The CIL-1 PI 5-Phosphatase Localizes TRP Polycystins to Cilia and Activates Sperm in C. elegans

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Summary

Background: C. elegans male sexual behaviors include chemotaxis and response to hermaphrodites, backing, turning, vulva location, spicule insertion, and sperm transfer, culminating in cross-fertilization of hermaphrodite oocytes with male sperm. The LOV-1 and PKD-2 transient receptor potential polycystin (TRPP) complex localizes to ciliated endings of C. elegans male-specific sensory neurons and mediates several aspects of male mating behavior. TRPP complex ciliary localization and sensory function are evolutionarily conserved. A genetic screen for C. elegans mutants with PKD-2 ciliary localization (Cil) defects led to the isolation of a mutation in the cil-1 gene.

Results: Here, we report that a phosphoinositide (PI) 5-phosphatase, CIL-1, regulates TRPP complex ciliary receptor localization and sperm activation. cil-1 does not regulate the localization of other ciliary proteins, including intraflagellar transport (IFT) components, sensory receptors, or other TRP channels in different cell types. Rather, cil-1 specifically controls TRPP complex trafficking in male-specific sensory neurons and does so in a cell-autonomous fashion. In these cells, cil-1 is required for normal PI(3)P distribution, indicating that a balance between PI(3,5)P2 and PI(3)P is important for TRPP localization. cil-1 mutants are infertile because of sperm activation and motility defects. In sperm, the CIL-1 5-phosphatase and a wortmannin-sensitive PI 3-kinase act antagonistically to regulate the conversion of sessile spermatids into motile spermatzoa, implicating PI(3,4,5)P3 signaling in nematode sperm activation.

Conclusion: Our studies identify the CIL-1 5-phosphatase as a key regulator of PI metabolism in cell types that are important in several aspects of male reproductive biology.

Introduction

Phosphoinositides (PIs) and their phosphatases and kinases play pivotal roles in receptor trafficking as well as in membrane organelle biogenesis and transport, endocytosis, cytoskeleton dynamics, signal transduction, cell motility, and channel activity modulation [1, 2]. Many sensory receptors localize to ciliary membrane, which serve as cellular antennae and function in development, signaling, and physiology. Whether PIs and PI-generating enzymes regulate ciliary receptor trafficking is unknown.

The nematode Caenorhabditis elegans is a powerful model for the study of molecular mechanisms required for ciliary receptor trafficking. The C. elegans transient receptor potential polycystin (TRPP) complex proteins LOV-1 (TRPP1) and PKD-2 (TRPP2) localize to sensory cilia [3, 4]. Autosomal-dominant polycystic kidney disease (ADPKD) is caused by mutations in the TRPP1 and TRPP2 genes [5]. Since the discovery of LOV-1 and PKD-2 in cilia of C. elegans male-specific sensory neurons, TRPPs have been found in primary cilia or flagella in organisms ranging from alga to man, hinting at an evolutionarily conserved mechanism regulating TRPP ciliary localization (reviewed in [6]).

In diverse species, TRPP family proteins serve reproductive functions [7–12]. C. elegans LOV-1 and PKD-2 function in male mating behaviors. pkd-2 appears to be expressed solely in the male nervous system and not sperm, as judged by antibody staining [4]. In contrast to mammalian and Drosophila sperm, nematode sperm are not flagellated and do not possess an actin or tubulin cytoskeleton (reviewed in [13]). Instead, each sperm extends a single pseudopod enriched with major sperm protein (MSP). MSP is used to assemble a filamentous network required for amoeboid motility and fertilization. Screens have identified genes required for spermatogenesis, hermaphrodite or male sperm activation, and sperm function. Signaling pathways involved in sperm activation in both males and hermaphrodites are not known.

PKD-2 ciliary localization requires vesicular trafficking and utilizes both general and cell-type-specific factors [14]. General factors include the clathrin-coated vesicle adaptor protein-1 (AP-1) UNC-101 and the intraflagellar transport (IFT) machinery. Cell-type-specific PKD-2 localization factors include LOV-1 and the STAM-Hrs (signal-transducing adaptor molecule and hepatocyte growth factor-regulated tyrosine kinase substrate) complex [14, 15]. We performed a genetic screen for C. elegans mutants with PKD-2 ciliary localization (Cil) defects [16]. Here, we identify CIL-1, a PI 5-phosphatase that regulates TRPP complex localization. cil-1 is also a positive regulator of sperm activation and motility. Using genetically encoded PI-indicators and pharmacological approaches, we determine that CIL-1 hydrolyses PI(3,5)P2 and PI(3,4,5)P3 in male-specific sensory neurons and sperm, respectively. We conclude that CIL-1 acts in multiple tissues that are important for male reproductive biology, thereby controlling diverse cellular processes as an in vivo PI-metabolizing enzyme.

Results

cil-1 Is Required for LOV-1 and PKD-2 Localization

In wild-type (WT), PKD-2::GFP localizes to cell bodies and ciliary endings of 21 male-specific neurons in the head (cephalic CEMs) and tail (ray RnBs and hook HOB) ([14], Figures 1A–1D). In my15 mutants, PKD-2::GFP is distributed throughout these male-specific neurons including dendrites, axons, cell bodies, and cilia (Figures 1E and 1F). cil-1 is not required for neuronal cell fate or development of pkd-2-expressing...
neurons as judged by reporters including transcriptional and soluble Ppkd-2::GFP, OSM-6::GFP (data not shown), and fluorescent-protein-tagged PI-markers (Figure S4). Ppkd-2::GFP and endogenous pkd-2 mRNA levels are unaltered in my15 animals as judged by qRT-PCR (data not shown), indicating that my15 may affect PKD-2 protein expression or stability but not gene expression. In WT and my15 dendrites, small PKD-2::GFP particles move bidirectionally (Movies S1 and S2), indicating that a pool of PKD-2::GFP is properly trafficked in my15 dendrites. Moreover, PKD-2::GFP distribution in the my15 ciliary region appears normal, despite abnormally increased dendritic and axonal distributions (Figures 1E and 1F, [16]).

We examined the distribution of additional GFP-tagged ciliary proteins, including functional LOV-1::GFP ([15], Figures S1A–S1D); a TRP-panvillloid, OSM-9; and a G protein-coupled receptor (GPCR), ODR-10; an IFT B-complex polypeptide, OSM-6; and an IFT modulator, BBS-5. Only LOV-1::GFP is abnormally distributed to dendritic and axonal processes in my15 sensory neurons (Figures S1C and S1D).

We also examined the localization of the presynaptic marker synaptobrevin Ppkd-2::SNB-1::GFP [17]. In WT and my15 males, SNB-1::GFP labels presynaptic puncta along axonal processes (Figures S1E and S1F), indicating that cil-1 does not grossly affect axonal targeting. my15 mutants are normal in lipophilic Dil dye filling of ciliated sensory neurons, chemotaxis to diacetyl, dauer formation, and osmotic avoidance (data not shown). We conclude that cil-1 is specifically required for localization of the TRPP complex but not for general receptor trafficking, ciliogenesis, neuronal polarization, or sensation.

lov-1, pkd-2, and a subset of Cil mutants are response and location of vulva (Lov) defective during male mating [3, 4, 16]. my15 males exhibit normal response and vulva location behaviors (Figure S1, [16]), which may be explained by the presence of Ppkd-2::GFP in cilia. cil-1 double- or triple-homozygous or transheterozygous mutants with pkd-2 and lov-1 did not alter male mating efficiency, indicating that cil-1 is not a genetic modifier of the C. elegans TRPP genes (data...
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not shown). Although mating behaviors appear normal, my15 males are largely infertile and produce few offspring because of a sperm (Spe) defect, which will be discussed later.

cil-1 Acts between lov-1 and stam-1 in RnB Ray Neurons
We previously showed that TRPP complex formation is important for trafficking [14]. In a lov-1 mutant, PKD-2::GFP forms aggregates in cell bodies and localizes to cilia at a reduced level (Figures 1G and 1H). In lov-1; cil-1 CEM neurons (Figure 1I), the PKD-2::GFP localization phenotype is additive: bright aggregates in the cell bodies (lov-1 phenotype; white arrows) and mislocalization to the dendrites and axons (cil-l phenotype), albeit at reduced levels (red arrows). In lov-1; cil-1 RnB neurons (Figure 1J), the PKD-2::GFP localization phenotype resembles that of lov-1 but not cil-1: aggregates in the cell body and absence from dendritic and ciliary compartments. This strict requirement of lov-1 in RnB as compared to CEM neurons has been previously shown for PKD-2 ciliary targeting [14]. The basis of this cell-type specificity is unknown, but may be due to differences in protein trafficking mechanisms or structural differences between CEM and RnB neurons.

PKD-2 ciliary abundance is tightly controlled. STAM and Hrs mediate PKD-2 and LOV-1 downregulation via transport from early endosomes to endosomal sorting complexes (ESCRT) [15]. Reducing stam-1 or hgrs-1 function results in PKD-2::GFP and LOV-1::GFP accumulation at the ciliary base (Figure 1K and 1L, [15]). In CEM neurons of a stam-1(ok4406); cil-1(my15) double mutant (Figure 1M), the PKD-2::GFP localization phenotype is additive. In stam-1; cil-1 RnB neurons (Figure 1N), PKD-2::GFP is distributed evenly throughout, similar to cil-1 single mutants. We conclude that, in RnB neurons, cil-1 acts after lov-1 but before stam-1. In CEMs, the lov-1; cil-1 and cil-1; stam-1 phenotype is complex, making it difficult to place lov-1, cil-1, and stam-1 in a linear or parallel pathway.

cil-1 Encodes a Phosphoinositide 5-Phosphatase that Acts Cell Autonomously
The smallest rescuing genomic fragment for both the Cil and Spe phenotypes (8160K-1, Figures 2A and 2B) contains two genes, C50C3.7 and bath-42 in the operon CEOP3484. The bath-42(tm2360) deletion mutant is nonCil and nonSpe (data not shown). A construct containing 2.2 kb 5'UTR of the bath-42 and the C50C3.7 genomic region including the 3'UTR rescues both Cil and Spe phenotypes of my15 (PCR-SOEed C50C3.7, Figures 2A and 2B). Sequencing analysis of my15 genomic DNA identified a G/A transition that converts Trp301 (TGG) to a stop codon (TAG) in the 5'th exon of C50C3.7 (Figure 2A). From both WT and my15 cDNA pools, reverse transcriptase PCR (RT-PCR) identified two cil-1 cDNAs (long C50C3.7a and short C50C3.7b). The short form (C50C3.7b) is generated by alternative splicing within the third intron, introducing a stop codon at the 124th amino acid before the my15-induced lesion. Ppkd-2::cil-1(C50C3.7a)::tdTomato fully rescues the cil-1(my15) Cil but not the Spe phenotype (Figures 2A and 2B), which is not surprising given that pkd-2 is not expressed in sperm. In contrast, expression of C50C3.7a with an intestinal promoter fails to rescue the Cil phenotype (data not shown). We did not attempt to rescue the cil-1(my15) Spe phenotype by using cell-type-specific promoters because transgenes are often silenced in the...
germline. We conclude that C50C3.7 is the gene mutated in cil-1(my15) animals and that CIL-1 acts autonomously in male-specific neurons to control TRPP localization.

cil-1 encodes a phosphoinositide (PI) 5-phosphatase (referred to as 5-phosphatase), which removes the D-5 phosphate from the inositol ring of membrane-associated PI or soluble inositol phosphates. The 5-phosphatase family comprises ten mammalian, four yeast, and five C. elegans enzymes. Phylogenetic analysis of the 5-phosphatase catalytic domain and/or of the presence or absence of adjacent domains reveals that CIL-1 is closely associated with two mammalian 5-phosphatases: SKIP (skeletal muscle and kidney-enriched inositol phosphatase) and PIPP (proline-rich inositol polyphosphate phosphatase). CIL-1, SKIP, and PIPP belong to the SKICH (SKIP carboxyl homology) subfamily, which contains a C-terminal SKICH-like domain (Figures S2A and S2D) and mediates protein localization [18].

The C. elegans genome encodes five 5-phosphatase genes: ipp-5, unc-26, ochr-1, cil-1/CS0C3.7, and T25E9.10. ipp-5 (type I) negatively regulates ovulation by inhibiting inositol 1,4,5-triphosphate (IP3) signaling in the spermatheca [19]. IP3 signaling also regulates mating behavior steps of turning, spicule insertion, and sperm transfer [20]. unc-26 (synaptotagmin) is required for synaptic vesicle endocytosis and recycling, with mutants exhibiting uncoordinated movements [21].

Neither the ipp-5 nor the unc-26 mutant is Cil or Spe defective, and cil-1(my15) mutants are normal in ovaulation, locomotion, male turning, spicule insertion, and sperm transfer, ruling out possible overlapping functions (data not shown).

CIL-1 contains two conserved 5-phosphatase motifs in its catalytic domain (Figure S2B). We introduced a missense mutation in a known critical residue of 5-phosphatase motif (CIL-1-N175A, Figure S2B, red arrowhead). Ppkd-2::cil-1-N175A and dtTomato failed to rescue the my15 Cil phenotype, indicating that phosphatase catalytic activity is required for CIL-1 function.

In male neurons, the rescuing Ppkd-2::cil-1::dtTomato is distributed in cilia, dendrites, axons, cell bodies with occasional small puncta, and weakly in nuclei (Figure S3D), and it is often visible as bright dots at ciliary bases of ray neurons (Figure S3E), suggesting CIL-1 function in ciliary regions. In the intestine, Pvh-a-6::cil-1::GFP localizes to cytoplasmic reticular structures (Figure S3F).

CIL-1 Regulates PI(3,4)P2/PI(3,4,5)P3 and PI(3)P but Not PI(4,5)P2 Levels

Seven PI species are generated by the reversible phosphorylation and dephosphorylation. To determine what PI species are CIL-1 substrates, we expressed genetically encoded biosensors to detect changes in specific PI lipid concentrations in male-specific sensory neurons and the intestine. We observed obvious differences in PI(3)P Hrs(2XFYVE) and PI(3,4)P2/PI(3,4,5)P3 AKT(PH), but not PI(4,5)P2 (PH domain of PLC-delta) markers between WT and cil-1(my15) animals. In the WT intestine, PI(3)P is primarily found in tubulovesicular structures without any plasma membrane (PM) enrichment, similar to the CIL-1 distribution pattern (compare Figure 3A with Figure S3F). In the cil-1 intestine, PI(3)P is severely disrupted, with a diffuse pattern in the cytoplasm (Figure 3D). In WT intestine, PI(3,4)P2/PI(3,4,5)P3 is found in tubulovesicular structures and enriched at the PM (Figure 3B, arrowheads and arrow). In cil-1(my15) mutants, the PI(3,4)P2/PI(3,4,5)P3 marker labels no distinct structure, appearing diffuse in the cytoplasm with no PM enrichment. A similar pattern has been reported in C. elegans let-512/vps-34 PI 3-kinase mutants, in which PI(3)P generation is reduced [22]. In both WT and cil-1(my15) intestine, PI(4,5)P2 is enriched at the apical PM lining the intestinal lumen as well as basolateral PM (Figures 3C–3F). These data indicate that CIL-1 displays in vivo phosphatase activity toward PI(3,4,5)P3 and PI(3,5)P2 in the intestine.

In male-specific sensory neurons, we observe differences in PI(3)P distribution (Figures 3G and 3H), but not PI(3,4)P2/PI(3,4,5)P3 or PI(4,5)P2 markers in cil-1(my15) males (Figure S4). In WT neurons, the PI(3)P marker is enriched in nuclei and small puncta in the cell bodies, but rarely in dendritic and ciliary regions (Figures 3G and 3H), with only 1/13 animals displaying detectable expression in dendrites and cilia. In cil-1(my15), the PI(3)P marker decorates dendritic processes and cilia (16/27 animals) in addition to the enrichment in the nuclei and cell bodies (Figures 3H and 4H). The PI(3)P marker is distinctly bright at cil-1(my15) ciliary bases (Figure 3H, inset), hinting that loss of CIL-1 perturbs PI(3)P distribution in this region. These data suggest that CIL-1 displays a tissue-specific substrate preference toward PI(3,5)P2 in male sensory neurons.

Because PI(3)P localizes to early endosomes, we examined the distribution of early endosomal proteins. Rab-5 is an early endosomal protein that recruits and activates PI(3)-generating PI 3-kinases [23]. STAM-1 colocalizes with Rab-5 in C. elegans male-specific sensory neurons and promotes polysaccharin trafficking to early endosomes to the ESCRT complex [15]. In WT and cil-1(my15) animals, Rab-5 and STAM-1 localize to small puncta in the cell bodies, axons, and dendrites (Figures S4G–S4J), indicating that cil-1 does not modify the overall organization of early endosomes.

cil-1 Mutants Are Sperm Defective

cil-1(my15) hermaphrodites exhibit a drastic reduction in brood size (4.85% of WT) (Figures 5A), which is due to a spermatogenesis defect (Spe) based on the following observations: (1) a my15 hermaphrodite lays 200–300 unfertilized eggs, which is comparable to the number of WT fertilized eggs (Figures 5A); (2) my15 male germ line architecture and early stages of spermatogenesis (data not shown), hermaphrodite oocyte maturation, and ovulation appear normal (Figures 5D and 5E); (3) my15 hermaphrodites contain endomitotic cells without eggshells in the uterus (Figures 5F and 5G); (4) the my15 fertility defect is completely rescued when my15 hermaphrodites are mated with WT males (Figures 5B); and (5) my15 males fail to sire cross progeny without overt behavioral defects in mating behaviors (Figures 5C and 1G). We conclude that cil-1 is required for sperm function in both hermaphrodites and males.

A Spe phenotype may arise from developmental defects in spermatogenesis, sperm activation (spermiogenesis), sperm motility, or sperm-egg interactions. my15 male gonads have normal DAPI staining patterns for each meiotic stage in the gonad and normal number of spermatids (inactive 1N sperm) (data not shown). Thus, cil-1 is not required for early spermatogenesis up to spermatid production. Defective sperm-egg interactions are the basis of Spe phenotypes in spe-9, spe-38, trp-3/spe-41, and spe-42 (Figure 4A) (reviewed in [13]). In these Spe mutants, male-derived sperm normally develop, activate, crawl, and compete with endogenous hermaphroditic sperm but cannot fertilize an oocyte. However, my15 male-derived sperm do not compete with endogenous hermaphrodite-derived sperm, as reflected by a large number
of self-progeny and extremely low mating efficiency (ME) (1.9%, Figure S5C). ME of WT males is higher with my15 hermaphrodites (~95%) than control hermaphrodites (58%) (Figure S5C), illustrating that my15 endogenous sperm are nearly incapable of competing with WT male-derived sperm. Hence, cil-1 acts in events between spermatid production and sperm-egg interactions.

**cil-1 Is Required for Sperm Activation and Motility**

During sperm activation, a round spermatid develops into a motile spermatozoon with a pseudopod (Figure 4A). This process can be mimicked in vitro by chemical activators such as the ionophore monensin or Pronase (reviewed in [13]). Within 15 min of Pronase application, the majority of WT male spermatids (63.4%, n = 347) extend pseudopods (Figures 4B and 4B'), and the average length of spermatozoa is 8.13 ± 1.11 μm (± standard deviation [SD], n = 95) (Figure 4B'). In contrast, only 47.8% (n = 128) of my15 male spermatids develop pseudopods after Pronase activation (Figures 4C and 4C'), with a significantly shorter spermatozoa length (6.37 ± 0.61 μm [n = 112], p = 1.09E-28, Figure 4C'). The diameter of my15 spermatids is also slightly smaller (6.22 ± 0.54 μm, n = 136) than WT (6.73 ± 0.65 μm, n = 110, p = 8.08E-07). We also observe in vivo sperm activation defects in my15 hermaphrodites. In wild-type, 83.3% of spermatozoa possess pseudopods (n = 36, Figure 4B'), whereas only 8.3% do in my15 mutants (n = 72, Figure 4C'). When present, my15 pseudopods are significantly shorter (average length of longest axes of sperm; 4.32 ± 0.38 μm, n = 6) than WT (5.43 ± 0.62 μm, n = 37, p = 1.14E-4). Thus, cil-1 positively regulates sperm activation in vitro and in vivo.

To measure my15 sperm motility, we performed a time-lapse sperm-tracking assay. WT male sperm crawl from the uterus to the spermatheca over time, with the majority localizing at the spermatheca in 16 hr (Figure 4D). In contrast, my15 sperm are not observed in the spermatheca at 16 hr despite being present at 4 hr (Figure 4D). These data suggest that my15 sperm display reduced motility and, consequently, are not retained in the hermaphrodite spermatheca. After in vitro chemical activation with monensin, WT sperm crawl at the rate of 0.33 ± 0.04 μm/s (n = 7) on glass slides (Movie S3), whereas isolated my15 sperm are immotile (Movie S4).

Sperm activation involves fusion of membranous organelles (MOs) to the PM, exocytosis, and pseudopod extension (reviewed in [13]). To examine MOs, we used the MO-specific monoclonal antibody 1CB4 [24] and the lipophilic dye FM1-43 [25]. In WT, the majority of MOs are found at the cell periphery of round spermatids (Figure 4H). After activation, MOs are excluded from the pseudopod (Figure 4H'). In my15 sperm, 1CB4 staining for MO morphology and localization before and after activation appears to be WT (Figures 4I and
Figure 4. *cil-1* Positively Regulates Sperm Activation and Motility

(A) *C. elegans* sperm activation summarized in this illustration depicting pathways and genes functioning during sperm activation and fertilization. In a spermatid, MOs containing a TRPC receptor, TRP-3, are located just below the PM. During WT activation (solid arrow), MOs fuse to the PM and a pseudopod develops, producing a motile spermatozoon. In *fer-1* mutant sperm (upper dotted arrow), MOs do not fuse with the PM and a short pseudopod forms, resulting in immotile sperm. A *cil-1* mutant sperm (lower dotted arrow) is normal in MO fusion but develops into immotile spermatozoon with a short pseudopod. TRPC TRP-3 translocation from MO to the PM appears normal in *cil-1* mutant sperm. *spe-9*, *spe-38*, *spe-41/trp-3*, and *spe-42* encode various membrane proteins required for sperm-egg interactions. Loss of any of these genes results in motile but infertile spermatozoa.

(B and C) Nomarski images of isolated male-derived sperm before and after in vitro activation and endogenously activated hermaphrodite-derived sperm. (B) shows a round WT spermatid. (B') shows WT spermatozoa after 15 min of pronase activation. Spermatozoa extend full-length pseudopods (yellow arrowheads). Yellow bars depict the length of WT spermatozoa measured. (B'') shows WT hermaphrodite-derived sperm that are endogenously activated. (B'''') WT male-derived sperm are activated to spermatozoa with pseudopods (arrow arrowheads) within 10 min of 100 nM wortmannin application. (C) *cil-1* mutant spermatids before activation are slightly smaller than WT spermatids. (C') Upon activation, *cil-1* mutant sperm develop stubby pseudopods. The length of sperm is indicated with orange bars shorter than WT (compare to yellow bars). (C'') Hermaphrodite-derived sperm from the *cil-1* mutant occasionally develop short pseudopods. (C''') *cil-1* male-derived sperm are not activated by 100 nM wortmannin but exhibit subtle morphological changes.

(D) Sperm-tracking assay. The majority of WT male-derived sperm are deposited and retained within the spermatheca in the hermaphrodite reproductive tract at 10 and 16 hr. In contrast, *cil-1* male-derived sperm are not found in the spermatheca at 16 hr. *spt* denotes spermatheca.

(E and E') Ultrastructure of *him-5* and *cil-1*/*him-5* spontaneously activated spermatozoa.

The following abbreviations are used: lm, laminar membranes; mo, membranous organelles; n, nucleus; and p, pseudopod. Although the cytoplasm in this *cil-1* pseudopod (compare E to E') appears denser than that of the WT control, the significance, if any, of this observation is unclear.

(F and G) Monitoring MO fusion during sperm activation with a lipophilic FM1-43 dye. (F) The PM of WT spermatids is stained with FM1-43. (F') In WT spermatozoa, the dye concentrates at the MO fusion sites. MO fusion events are restricted to the PM of cell body (arrowheads) but not pseudopod (bracket). (G) FM1-43 dye marks the PM of *cil-1* spermatids. (G') In short *my15* spermatozoa with visible pseudopods, MO fusion sites are concentrated on the cell body (arrowheads) and excluded from the pseudopod PM (bracket).

(H and I) Immunohistochemistry of sperm with the MO antibody 1CB4 (red), anti-TRP-3 (green), and DAPI (blue). The triple-labeled images were generated by overlaying three confocal images from the same Z section. (H) In WT spermatids, MOs (red) are located around the cell periphery below the PM. The location
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4E). In both WT and my15, FM1-43 labels the PM of the round spermatids (Figures 4F and 4G) and, after activation, concentrates at the site of membrane fusion on the spermatoozoon cell body (Figures 4F′ and 4G′, arrowheads). cil-1 is not essential for MO morphology, localization, or fusion.

The ultrastructure of my15 sperm is largely unaffected as determined by transmission electron microscopy (TEM). The early stages of spermatogenesis in WT and my15 appear very similar (data not shown). Occasionally, dissected males will produce spontaneously activated spermatozoa, and we examined such cells in both WT and my15 (Figures 4E and 4E′). In both cases, a pseudopod is extended and separated from the cell body by lamellar membranes. MOs successfully fuse with the PM in my15, consistent with immunostaining and MO fusion assay (Figures 4G′ and 4I′).

cil-1 may regulate membrane receptor localization in sperm. TRP-3/SPE-41 acts in sperm, translocates from MOs to the PM upon activation, and is required for fertilization [26]. In WT and my15 spermatids, anti–TRP-3 labels cytoplasmic puncta partially overlapping with MOs (Figures 4H and 4I). In WT and my15 spermatoozoon, TRP-3 is detected ubiquitously on the PM of the pseudopod and cell body (Figures 5H′ and 5I′). cil-1 is not required for TRP-3/TRPC translocation to the PM.

CIL-1 and PI 3-Kinase Activity Antagonistically Regulate Sperm Activation
Because PI biosensors show that CIL-1 hydrolyzes PI(3,4,5)P3 and PI(3,5)P2, we asked whether 3-kinase activity antagonizes CIL-1 5-phosphatase function. We applied 1 nM, 10 nM, and 100 nM wortmannin, a pharmacological inhibitor of PI 3-kinases that produce PI(3,4,5)P3 from PI(4,5)P2 [27], to WT and my15 spermatids. At all concentrations tested, wortmannin acts as an in vitro activator of WT but not my15 spermatids, with 100 nM being the most effective (compare Figure 4B′ with Figure 4C′). The low dose (1 nM) of wortmannin suggests specificity and indicates that PI 3-kinase activity and cil-1 act antagonistically in sperm activation (Figures 5C and 5D).

Discussion
The PI 5-phosphatase CIL-1 mediates multiple aspects of C. elegans male reproductive biology (Figure 5). In male neurons, cil-1 acts cell autonomously to control LOV-1 and PKD-2 localization but not function. Our data support a model whereby cil-1 acts in early steps in TRPP complex downregulation by regulating PI(3)P distribution but not RAB-5 and STAM localization (Figures 5A and 5B). In sperm, cil-1 positively regulates sperm activation and motility without affecting major membrane trafficking events. The 3-kinase inhibitor wortmannin is a potent sperm activator, which indicates that PI(3,4,5)P3 is a major CIL-1 substrate in sperm. We propose that one of the three 3-kinases encoded in the C. elegans genome (VPS-34, AGE-1, or F39B1.1) acts antagonistically to CIL-1 in a sperm activation pathway (Figures 5C and 5D).

cil-1 may be required for cellular polarity because distinct PI enrichment is a hallmark of PM domains [28, 29]. However, cil-1 mutants properly localize several other ciliary and presynaptic markers (Figure S1) and exhibit normal sensory behaviors, reflecting properly polarized and functional neurons. Alternatively, cil-1 may modulate the activity of the TRPP complex. PI(4,5)P2 is a regulator of both TRP channel activity and trafficking [2]. Because cil-1 mutant males exhibit normal mating behaviors, cil-1 appears to regulate TRPP trafficking but not activity.

In neurons, cil-1 regulates the balance between PI species that is important for intracellular polycystin trafficking. Although cil-1 may negatively regulate polycystin insertion into the PM after initial targeting, our data are consistent with this model. A similar PKD-2::GFP Cil phenotype is observed in the adaptor protein 1 (AP-1) unc-101(m1) mutant background [14]. However, PKD-2::GFP particles are visibly moving along dendrites of both WT and my15 (Movies S1 and S2) but not unc-101 male sensory neurons (data not shown). Finally, PKD-2 abundance within ciliary regions is comparable between WT and my15 (Figure 1, [16]).

Our data are most consistent with a requirement for CIL-1 in TRP-polycystin trafficking to early endosomes after endocytosis (Figure 5A and 5B). In RnB neurons, cil-1 acts before STAM/Hrs-mediated receptor downregulation, which transport PKD-2 and LOV-1 from early endosomes to ESCRT [15]. In CEMs, it is difficult to place lov-1, cil-1, and stam-1 in a pathway because of the additive phenotypes of double mutants. PI(3)P and PI(3,5)P2 balance in endocytic compartments is important for organelle maturation and receptor trafficking. In cil-1(my15), pre-early endosomal vesicles lacking the normal destination, the PI(3)P-enriched early endosomes, may simply accumulate and disperse along sensory neurons. Alternatively, cil-1 may be required for maturation of endocytic compartments by affecting PI(3)P distribution, although this is unlikely given the normal localization of RAB-5 and STAM-1 in my15 male sensory neurons. We propose that maintaining the balance between PI(3)P and PI(3,5)P2 by the CIL-1 5-phosphatase plays an essential role in polycystin trafficking between endocytic compartments.

Our data also reveal that C. elegans sperm activation is coordinated by the antagonistic actions of the CIL-1 5-phosphatase and an unidentified wortmannin-sensitive target. Although we cannot rule out off-target effects of wortmannin, we propose that CIL-1 and a PI3-kinase control PI(4,5)P3 levels. PI(3,4,5)P3 recruits and activates various effectors such as serine/threonine kinases and tyrosine kinases [30]. Cytoplasmic protein kinases and protein phosphatases are significantly overrepresented in C. elegans sperm [31]. Molecules such as SPE-6 serine/threonine kinase [32] may regulate sperm activation downstream of CIL-1-mediated turnover of PI(3,4,5)P3.

TRPP family members play important roles in mating and fertilization of numerous species [3, 7–12]. ADPKD male patients may exhibit infertility due to sperm immotility [33]. Sea urchin surREJ (receptor for egg jelly, a polycystin-1 homolog) and suPC2 (polycystin-2) localize to the PM proximal to acrosomal vesicles in sperm [8, 34]. In mice, PKD-REJ controls postcoitalatory reproductive selection via effects of TRP-3 (green) partially overlaps with 1CB4-labeled MOs. (H′) In WT spermatozoa, 1CB4-positive MOs are primarily located around the PM but absent from pseudopods (compare with bracket area in H′). (H″) In WT spermatozoa, anti–TRP-3 staining is detectable in the cell body and pseudopod PM (bracket). (H‴) An overlay of WT spermatozoa is shown. (I) In my15 spermatids, MOs (red) are localized to the cell periphery just below the PM as in WT. Anti–TRP-3 staining (green) overlaps with MOs. (I′) In my15 spermatoozoon, similar to WT, MOs are found in the cell body but not pseudopod. (I″) An overlay of (H″) and (H‴) with the DAPI image is shown.

The scale bars represents 5 μm.
on sperm transport and exocytic competence during the acrosome reaction [11]. In the hermaphroditic chordate Ciona intestinalis, the PKD1 homolog is expressed in testes and may act on sperm to control self-incompatibility [12]. In Drosophila, PKD2 may mediate sperm directional movement [9, 10]. Intriguingly, mutations in the type IV 5-phosphatase INPP5E gene cause human ciliary diseases [35, 36]. According to our phylogeny tree (Figure S2A), cil-1 is the closest C. elegans homolog of INPP5E. Further experiments are needed to determine whether CIL-1 plays an evolutionarily conserved role in TRPP complex trafficking and sperm function in other species.

Supplemental Data

Supplemental Data include four figures, Supplemental Experimental Procedures, and four movies and can be found with this article online at http://www.cell.com/current-biology/supplemental/S0960-9822(09)01621-2.

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