

(B) GFP:CHS-1(nnIs2) and mCherry:EGG-3 is colocalized in newly fertilized embryos. Green, GFP:CHS-1; magenta, mCherry:EGG-3.

Scale bars represent 10 μm.

that may be relevant to EGG-3 function during egg activation [21]. One idea is that PTPL proteins could function as noncatalytic binding domains and work as molecular "adaptors" or "scaffolds" much like Src-homology 2 (SH2) domains [21]. Based on the molecular epistasis of EGG-3, CHS-1, and MBK-2 with regards to their subcellular localization, we favor this hypothesis for EGG-3 function. EGG-3 may be regulating the localization and/or activity of the egg activation machinery by forming a plasma membrane-associated complex. Indeed, it has been recently determined that EGG-3 can bind directly to MBK-2 both in vitro and in vivo (see accompanying paper in this issue of *Current Biology* [36]).

The polarized distribution of F-actin during egg activation appears to be an evolutionarily conserved oocyte feature [37]. EGG-3 mutant oocytes showed normal actin cap formation that is associated with sperm entry (N.V.V. and F.P., unpublished data) but had defects in the polarized dispersal of F-actin after fertilization. The meiosis defects in *egg-3* mutants might be related to this actin reorganization defect at the cortex because the meiosis phenotypes of *egg-3* mutants are notably similar to the phenotypes of actin filament-depleted worms [30]. However, F-actin in *egg-3* mutant oocytes during telophase of meiosis looked similar to wild-type (data not shown). Therefore, the precise mechanism of

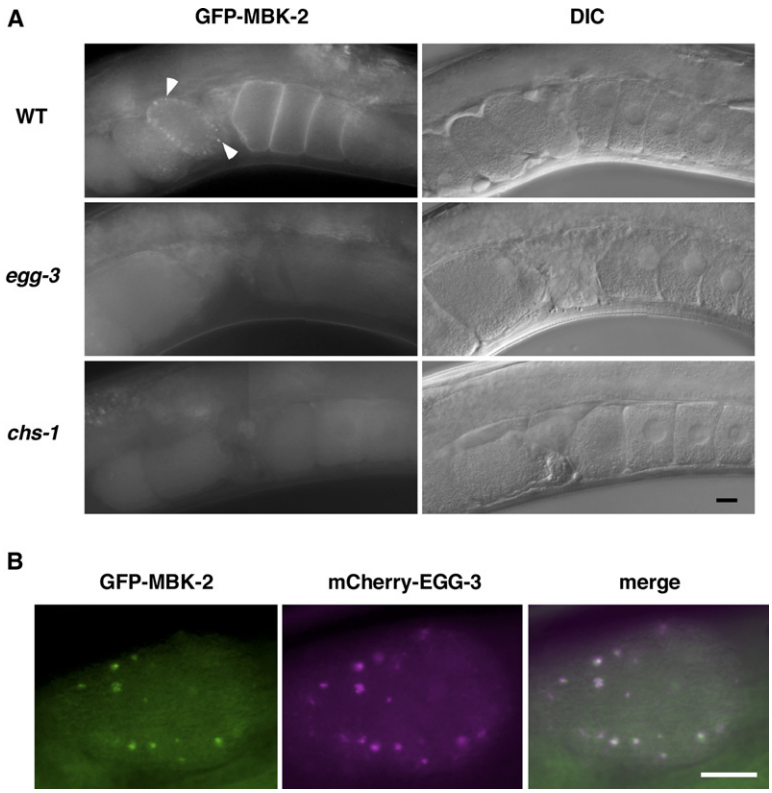

Figure 4. EGG-3 and CHS-1 Are Required for the Proper Membrane-Associated Localization of GFP:MBK-2

(A) GFP:MBK-2 is not localized to oocyte plasma membranes in *egg-3(tm1191)* and *chs-1(ok1120)* mutants. Arrowheads indicate foci.

(B) GFP:MBK-2 and mCherry:EGG-3 is colocalized in newly fertilized embryos. Green, GFP:MBK-2; magenta, mCherry:EGG-3.

Scale bars represent 10 μm.

how EGG-3 influences polar body formation remains to be determined.

Although the reorganization of F-actin, eggshell formation, and polar body formation were abnormal in *egg-3* mutant oocytes, the block to polyspermy and meiotic progression occurred. As noted above, EGG-3 might function in stabilizing CHS-1 and MBK-2 associations with the oocyte plasma membrane. If this was the case, many defects seen in *egg-3* mutants could be due to a decrease in proper CHS-1 localization. CHS-1 would then be required for the polarized reorganization of F-actin and polar body formation. Another possibility is that EGG-3 might play a role in transmitting a sperm entry signal to key downstream events required for the egg-to-embryo transition. The block to polyspermy and meiotic progression from anaphase I are sperm-dependent events as well as the events that are defective in *egg-3* mutants. This indicates that sperm entry might not be detected properly in *egg-3* oocytes and that *egg-3* might function in one of the signal cascades that triggers parts of egg activation events in response to sperm entry. An exciting candidate for a sperm signal is encoded by the paternal effect lethal *spe-11* gene [38]. Although SPE-11 is a sperm-supplied protein, *spe-11* mutants have identical defects as displayed by maternal effect *egg-3* mutants [27, 39]. Therefore, this work provides the first glimpse of the molecules required for the flow of information from the union of sperm and egg to the cascade of events required for embryonic development.

Genome sequencing has established that PTPL family encoding genes are evolutionarily conserved from plants to humans and carry out vital functions in many of these organisms [15]. For instance, there are at least 14 predicted catalytically inactive PTPL domain-containing genes in humans [15]. The biological functions of the vast majority of these genes still need to be elucidated. However, several PTPL family genes have been implicated in human genetic disease. Consistent with these links, another *C. elegans* PTPL family protein, SDF-9, is a regulator of insulin-like signaling [19, 40]. Like EGG-3, SDF-9 is localized to the plasma membrane of the cells where it is expressed [19]. It is possible that membrane localization is a common feature of PTPL proteins.

This study represents the first link of a PTPL family protein (EGG-3) to the events of egg activation. EGG-3 plays a central role in egg activation by influencing polarized F-actin dynamics and the localization and activity of molecules that are directly involved in executing the egg-to-embryo transition. Therefore, we not only establish a new role for this class of molecule but also provide new insights into the events of egg activation. This work could eventually lead to clinical advances directed at improved diagnostics and treatments for infertility cases in humans that are associated with defective egg activation.

Supplemental Data

Three figures, two tables, and Experimental Procedures are available at http://www.current-biology.com/cgi/content/full/17/18/1555/DC1/.

Acknowledgments

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