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MECHANISMS OF DEVELOPMENT

DPL-1 (DP) acts in the germ line to coordinate ovulation and fertilization in *C. elegans*

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ABSTRACT

Proper coordination of oogenesis, ovulation, and fertilization is essential for successful reproduction. In *Caenorhabditis elegans*, a strong loss-of-function mutation in *dpl-1*, which encodes a subunit of the E2F heterodimeric transcription factor EFL-1/DPL-1, causes severe defects during ovulation and fertilization. Here we demonstrate that the somatic gonad structure and sheath cell contraction rate appear normal in *dpl-1* mutants, but that dilation of the spermatheca valve does not occur properly, causing oocytes to become trapped in the proximal gonad arm and enter endomitosis. This ovulation defect can be partially suppressed by increasing the activity of ITR-1, an inositol triphosphate receptor in the spermatheca that promotes dilation in response to IP_3 signaling. Tissue-specific rescue experiments demonstrate that expression of DPL-1 in germ cells but not the spermatheca can restore both ovulation and fertilization in *dpl-1* mutants, indicating that the absence of DPL-1 likely disrupts a pro-ovulation signal originating in the oocyte that in turn stimulates the spermatheca. Moreover, we found that expression of a single EFL-1/DPL-1-responsive gene, *rme-2*, in the germ line of *dpl-1* mutants significantly rescues ovulation, but not fertilization. Instead, other EFL-1/DPL-1-responsive genes function to promote successful fertilization. We propose that DPL-1 acts with EFL-1 in developing oocytes to directly regulate a transcriptional program that couples the critical events of ovulation and fertilization.

1. Introduction

Germ cells are the unique population of cells that specialize in transmitting the genetic information to the next generation, through fertilization of oocytes by sperm. For a wide range of sexually reproducing animals, maintenance of a close association between germ cells and surrounding somatic cells is critical for successful fertility (Eppig et al., 2002; Matzuk et al., 2002). In female mammals, for example, gap junction-mediated association between granulosa cells and the oocyte is required for oocyte growth and meiotic arrest (Kidder and Mhawi, 2002). The oocyte in turn promotes granulosa cell proliferation and differentiation (Gilchrist et al, 2006).

Ovulation in *C. elegans* provides an excellent system for the study of interactions between germ cells and somatic cells, owing to the powerful genetic tools that are available, and the ability to monitor oocyte development and maturation in real time within living animals (reviewed in Greenstein, 2005). The *C. elegans* hermaphrodite gonad is arranged in two U-shaped tubes, each containing undifferentiated mitotic germ cells that progress to fully differentiated gametes along the distal to proximal axis. Germ cells differentiate into sperm in the fourth larval stage, while in the adult, germ cells differentiate into oocytes. The proximal portion of each gonad arm, which contains the gametes, is surrounded by a sheath made of a non-striated myoepithelium. This sheath is attached to the spermatheca, a sac-like structure where

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sperm are stored and fertilization occurs. The sheath cells, spermatheca, and uterus are all composed of somatic cells.

During ovulation, oocyte-mediated signaling induces rapid contraction of the myoepithelial sheath and dilation of the distal spermatheca. The mature oocyte is then propelled from the gonad arm into the lumen of the spermatheca, and fertilization occurs shortly thereafter. A cycle of ovulation and fertilization in the adult hermaphrodite germ line occurs about every 23 min until all the sperm have been utilized. Fertilization triggers rapid eggshell formation and completion of meiosis I and II. The fertilized embryos pass through the proximal valve of the spermatheca into the uterus, where they undergo several rounds of cell division before being laid.

Some molecular components of the signaling pathway governing ovulation in *C. elegans* have been identified. Sperm signal their presence to the oocyte through the release of major sperm protein (MSP), which initiates oocyte maturation through interaction with EPH and NMDA receptors, triggering calcium release and MAP kinase activation (Miller et al., 2001, 2003; Corrigan et al., 2005). The oocyte and surrounding sheath cells communicate via gap junctions to coordinate sheath cell contraction and successful ovulation through regulation of Gα signaling (Govindan et al., 2006; Whitten and Miller, 2007). The maturing oocyte also signals via the EGF ligand LIN-3 to the receptor tyrosine kinase LET-23 on the spermatheca cell membrane to stimulate inositol-1,4,5-triphosphate (IP_3) production in the spermatheca (Clandinin et al., 1998). In turn, IP_3 binds to its receptor, ITR-1, on the surface of the endoplasmic reticulum, causing the release of intracellular calcium ions into the cytosol. This burst of calcium presumably stimulates spermatheca dilation, through mechanisms currently unknown.

Defective ovulation is the major phenotype of animals carrying strong loss-of-function mutations in *dpl-1* or *efl-1*, which encode the two components of the heterodimeric transcription factor E2F (Ceol and Horvitz, 2001; Page et al., 2001; Chi and Reinke, 2006). However, which aspect of ovulation is disrupted in these mutants has not been determined. Previous investigations either examined only partial loss-of-function alleles (Page et al., 2001) or provided only preliminary descriptions of the phenotype (Ceol and Horvitz, 2001; Chi and Reinke, 2006). We have previously identified genes whose expression is regulated by E2F (Chi and Reinke, 2006). Many of these EFL-1/DPL-1-responsive genes are known or predicted to play roles in various aspects of oocyte and early embryo development, but which genes directly mediate ovulation was not experimentally addressed. Assigning target genes to specific physiological processes such as ovulation will not only permit a better understanding of the processes themselves, but will also further illuminate how a commonly used transcription factor such as E2F acts on distinct effector genes to regulate tissue-specific aspects of development.

Here, we investigated the mechanisms underlying the ovulation defect of *dpl-1(n3316)* mutants. We found that ovulation is defective because of a failure to properly coordinate oocyte maturation with spermatheca dilation. This phenotype could occur because *dpl-1* could function in the soma as well as the germ line. However, expression of DPL-1 specifically in germ cells rescued the ovulation defect, whereas expression in the spermatheca did not. Moreover, expression of a single EFL-1/DPL-1-responsive gene, *rme-2*, in the germ line was also sufficient to rescue ovulation, providing direct experimental evidence that the absence of *rme-2* expression is a primary contributor to the ovulation defects of *dpl-1* mutants. We also show that both *dpl-1* and *rme-2* act upstream of the IP_3 signaling pathway to control spermatheca dilation, indicating that the ovulation defects of *dpl-1* mutants originate from the oocyte. Finally, we demonstrate that DPL-1 activity is also required specifically in germ cells for successful fertilization, independently of its role in ovulation. Taken together, we propose that DPL-1, along with EFL-1, promotes the expression of key target genes in germ cells to coordinate multiple molecular events necessary for ovulation and fertilization in *C. elegans*.

2. Materials and methods

2.1. Strains and maintenance

Nematode strain maintenance was as described (Sulston and Hodgkin, 1988). All strains were grown at 20 °C. The following variants were used: N2 (wild type), LG II: *dpl-1(n3316)* (Ceol and Horvitz, 2001), LG III: *unc-119(ed3)*, LG IV: *rme-2(b1005)* (Grant and Hirsh, 1999), *itr-1(sy290)* (Clandinin et al., 1998), *lfe-2(sy326)* (Clandinin et al., 1998), LG V: *efl-1(n3639)* (Ceol and Horvitz, 2001).

2.2. Time-lapse microscopy

For time-lapse observation of ovulation, worms were anesthetized for 20–30 min in M9 solution with 0.1% tricaine and 0.01% tetramisole (Sigma, Inc.) before viewing (McCarter et al., 1997). Anesthetized worms were mounted on a 2% agarose pad, and a small amount of vaseline was applied to the edges of the pads to minimize pressure from the coverslip. Mounted animals were viewed on a Zeiss Axioplan 2 microscope with low light using the 40× lens under differential image contrast (DIC). Images were recorded using Zeiss Axiovision software. Sheath cell contraction analysis was also performed under these mounting conditions.

2.3. Construction of transgenic strains

efl-7::GFP:DPL-1 and *sth-1*::GFP:DPL-1 transgenic strains were generated by standard injection methods that produce high copy number extrachromosomal arrays (Mello et al., 1991). *efl-7* corresponds to F49E12.6. Both transgenes were constructed using a PCR stitching method (Hobert, 2002). Sequences 3.3 kb upstream of *efl-7* or 1.4 kb upstream of *sth-1*, including the ATG start, were stitched in frame to GFP. The genomic sequence of *dpl-1*, beginning with the second amino acid and including the 3′ UTR, was fused downstream of GFP. Each construct was injected at 20 ng/μL into wild type young adult hermaphrodites, along with the transformation marker $P_{myo\text{-}2}$::GFP (pJKL449.1; 5 ng/μL) and empty vector pGEM-7Z (40 ng/μL). At least two lines were obtained for each transgene based on $P_{myo\text{-}2}$::GFP expression in the pharynx. Both lines were crossed into the *dpl-1* mutant background to test for suppression of the mutant phenotype.

To obtain a line that expresses *dpl-1* in the germ line, the *dpl-1* gene including its 3′ UTR was cloned into Gateway vector pID3.01B, which contains the *pie-1* germ line-specific promoter, 5′ GFP coding sequence, and *unc-119* rescuing construct (Reese et al., 2000), and its sequence was verified. The resulting plasmid was integrated into *unc-119(ed3)* worms by microparticle bombardment as described (Praitis et al., 2001). Transgenic animals had stable expression of GFP:DPL-1 in germ cells and embryos, but not in other tissues.

2.4. RNAi screening for ovulation and fertilization defects

Genes previously defined as requiring both DPL-1 and EFL-1 for their expression in the germ line (Group I genes; Chi and Reinke, 2006) were subjected to RNAi by the feeding method using an available RNAi library (Kamath et al., 2003). This library contained representatives of 66 out of the 75 Group I genes. Wild type worms were synchronized at the L1 stage and grown on NGM plates that were seeded with bacteria expressing dsRNA corresponding to each gene. Ten egg-laying adult worms per gene were picked and allowed to lay eggs for an hour. After removing the adults, the number of eggs laid was counted. The effectiveness of RNAi was checked based on the known phenotypes among the target genes. RNAi was repeated at least twice for each gene. Candidates for an ovulation defect were selected if the number of eggs laid was 70% or less than wild type.

A subset of seven Group I genes were selected for testing for possible fertilization defects: *cyb-2.1*, *cyb-3*, *rme-2*, F01F1.12, *rnr-2*, *mex-1*, and T21E3.1, based on previously described sterile phenotypes by RNAi (http://www.wormbase.org). After exposure to dsRNA as described above, the plates for each test dsRNA were examined for the presence of unfertilized oocytes, which are easily distinguishable from fertilized embryos under a dissecting microscope by virtue of being flat, grainy, square and gray, in contrast to embryos, which are ovoid, clear, possess a visible eggshell, and are multicellular.

dpl-1(RNAi) was conducted by injection of *dpl-1* dsRNA (concentration > 2 μg/μl) into young adult wild type animals. To obtain progeny with a maximal RNAi effect, initial progeny produced by injected animals during the first 6 h were discarded, and only subsequent progeny were counted to determine sterility and ovulation defects.

3. RESULTS

3.1. Aberrant spermatheca dilation underlies the dpl-1(n3316) ovulation defect

We wished to investigate the mechanisms underlying defective ovulation in animals lacking E2F activity. Because the *efl-1* locus is not covered by a balancer chromosome, *efl-1* homozygous mutants are impossible to distinguish from heterozygote siblings early enough for most of our assays. We therefore focused on *dpl-1*. A likely null allele of *dpl-1*, *n3316*, displays defective ovulation as indicated by the accumulation of endomitotic (Emo) oocytes in the proximal gonad, similar to *efl-1* mutants (Fig. 1A and B; see also Chi and Reinke, 2006 for additional images) (Ceol and Horvitz, 2001; Page et al., 2001). Sperm provided by mating wild type males to *dpl-1(n3316)* hermaphrodites did not suppress the ovulation phenotype, indicating that the defect likely occurs in either oocytes or the somatic gonad, but not sperm (Chi and Reinke, 2006).

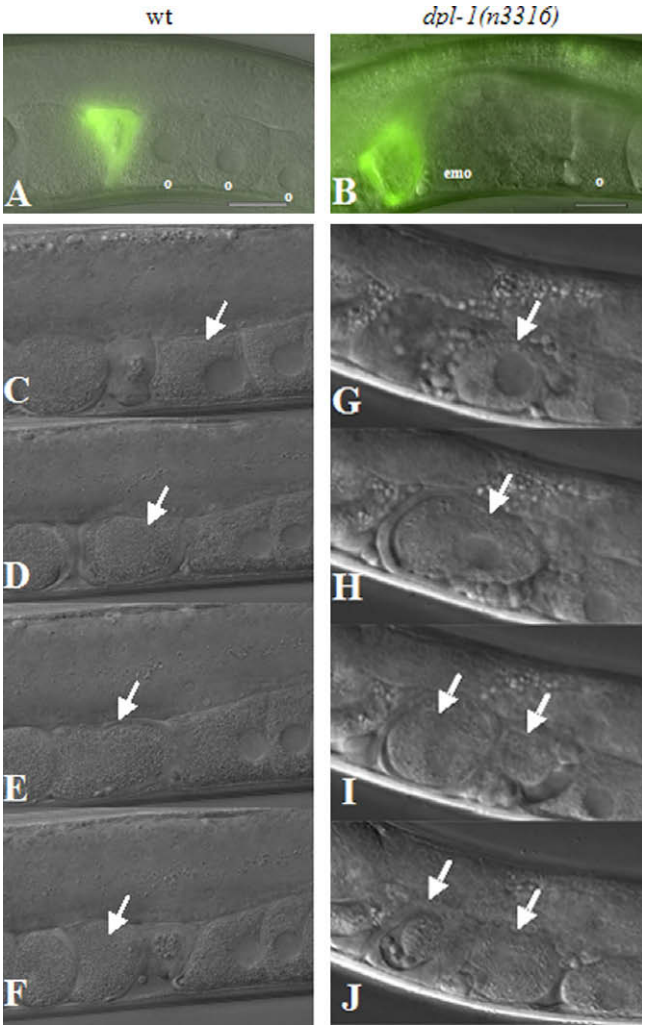

Fig. 1 – *dpl-1(n3316)* mutants have ovulation defects. Representative DIC images of the proximal gonads of wild type and *dpl-1(n3316)* mutant hermaphrodites. (A, B) The proximal gonads of mature wild type (A) and *dpl-1(n3316)* mutants (B), showing accumulation of oocytes in the mutant. The spermatheca is marked with GFP (green); o = immature oocyte; emo = endomitotic oocytes. Scale bar = 20 μm. (C–J) Time-lapse still images of the first ovulation in wild type (C–F) and *dpl-1(n3316)* mutant hermaphrodites (G–J). The white arrow follows the most proximal oocyte undergoing ovulation.

To further dissect the complex ovulation defects of *dpl-1(n3316)* mutants, we wished to determine the earliest detectable abnormality during ovulation. We therefore used time-lapse microscopy to visualize oocyte maturation, ovulation, and fertilization in vivo. In wild type hermaphrodites (Fig. 1C–F; Movie 1), the most proximal oocyte initiated maturation and ovulation after exposure to major sperm protein,

or MSP, from sperm (Miller et al., 2001). Oocyte maturation is characterized by nuclear envelope breakdown (NEBD) and cortical rearrangement, which causes the maturing oocyte to adopt a circular shape. Prior to ovulation, the proximal pair of somatic sheath cells that surrounds the oocytes underwent vigorous contraction, and the distal spermathecal valve dilated. During ovulation, the oocyte entered into the spermatheca and became fertilized as it came into contact with sperm (Fig. 1A, C–F; Movie 1).

In *dpl-1(n3316)* hermaphrodites, defects were apparent during ovulation of the very first oocyte produced after the sperm-to-oocyte switch (Fig. 1G–J; Movie 2). Time-lapse microscopy revealed that the spermatheca partially dilated before the completion of oocyte maturation, although the nuclear envelope of the oocyte eventually did break down, indicating that oocyte maturation does occur. We cannot distinguish whether the spermatheca dilated too early, or whether oocyte maturation was delayed. The distal spermatheca valve then closed prematurely, prior to complete entry of the oocyte, either tearing the oocyte or squeezing it back into the proximal gonad. This defect led to the accumulation of trapped and degenerating oocytes, causing mature *dpl-1(n3316)* adults to have an enlarged proximal germ line. Many of the trapped oocyte fragments initiated endomitosis (Fig. 1B). Rare oocyte fragments that did enter the uterus remained unfertilized and over time also initiated endomitosis. These observations suggest that DPL-1 serves an important role in coordinating oocyte maturation and spermatheca dilation. Similar ovulation defects were also seen in *efl-1(n3639)* mutants (Supplemental Figure S1, Movie 3).

3.2. *Increased activity of itr-1 in the spermatheca restores ovulation but not fertilization in dpl-1(n3316) mutants*

Our time-lapse microscopy studies do not distinguish whether a defect in oocyte maturation and signaling results in aberrant spermatheca function, or whether the spermatheca itself has an intrinsic defect, rendering it incapable of responding to a pro-ovulatory signal from the oocyte. We therefore examined whether the spermatheca can function properly in *dpl-1* mutants if the requirement for a signal from the oocyte is bypassed. A maturing oocyte is thought to signal to the adjacent spermatheca to dilate via production of the epidermal growth factor (EGF)-like protein LIN-3, which binds the receptor tyrosine kinase LET-23 on the membrane of spermathecal cells and activates a downstream phosphatidyl inosital-mediated signaling cascade (Clandinin et al., 1998). *itr-1* encodes an IP_3 receptor that triggers intracellular calcium release and spermathecal dilation in response to LET-23 activation (Bui and Sternberg, 2002; Clandinin et al., 1998). A gain-of-function mutation in *itr-1*, *itr-1(sy290)*, suppresses the ovulation defect in *lin-3* and *let-23* mutants, possibly by increasing sensitivity to IP_3 in the spermatheca (Clandinin et al., 1998).

itr-1(sy290) mutant hermaphrodites have normal ovulation and spermatheca dilation ($n = 23$) (Fig. 2A; Clandinin et al., 1998). We therefore tested if the ovulation defect in *dpl-1(n3316)* could be suppressed by *itr-1(sy290)*. While essentially no ovulation occurs in *dpl-1(n3316)* worms (Fig. 2B), 88% ($n = 24$) of the *itr-1(sy290)*; *dpl-1(n3316)* double mutants successfully ovulated oocytes into the uterus (Fig. 2C). Furthermore, torn pieces of oocytes were no longer found in the proximal gonad of most *itr-1(sy290)*; *dpl-1(n3316)* mutants, indicating that increased activation of ITR-1 rescues the ovulation defects. Indeed, time lapse microscopy of ovulation in *itr-1(sy290)*; *dpl-1(n3316)* mutants revealed that spermatheca dilation and oocyte entrance occurred normally (Movie 4). We conclude that *dpl-1* acts upstream of *itr-1*, and suggest that *dpl-1(n3316)* mutant oocytes fail to signal to the spermatheca, leading to inefficient IP_3 activation in the spermatheca. When the requirement for the missing oocyte signal is bypassed, the spermatheca can function normally.

Notably, even though ovulation was rescued, *itr-1(sy290)*; *dpl-1(n3316)* mutants remained sterile. We found that successfully ovulated oocytes undergo endomitosis in the uterus (Fig. 2C), suggesting that *dpl-1(n3316)* oocytes cannot be fertilized, even if ovulated into the spermatheca. To investigate whether fertilization is indeed defective, we examined whether both maternal and paternal pronuclei could be found in the rare *dpl-1(n3316)* ovulated oocytes, as well as in oocytes from *dpl-1(n3316); itr-1(sy290)* double mutants. While pronuclei in wild type or *itr-1(sy290)* one-cell embryos are clearly visible upon DNA staining with DAPI, we were unable to observe pronuclei in either *dpl-1(n3316)* or *itr-1(sy290)*; *dpl-1(n3316)* mutants (Fig. 3A). Additionally, we examined eggshell formation, which is one of the earliest events following fertilization. Embryos that form an eggshell are impervious to penetration by the dye FM4-64 (Rappleye et al., 1999). We incubated embryos and oocytes collected from wild type or *dpl-1(n3316)* mutant hermaphrodite gonads in FM4-64. Wild type embryos did not incorporate the dye, but the rare *dpl-1(n3316)* ovulated oocytes were unable to block dye penetration (Fig. 3B), indicating that eggshell formation was impaired. Moreover, the ovulated oocytes never showed any signs of normal cell division. Thus, our inability to detect any pronuclei along with the absence of both eggshell formation and cell division is consistent with the inability of *dpl-1(n3316)* oocytes to complete fertilization.

Finally, we tested whether wild type sperm could restore fertilization in the *dpl-1(n3316)*; *itr-1(sy290)* double mutant. Even when wild type sperm were provided through mating, ovulated oocytes in the uterus of *dpl-1(n3316)*; *itr-1(sy290)* animals were still unfertilized and underwent endomitosis ($n = 4$), consistent with the idea that *dpl-1* mutant oocytes were incapable of being fertilized, even if ovulated. Combined, our results indicate that *dpl-1(n3316)* is required for successful fertilization as well as ovulation.

3.3. *The maternal contribution of DPL-1 is required for proximal gonad formation*

Because all *dpl-1(n3316)* homozygous mutants are born of heterozygotes, we assessed whether maternally contributed DPL-1 in these mutants masked earlier requirements for DPL-1 activity. We injected *dpl-1* dsRNA into wild type young adult worms and observed the development of F1 progeny, which should have reduced maternal and zygotic *dpl-1*. *dpl-1(RNAi)* did not significantly hinder larval growth and the worms reached adulthood, but were sterile. Microscopic examination revealed that the germline defects were more

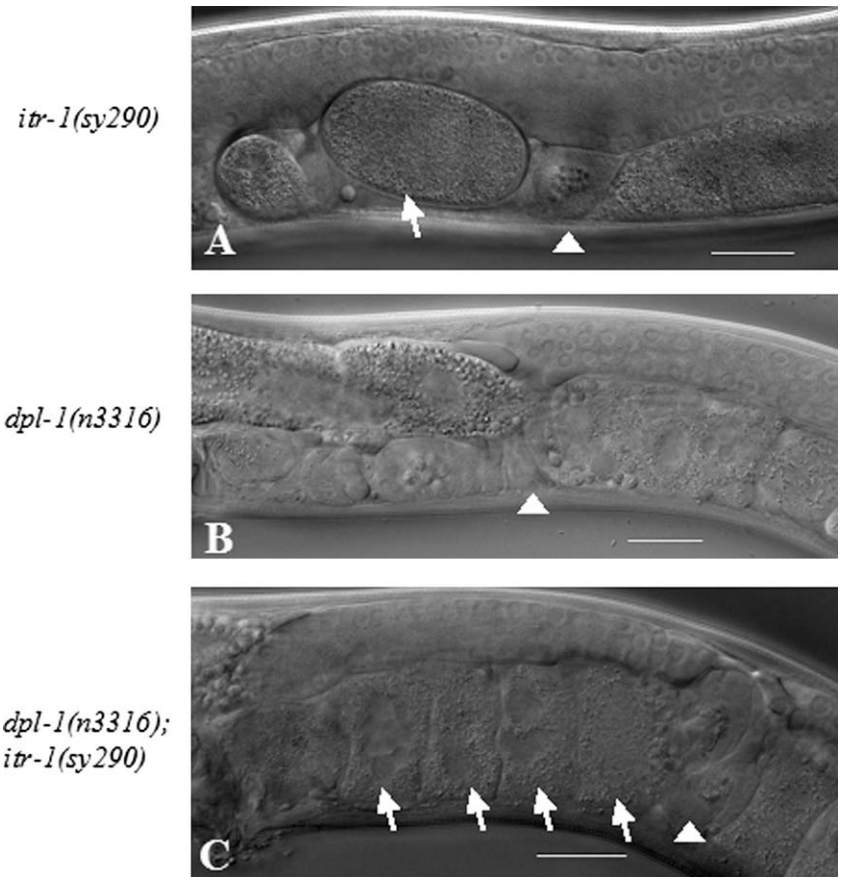

Fig. 2 – itr-1(gf) rescues dpl-1(n3316) ovulation but not fertilization. Representative DIC images of the proximal gonad. Arrowhead indicates spermatheca. (A) itr-1(sy290) gain-of-function mutants have normal ovulation and fertilization as indicated by the presence of an embryo (arrow). (B) No oocytes are present in the uterus in dpl-1(n3316) animals. (C) dpl-1; itr-1(gf) double mutants can undergo ovulation, but the oocytes in the uterus are unfertilized (arrows). Scale bar = 20 μm.

severe in *dpl-1(RNAi)* animals than in *dpl-1(n3316)* mutants (Fig. 4A and B). Although *dpl-1(RNAi)* worms possessed developing germ cells and somatic sheath cells and had a normally shaped gonad arm, the proximal end of the gonad was severely disrupted, and the spermatheca and uterus were not identifiable. Both sperm and oocytes were sometimes found floating freely in the body cavity, having presumably escaped from the gonad through structural defects in the spermatheca. The stronger phenotype in *dpl-1(RNAi)* compared to the *dpl-1(n3316)* mutant suggests that maternally supplied DPL-1 from heterozygotes plays a significant role in development of the somatic gonad.

3.4. Gonadal sheath cells and spermatheca develop normally in dpl-1(n3316) mutants

Because our RNAi experiments indicated that DPL-1 may function in somatic gonad formation, we wondered whether subtle defects in either spermatheca or sheath cell structure or function contributed to the defective ovulation phenotype of *dpl-1(n3316)* mutants. We therefore analyzed the development of the spermatheca in more detail in *dpl-1(n3316)* mutants using *efl-7::GFP*, which is expressed in the spermatheca from the L4 stage onward (B. Lin and V.R., unpublished results; Fig. 5A and B). Expression of *efl-7::GFP* in *dpl-1(n3316)* mutants was also limited to the spermatheca and indicated a normal spermatheca morphology (Fig. 5C and D). We also examined the morphology of the somatic gonad by rhodamine-phalloidin staining of actin (Strome, 1986). The arrangement of actin fibers in the spermatheca looked similar between young adult wild type and *dpl-1(n3316)* mutant animals (Fig. 5E and F).

Successful ovulation also requires vigorous, rhythmic contraction of the myo-epithelial sheath cells that surround the germ cells (McCarter et al., 1999). To assess whether the sheath cells develop normally, we analyzed expression of the sheath cell-specific marker, *lim-7::GFP*, which marks sheath cell pairs 1–4, beginning at the L3 stage and into adulthood (Hall et al., 1999). We found that temporal and spatial expression of *lim-7::GFP* in the *dpl-1(n3316)* mutant was similar to that of wild type (Fig. 5G–J). Moreover, the actin fiber morphology appeared normal (Fig. 5K and L). We also analyzed the contractility and integrity of sheath cells in the mutant. Wild type sheath cells contracted at an average rate of 10.69 ± 1.7 per minute ($n = 13$) compared to 10.27 ± 1.8 in *dpl-1(n3316)* ($n = 11$), indicating that the sheath cell functions normally in *dpl-1(n3316)* mutants. The absence of zygotic DPL-1 activity in the *dpl-1(n3316)* mutant therefore does not appear to overtly affect sheath cell or spermatheca function and development, and the maternally provided DPL-1 from

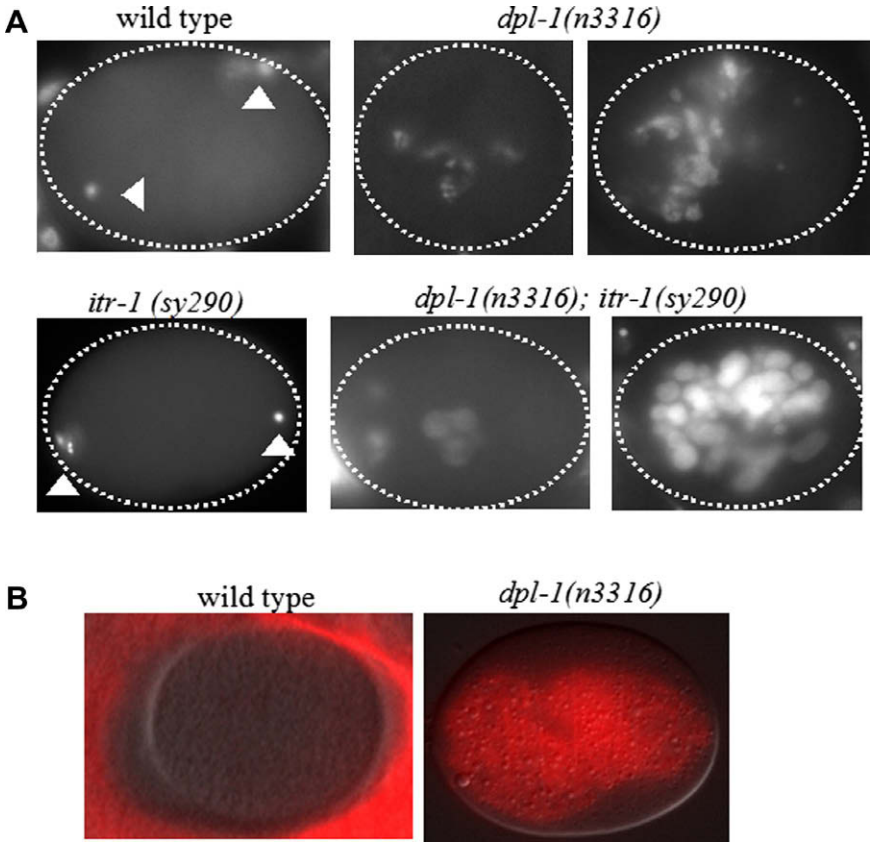

Fig. 3 – *dpl-1(n3316)* mutants have a defect in fertilization. (A) DAPI images of wild type, *dpl-1(n3316)*, *itr-1(sy290)*, and *dpl-1(n3316); itr-1(sy290)* ovulated single-cell embryos and/or oocytes. Pronuclei are clearly visible in wild type and *itr-1* mutants (arrowheads), whereas *dpl-1* and *itr-1; dpl-1* ovulated oocytes are multi-nucleated. Dotted lines indicate eggshell. (B) Incubation of wild type embryos and *dpl-1(n3316)* oocytes in FM464 (red). The dye is excluded from wild type embryos but incorporated by *dpl-1* mutant oocytes, indicating the lack of an eggshell.

heterozygous *dpl-1(n3316)/+* mothers apparently is sufficient to establish proper somatic gonad development.

3.5. *Expression of wild type DPL-1 in germ cells but not the spermatheca rescues the ovulation and fertilization defects of dpl-1 mutants*

Our observations that maternal DPL-1 plays a role in the soma led us to question whether DPL-1 acts in the soma or in the germ line to control ovulation. Previous mosiac studies indicated that EFL-1, the partner of DPL-1, functions in the germ line to promote successful embryogenesis (Page et al., 2001), but these studies utilized a partial loss-of-function *efl-1* mutant that has fairly normal ovulation, leaving open the question of whether either EFL-1 and/or DPL-1 control ovulation in the soma or the germline. We therefore decided to determine the site of action of DPL-1 in ovulation by carrying out tissue-specific transgene rescue experiments. We drove the expression of DPL-1 under either spermatheca-specific (*efl-7* and *sth-1*) or germline-specific (*pie-1*) promoters (Supplemental Figure S2). GFP was fused in frame to DPL-1 to monitor expression in vivo. Injection of *efl-7::gfp:dpl-1* DNA resulted in two extrachromosomal transgenic lines that express GFP:DPL-1 in the nuclei of spermatheca cells, but not in germ cells or sheath cells (Supplemental Figure S2B). *efl-7:: gfp:dpl-1* animals did not exhibit any apparent defects. When *efl-7::gfp:dpl-1* was crossed into the *dpl-1(n3316)* background, the *dpl-1(n3316)* ovulation defects still occurred (Fig. 6A and Table 1).

To independently verify this result, we used a second spermatheca-specific promoter from the *sth-1* gene (Bando et al., 2005) to drive DPL-1 expression. Injection of *sth-1::gfp:dpl-1* DNA also resulted in two extrachromosomal lines, and both lines displayed DPL-1 expression in the nucleus of spermathecal cells (Supplemental Figure S2B). These transgenic worms were fertile and appeared wild type. Similar to the results obtained with *efl-7*-driven GFP:DPL-1, *sth-1*-driven GFP:DPL-1 expression also did not suppress the ovulation defect of *dpl-1(n3316)* mutants (Table 1). Therefore, spermatheca-specific expression of DPL-1 did not improve the ovulation phenotype of *dpl-1(n3316)* mutants.

To test whether DPL-1 functions in the germ line to regulate ovulation, we used the *pie-1* promoter to drive expression of DPL-1 in a germline-specific manner (Mello et al., 1996; Seydoux et al., 1996). Using microparticle bombardment, we obtained one integrated line and one extrachromosomal line, both of which expressed GFP:DPL-1 in the nucleus of germ cells in mitotic and pachytene stages, as well as in oocytes and embryos (Fig. 6B; Supplemental Figure S2D). This expression recapitulates previously published DPL-1 expression patterns determined by antibody staining (Ceol and Horvitz,

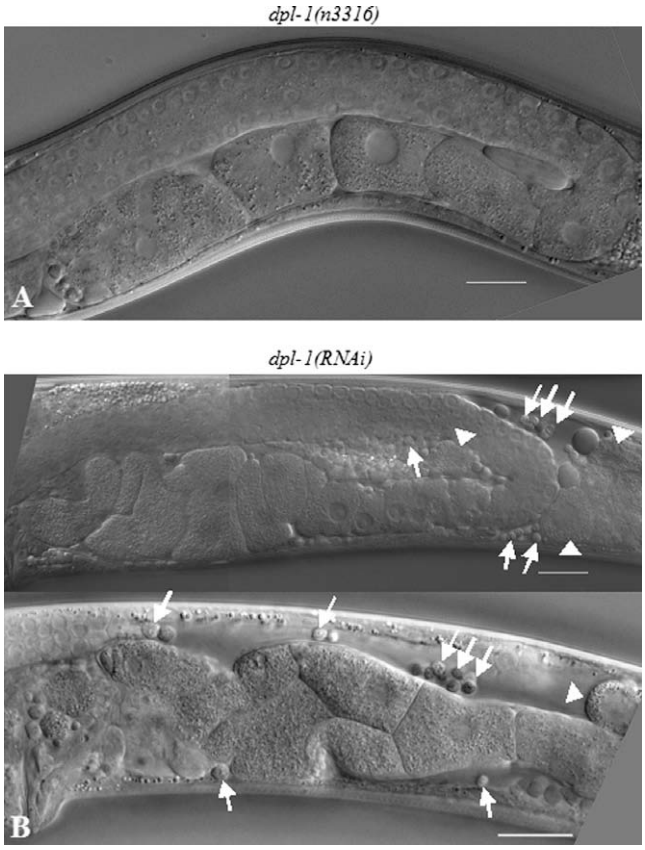

Fig. 4 – *dpl-1(RNAi)* results in structural defects in the somatic gonad. DIC images of (A) a *dpl-1(n3316)* adult hermaphrodite and (B) two representative *dpl-1(RNAi)* adult hermaphrodites, in which the spermatheca and uterus cannot be identified. White arrows indicate ectopically localized sperm, arrowheads indicate ectopic oocytes. Scale bar = 20 μm.

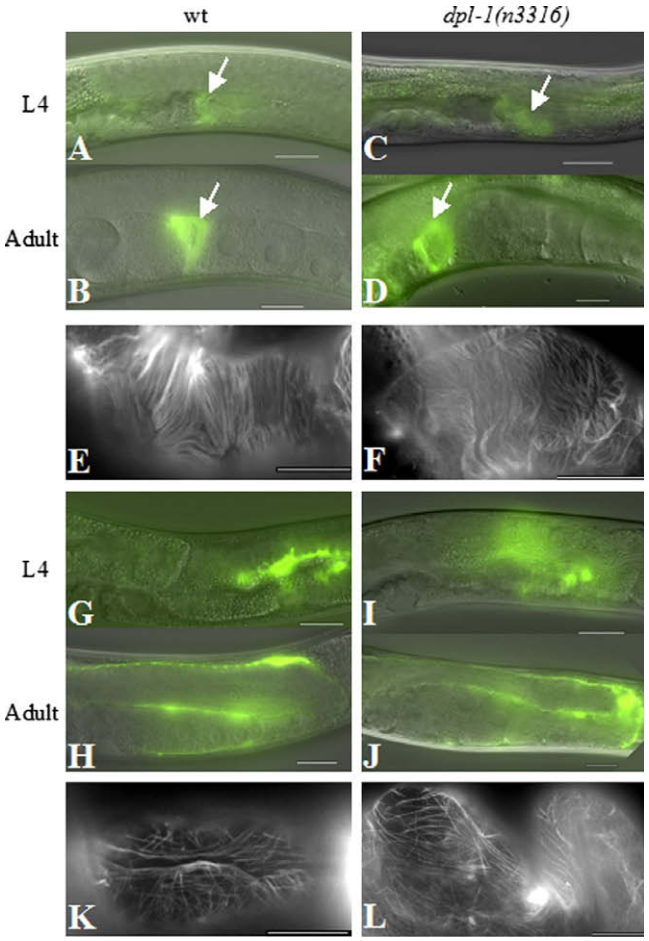

Fig. 5 – Somatic gonad development is normal in *dpl-1(n3316)* mutants. *efl-7::GFP* expression is apparent in the developing spermatheca while *lim-7::GFP* marks the somatic sheath cells surrounding the germ line. Low-level autofluorescence can also be seen in the intestine. (A–D) The onset of *efl-7::GFP* expression in the spermatheca in wild type (A, B) and *dpl-1(n3316)* (C, D) is similar; white arrows indicate spermatheca. (E–F) Rhodamine phalloidin staining of actin and myosin filament arrays in the spermatheca of wild type (E) and *dpl-1(n3316)* mutants (F). (G–J) *dpl-1(n3316)* animals display normal expression of the sheath cell marker *lim-7::GFP*. Wild type (G,H) and *dpl-1(n3316)* (I, J) sheath cells cover both distal and proximal gonad. (K, L) Rhodamine phalloidin staining of the sheath cells of wild type (K) and *dpl-1(n3316)* mutants (L). Scale bar = 20 μm.

2001). DPL-1 was also expressed in the nuclei of early embryos. When this transgene was crossed into the *dpl-1(n3316)* background, expression of GFP:DPL-1 in germ cells was sufficient to rescue ovulation in 100% of the worms ($n = 21$). Rescue was specific to the presence of the transgene, as sibling worms that did not carry the transgene exhibited the severe ovulation defect of *dpl-1(n3316)* mutants (Fig. 6C). However, we found that 16 of the 21 rescued worms still contained a few torn oocytes in the proximal gonad, suggesting that the rescue was not complete (Table 1).

Notably, the ovulated oocytes in *dpl-1(n3316)* worms carrying *pie-1*::GFP:DPL-1 were fertilized, and the resulting embryos underwent normal cell divisions. Thus, expression of DPL-1 in the germ line is sufficient to rescue both the ovulation and fertilization defects of *dpl-1(n3316)* mutants.

3.6. *The EFL-1/DPL-1-responsive gene rme-2 is required in oocytes for proper ovulation*

Previously, we have shown that DPL-1 acts with its partner EFL-1 to promote expression of a battery of genes that are likely to function in oocyte and embryo development (Chi and Reinke, 2006). We hypothesized that EFL-1/DPL-1 functions in the germ line to regulate one or more downstream target genes required for ovulation. One well-validated EFL-1/DPL-1-responsive gene with a likely role in ovulation is *rme-2*. RME-2 is a member of the LDL receptor superfamily that mediates yolk endocytosis and fatty acid transport into oocytes (Grant and Hirsh, 1999). RME-2 is specifically localized to the oocyte membrane and is not detected in the spermatheca or sheath cells (Grant and Hirsh, 1999). *rme-2* loss-of-function mutants display ovulation defects, including endomitotic and torn oocytes in the proximal gonad (Fig. 7; Grant and Hirsh, 1999), although these defects are less severe than those seen in *dpl-1(n3316)* mutants. We wished to test

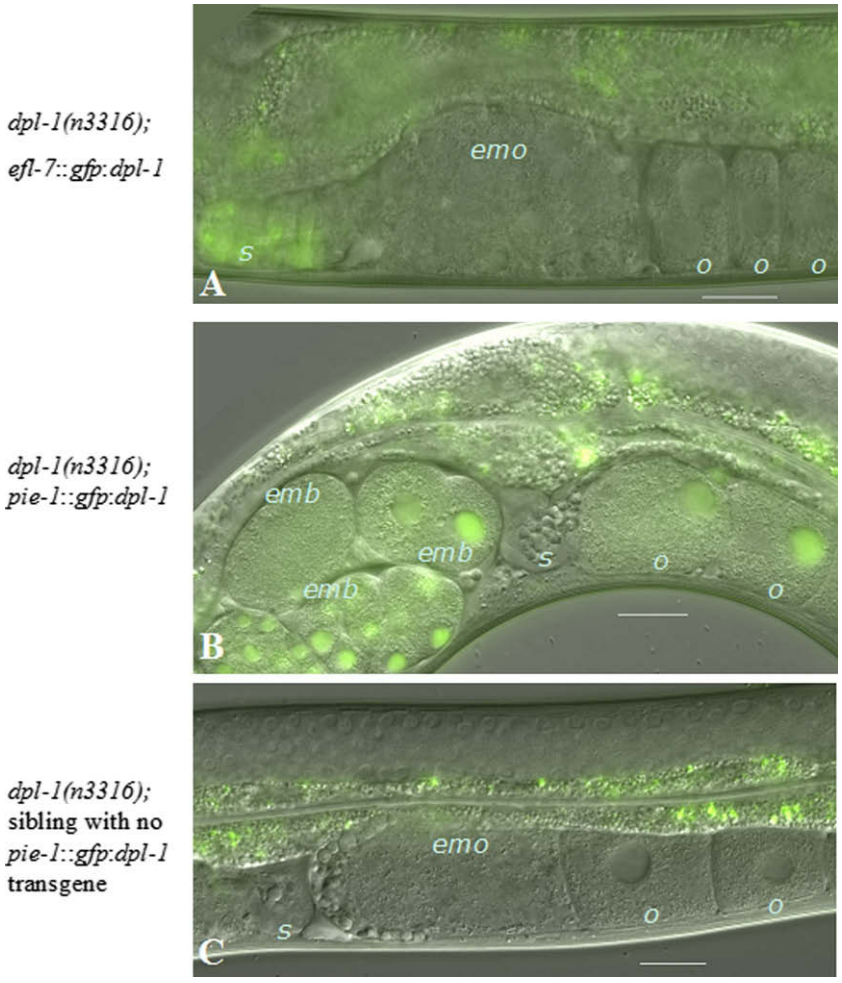

Fig. 6 – Germline-specific expression of DPL-1 rescues ovulation and fertilization. Expression of GFP:DPL-1 (green) using spermtheca-specific and germline-specific promoters in *dpl-1(n3316)* mutants. Low-level autofluorescence can also be seen in the intestine. emb, developing embryos in the uterus; s, spermatheca; o, oocytes; emb, embryos; emo, endomitosis. (A) DPL-1 expression in spermatheca does not suppress ovulation; oocytes remain in the gonad arm and enter endomitosis. (B) *dpl-1(n3316)* mutants expressing GFP:DPL-1 in the germ line have normal ovulation and fertilization, as indicated by presence of GFP+ developing embryos in the uterus. (C) *dpl-1(n3316)* mutant siblings that do not carry the GFP:DPL-1 transgene remain defective in ovulation and are sterile. Scale bar = 20 μm.

Table 1 – Assessment of ovulation and fertilization in strains used in this study.

Genotype	Oocytes trapped in proximal gonad	Fragmented oocytes	Fertilization defects
wild type (N2)	0/30 (0%)	0/30 (0%)	0/30 (0%)
itr-1(sy290)	0/23 (0%)	0/23 (0%)	0/23 (0%)
rme-2(b1008)	0/21 (0%)	16/21 (76.2%)	6/21 (28.6%)
dpl-1(n3316)	54/60 (90%)	N/D	60/60 (100%)
dpl-1(n3316); itr-1(sy290)	3/24 (12.5%)	10/24 (41.6%)	24/24 (100%)
dpl-1(n3316); pie-1::rme-2	16/51 (31.3%)	45/51 (88.2%)	51/51 (100%)
rme-2(b1008); itr-1(sy290)	0/18 (0%)	4/18 (22.2%)	0/18 (0%)
dpl-1(n3316); efl-7::gfp:dpl-1	22/26 (84.6%)	N/D	26/26 (100%)
dpl-1(n3316); sth-1::gfp:dpl-1	35/42 (83.3%)	N/D	42/42 (100%)
dpl-1(n3316); pie-1::gfp:dpl-1	0/21 (0%)	5/21 (33.8%)	0/21 (0%)

Ovulation and fertilization were examined using DIC microscopy at 400× magnification. The presence of endomitotic, fragmented, or degenerating oocytes in the proximal gonad and lack of oocytes/embryos in the uterus indicated failed ovulation. Fertilization was assessed by monitoring the presence of developing embryos (two-cell stage or beyond) and eggshell formation. N/D = not determined.

whether additional EFL-1/DPL-1 responsive genes might be required for ovulation as well.

In order to identify additional candidate ovulation gene(s), we performed an RNAi screen examining whether any of 66

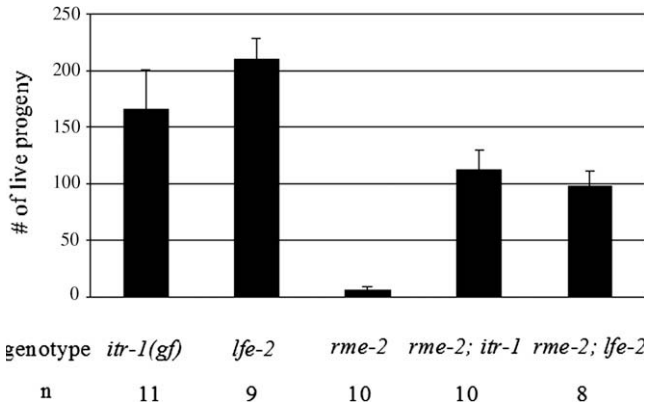

Fig. 7 – *rme-2* brood size is partially suppressed by *itr-1(gf)* or *lfe-2(lf)*. Total number of live progeny from each mutant were assessed. *itr-1* and *lfe-2* mutants were fertile and produce over 150 progeny. *rme-2* mutants have ovulation defects and produce less than 10 live progeny, along with damaged embryos. When *itr-1(gf)* or *lfe-2* mutations were introduced into the *rme-2* mutant background to increase IP3 sensitivity and intracellular calcium release in the spermatheca, the brood size increased to ~100.

genes that were down-regulated in the *dpl-1* mutant gonad have a role in ovulation (see Section 2.4). During a 1-h interval, vector-fed control worms produced an average of 8.8 eggs per worm ($n = 40$ worms). Of the 66 candidates, we found that RNAi of *rme-2, rnr-2, cyb-3,* or *tbb-2* consistently displayed a reduction in ovulation rates (<70% of control rates). However, RNAi of *rnr-2, cyb-3,* and *tbb-2* caused defects in germ cell development or proliferation at stages prior to oogenesis, making their effect on ovulation impossible to analyze. Thus, *rme-2* was the only gene tested with a detectable contribution to the *dpl-1(n3316)* ovulation phenotype. Possibly, the egg-laying assay was not sufficiently sensitive to identify candidate genes with only minor effects on ovulation. We therefore decided to test whether *rme-2* was the critical component downstream of EFL-1/DPL-1 in ovulation.

To first determine whether defective signaling from the oocyte might contribute to the ovulation phenotype in *rme-2(b1008)*, as seen in *dpl-1(n3316)* mutants, we crossed *rme-2(b1008)* with either *itr-1(sy290gf)* or *lfe-2(sy326)* mutants, both of which permit spermatheca dilation in the absence of a signal from the oocyte (Clandinin et al., 1998). A mutation in *itr-1* or *lfe-2* greatly increased the brood size of *rme-2* mutants, from 5.2 progeny/parent to either 165 or 210, respectively (Fig. 7). Therefore, like DPL-1, RME-2 acts upstream of the *itr-1* and *lfe-2* pathway that regulates spermatheca dilation by IP3 signaling. Our results suggest that much of the phenotype of *rme-2* mutants can be explained by oocyte defects that affect ovulation, because once ovulated, *rme-2* mutant oocytes can be fertilized, undergo embryonic development and hatch into viable larvae.

To see whether the *dpl-1(n3316)* ovulation defects could be rescued by forced expression of RME-2, we mated animals expressing *pie-1*-driven RME-2:GFP (Kadandale et al., 2005) into the *dpl-1* background. The percentage of *dpl-1(n3316)* animals with accumulated oocytes in the proximal gonad, indicative of ovulation defects, dropped from 90% to 31.3% when RME-2:GFP was expressed ($n > 50$; Table 1). This result indicates that lack of RME-2 activity in the oocytes of *dpl-1* mutants is a major cause of ovulation failure. Although *rme-2* appears to be the major EFL-1/DPL-1-responsive gene for controlling ovulation, the incomplete rescue combined with a milder ovulation phenotype in *rme-2* null mutants suggests that additional EFL-1/DPL-1-responsive genes are necessary for ovulation.

4. DISCUSSION

Gametes must be brought together only upon completion of differentiation and maturation, and not before, in order for fertilization to be successful. To coordinate these events, multiple signals pass between sperm, oocytes, and the somatic gonad. In *C. elegans*, immature oocytes suppress sheath cell contractions (Miller et al., 2003) and send a signal made of polyunsaturated fatty acids to attract sperm to the spermatheca (Kubagawa et al., 2006). In turn, sperm indicate their presence to the oocyte via a signal composed of the small protein MSP, which triggers oocyte maturation and releases the block to sheath cell contraction (Miller et al., 2001, 2003). Mature oocytes then send a signal through an EGF (LIN-3) pathway to the somatic gonad to cause dilation of the distal spermatheca (Clandinin et al., 1998; McCarter et al., 1999). To better understand how a single transcription factor can control complex developmental and signaling events during oogenesis and ovulation through the regulation of a set of tissue-specific genes, we investigated the underlying mechanisms by which the E2F transcription factor EFL-1/DPL-1 is required for proper ovulation. Our results define where E2F functions and demonstrate that it acts through one critical target gene to promote ovulation.

4.1. *DPL-1 primarily functions in the germ line to regulate ovulation*

The earliest defect that we could find in *dpl-1(n3316)* and *efl-1(n3639)* mutants was premature and incomplete spermatheca dilation. This observation, along with the observation that *dpl-1(RNAi)* resulted in aberrant spermatheca development and morphology, suggested that *dpl-1* might function in the somatic gonad. However, we did not find any visible defects in the spermatheca in *dpl-1(n3316)* mutants. Additionally, we demonstrated that proper spermatheca function could be restored in *dpl-1* mutants if the requirement for the pro-ovulatory signal from the oocyte is bypassed by activating IP3 signaling in the spermatheca. Finally, we found that expressing DPL-1 in the germ line is sufficient to restore ovulation in *dpl-1* mutants, whereas expression of DPL-1 in the spermatheca is not. Based on our data and that of Page et al. (2001) which indicates that *efl-1* acts in the germ line, we conclude that the EFL-1/DPL-1 heterodimer primarily acts in the germ line to control ovulation. Moreover, our results indicate that *dpl-1* mutant oocytes fail to signal appropriately to the surrounding somatic gonad to initiate ovulation.

Although the predominant action of DPL-1 to regulate ovulation occurs in the germ line, at least a minor contribution of DPL-1 in the somatic gonad cannot be absolutely ruled out. In

dpl-1(RNAi) animals, the spermatheca shows significant structural defects that permit gametes to escape the proximal gonad and float freely in the body cavity. This defect is not detectable in *dpl-1(n3316)* mutants, probably because maternal *dpl-1* is sufficient to ensure proper spermatheca development. Indeed, we found that the spermatheca and sheath cells are formed normally in *dpl-1(n3316)* mutants. However, a subtle functional defect in the spermatheca could still exist, although it escaped our detection. Indeed, rescue of the ovulation defects by expressing DPL-1 in the germ line, while very extensive, is not complete. Possibly, DPL-1 is not expressed at the right level because of the use of the heterologous *pie-1* promoter, or defects are present outside of the germ line, possibly in the somatic gonad.

4.2. *RME-2 is a key regulator of ovulation downstream of DPL-1*

One of the most striking observations in our studies is that a single EFL-1/DPL-1-responsive gene, *rme-2*, plays a key role in mediating ovulation. RME-2, a yolk receptor expressed in oocytes, is required for proper ovulation (Grant and Hirsh, 1999), and its expression in the germ line is sufficient to extensively rescue the ovulation defects of *dpl-1(n3316)* mutants. However, the rescue was not complete, suggesting that either RME-2 driven by the *pie-1* promoter is not expressed at wild type levels, or that other targets of EFL-1/DPL-1 provide minor supporting roles to RME-2 in this process.

How could a yolk receptor be playing a role in ovulation? One possibility is simply that yolk accumulation is required for appropriate oocyte maturation, and that immature oocytes do not trigger ovulation because they fail to reach the stage where they can signal to the spermatheca. However, *dpl-1* mutant oocytes, which lack detectable RME-2, still express the signal LIN-3 on their membrane surface (Chi and Reinke, 2006). This observation suggests that *dpl-1* mutants can still produce the known pro-ovulatory signal, although LIN-3 might not be processed appropriately to act as a signal, despite its presence at the cell surface. Alternatively, a LIN-3-independent ovulation signal could be missing in *dpl-1* mutants. Similar to LIN-3, RME-2 is a surface protein that has EGF repeats that could be used as an ovulatory signal from the oocyte. Another possibility is that the yolk taken up by RME-2 is processed to generate a second signal. Yolk is processed into polyunsaturated fatty acids that are then used as a sperm attractant (Kubagawa et al., 2006). Other breakdown products such as monounsaturated fatty acids could potentially act as signals for other processes, such as ovulation. Using a common precursor such as yolk to generate multiple signals is an advantageous way to coordinate the diverse events that must occur in a time-appropriate manner to ensure that ovulation occurs only when oocytes have undergone maturation and sperm are present.

4.3. *DPL-1 promotes fertilization by regulating multiple egg genes*

Our results demonstrate that DPL-1 also has an important role in fertilization, since oocytes that lack DPL-1 activity are unable to be fertilized, even if ovulation is successful. Surprisingly few oocyte-specific genes required for fertilization have been identified in any organism. In *C. elegans*, the only identified oocyte factors required for fertilization are the LDL repeat-containing proteins EGG-1 and EGG-2, which are present on the oocyte plasma membrane and act redundantly (Kadandale et al., 2005). EGG-3, an anti-phosphatase, is required in the oocyte for egg activation immediately following fertilization (Maruyama et al., 2007). We have previously found that *egg-2* expression is dependent on EFL-1/DPL-1 (Chi and Reinke, 2006). Another EFL-1/DPL-1-regulated gene that might be important for fertilization or egg activation is T21E3.1, a protein tyrosine phosphatase that has recently been named *egg-4*, although no function has been currently assigned to this gene (http://www.wormbase.org). An essentially identical gene R12E2.10 (*egg-5*) is likely functionally redundant with *egg-4*, because mutation of either gene singly does not result in sterility (http://www.wormbase.org; unpublished data), but we found that RNAi targeting both gene products results in failure of one or more developmental events occurring during or immediately after fertilization (V.R. and W.C., unpublished data). The presence of canonical E2F binding sites in the *egg-4* and *egg-5* promoters, and reduced expression of *egg-4* in *efl-1* or *dpl-1* mutants (Chi and Reinke, 2006) suggests that EFL-1/DPL-1 promotes their expression in the germ line. Thus, EFL-1/DPL-1 likely regulates multiple genes required for fertilization and/or egg activation in the oocyte. Functional analysis of the currently untested EFL-1/DPL-1-responsive genes should identify additional candidate fertilization factors.

5. Conclusions

Our studies trace the path that the E2F transcription factor utilizes to control multiple events in the proximal gonad, by dissecting the proximate causes of the mutant phenotype, and assigning function to one of the key target genes. Placing multiple developmental processes under the control of a common regulator, in this case E2F, ensures that their execution is properly coordinated both spatially and temporally.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.mod.2009.01.008.

REFERENCES

Bando, T., Ikeda, T., Kagawa, H., 2005. The homeoproteins MAB-18 and CEH-14 insulate the dauer collagen gene *col-43* from

activation by the adjacent promoter of the spermatheca gene *sth-1* in *Caenorhabditis elegans*. J. Mol. Biol. 348, 101–112.
Bui, Y.K., Sternberg, P.W., 2002. *Caenorhabditis elegans* inositol 5-phosphatase homolog negatively regulates inositol 1, 4, 5-triphosphate signaling in ovulation. Mol. Biol. Cell 13, 1641–1651.
Ceol, C.J., Horvitz, H.R., 2001. Dpl-1 DP and efl-1 E2F act with lin-35 Rb to antagonize Ras signaling in *C. elegans* vulval development. Mol. Cell 7, 461–473.
Chi, W., Reinke, V., 2006. Promotion of oogenesis and embryogenesis in the *C. elegans* gonad by EFL-1/DPL-1 (E2F) does not require LIN-35 (pRB). Development 133, 3147–3157.
Clandinin, T.R., DeModena, J.A., Sternberg, P.W., 1998. Inositol triphosphate mediates a RAS-independent response to LET-23 receptor tyrosine kinase activation in *C. elegans*. Cell 92, 523–533.
Corrigan, C., Subramanian, R., Miller, M.A., 2005. Eph and NMDA receptors control Ca2+/calmodulin-dependent protein kinase II activation during *C. Elegans* oocyte meiotic maturation. Development 132, 5225–5237.
Eppig, J.J., Wigglesworth, K., Pendola, F.L., 2002. The mammalian oocyte orchestrates the rate of ovarian follicular development. Proc. Natl. Acad. Sci. USA. 99, 2890–2894.
Gilchrist, R.B., Ritter, L.J., Myllymaa, S., Kaivo-Oja, N., Dragovic, R.A., Hickey, T.E., Ritvos, O., Mottershead, D.G., 2006. Molecular basis of oocyte-paracrine signalling that promotes granulosa cell proliferation. J. Cell Sci. 119, 3811–3821.
Govindan, J.A., Cheng, H., Harris, J.E., Greenstein, D., 2006. Galphao/i and Galphas signaling function in parallel with the MSP/Eph receptor to control meiotic diapause in *C. Elegans*. Curr. Biol. 16, 1257–1268.
Grant, B., Hirsh, D., 1999. Receptor-mediated endocytosis in the *Caenorhabditis elegans* oocyte. Mol. Biol. Cell 10, 4311–4326.
Greenstein, D., 2005. Control of Oocyte Meiotic Maturation and Fertilization. In: WormBook, 28 December 2005. pp. 1–12.
Hall, D.H., Winfrey, V.P., Blaeuer, G., Hoffman, L.H., Furuta, T., Rose, K.L., Hobert, O., Greenstein, D., 1999. Ultrastructural features of the adult hermaphrodite gonad of *Caenorhabditis elegans*: relations between the germ line and soma. Dev. Biol. 212, 101–123.
Hobert, O., 2002. PCR fusion-based approach to create reporter gene constructs for expression analysis in transgenic *C. elegans*. Biotechniques 32, 728–730.
Kadandale, P., Stewart-Michaelis, A., Gordon, S., Rubin, J., Klancer, R., Schweinsberg, P., Grant, B.D., Singson, A., 2005. The egg surface LDL receptor repeat-containing proteins EGG-1 and EGG-2 are required for fertilization in *Caenorhabditis elegans*. Curr. Biol. 15, 2222–2229.
Kamath, R.S., Fraser, A.G., Dong, Y., Poulin, G., Durbin, R., Gotta, M., Kanapin, A., Le Bot, N., Moreno, S., Sohrmann, M., Welchman, D.P., Zipperlen, P., Ahringer, J., 2003. Systematic functional analysis of the *Caenorhabditis elegans* genome using RNAi. Nature 421, 231–237.
Kidder, G.M., Mhawi, A.A., 2002. Gap junctions and mammalian folliculogenesis. Reproduction 123, 613–620.
Kubagawa, H.M., Watts, J.L., Corrigan, C., Edmonds, J.W., Sztul, E., Browse, J., Miller, M.A., 2006. Oocyte signals derived from polyunsaturated fatty acids control sperm recruitment in vivo. Nat. Cell Biol. 8, 1143–1148.
Maruyama, R., Velarde, N.V., Klancer, R., Gordon, S., Kadandale, P., Parry, J.M., Hang, J.S., Rubin, J., Stewart-Michaelis, A., Schweinsberg, P., Grant, B.D., Piano, F., Sugimoto, A., Singson, A., 2007. EGG-3 regulates cell-surface and cortex rearrangements during egg activation in *Caenorhabditis elegans*. Curr. Biol. 17, 1555–1560.
Matzuk, M.M., Burns, K.H., Viveiros, M.M., Eppig, J.J., 2002. Intercellular communication in the mammalian ovary: oocytes carry the conversation. Science 296, 2178–2180.
McCarter, J., Bartlett, B., Dang, T., Schedl, T., 1997. Soma-germ cell interactions in Caenorhabditis elegans: multiple events of hermaphrodite germline development require the somatic sheath and spermathecal lineages. Dev. Biol. 181, 121–143.
McCarter, J., Bartlett, B., Dang, T., Schedl, T., 1999. On the control of oocyte meiotic maturation and ovulation in *Caenorhabditis elegans*. Dev. Biol. 205, 111–128.
Mello, C.C., Kramer, J.M., Stinchcomb, D., Ambros, V., 1991. Efficient gene transfer in *C. elegans*: extrachromosomal maintenance and integration of transforming sequences. EMBO J. 10, 3959–3970.
Mello, C.C., Schubert, C., Draper, B., Zhang, W., Lobel, R., Priess, J.R., 1996. The PIE-1 protein and germline specification in *C. elegans* embryos. Nature 382, 710–712.
Miller, M.A., Nguyen, V.Q., Lee, M.H., Kosinski, M., Schedl, T., Caprioli, R.M., Greenstein, D., 2001. A sperm cytoskeletal protein that signals oocyte meiotic maturation and ovulation. Science 291, 2144–2147.
Miller, M.A., Ruest, P.J., Kosinski, M., Hanks, S.K., Greenstein, D., 2003. An Eph receptor sperm-sensing control mechanism for oocyte meiotic maturation in *Caenorhabditis elegans*. Genes Dev. 17, 187–200.
Page, B.D., Guedes, S., Waring, D., Priess, J.R., 2001. The *C. elegans* E2F- and DP-related proteins are required for embryonic asymmetry and negatively regulate Ras/MAPK signaling. Mol. Cell 7, 451–560.
Praitis, V., Casey, E., Collar, D., Austin, J., 2001. Creation of low-copy integrated transgenic lines in *Caenorhabditis elegans*. Genetics 157, 1217–1226.
Rappleye, C.A., Paredez, A.R., Smith, C.W., McDonald, K.L., Aroian, R.V., 1999. The coronin-like protein POD-1 is required for anterior–posterior axis formation and cellular architecture in the nematode *Caenorhabditis elegans*. Genes Dev. 13, 2838–2851.
Reese, K.J., Dunn, M.A., Waddle, J.A., Seydoux, G., 2000. Asymmetric segregation of PIE-1 in *C. elegans* is mediated by two complementary mechanisms that act through separate PIE-1 protein domains. Mol. Cell 6, 445–455.
Seydoux, G., Mello, C.C., Pettitt, J., Wood, W.B., Priess, J.R., Fire, A., 1996. Repression of gene expression in the embryonic germ lineage of *C. elegans*. Nature 382, 713–716.
Strome, S., 1986. Fluorescence visualization of the distribution of microfilaments in gonads and early embryos of the nematode *Caenorhabditis elegans*. J. Cell Biol. 103, 2241–2252.
Sulston, J., Hodgkin, J., 1988. Methods. In: Wood, W.B. (Ed.), The Nematode Caenorhabditis elegans. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 587–606.
Whitten, S.J., Miller, M.A., 2007. The role of gap junctions in *Caenorhabditis elegans* oocyte maturation and fertilization. Dev. Biol. 301, 432–446.