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Oocyte signals derived from polyunsaturated fatty acids control sperm recruitment *in vivo*

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A fundamental question in animal development is how motile cells find their correct target destinations. During mating in the nematode *Caenorhabditis elegans*, males inject sperm through the hermaphrodite vulva into the uterus. Amoeboid sperm crawl around fertilized eggs to the spermatheca – a convoluted tube where fertilization occurs[1,2]. Here, we show that polyunsaturated fatty acids (PUFAs), the precursors of eicosanoid signalling molecules, function in oocytes to control directional sperm motility within the uterus. PUFAs are transported from the intestine, the site of fat metabolism, to the oocytes yolk, which is a lipoprotein complex. Loss of the RME-2 low-density lipoprotein (LDL) receptor, which mediates yolk endocytosis[3] and fatty acid transport into oocytes, causes severe defects in sperm targeting. We used an RNAi screen to identify lipid regulators required for directional sperm motility. Our results support the hypothesis that PUFAs function in oocytes as precursors of signals that control sperm recruitment to the spermatheca. A common property of PUFAs in mammals and *C. elegans* is that these fats control local recruitment of motile cells to their target tissues.

To investigate the mechanism by which sperm target the spermatheca, we developed a method to track sperm movement in the hermaphrodite reproductive tract (Fig. 1a). When wild-type males labelled with MitoTracker were mated to non-labelled wild-type hermaphrodites, fluorescent sperm accumulated in the spermatheca over time[4,5] (Fig. 1b). After mating, sperm deposited within the uterus near zone 1 migrated through zone 2 (see Fig. 1a for zone locations), and rarely reversed their direction (Fig. 1c; Table 1, line 1). During this journey, the average vectorial velocity toward the spermatheca was less than the average velocity, primarily due to migration around fertilized eggs (Table 1, line 1). One hour after mating, more than 95 percent of sperm reached zone 3 (Fig. 1a; see Supplementary Information, Movie 1), where a bottleneck occurred while sperm search for the spermatheca–uterine valve.

Within the spermatheca, sperm density was greatest at the distal end closest to the oocytes (Fig. 1b). We hypothesized that oocytes are the source of a sperm attractant[6]. To examine this hypothesis, MitoTracker-labelled males were mated to mutant hermaphrodites lacking oocytes. *glp-4(bn2)* hermaphrodites generate few germ cells and no oocytes at the restrictive temperature[7]. Sperm from wild-type males accumulated in the uterus and rarely reached the spermatheca in *glp-4(bn2)* mutants (Fig. 1b). These same sperm targeted the spermatheca when *glp-4(bn2)* adults were shifted back to the permissive temperature for 24 h to promote oogenesis (Fig. 1b). Male sperm failed to accumulate in the spermatheca of *fem-3(q20)* and *mog-5(q449)* mutants, whose germ cells only differentiate into sperm[8,9], and of *gld-1(q485)* mutants, whose germ cells fail to differentiate into sperm or oocytes[10] (Fig. 1b and data not shown). Quantitative analysis of sperm motility in *glp-4(bn2)* and *fem-3(q20)* uteri indicated that sperm moved with reduced velocity, no vectorial velocity and a high rate of reversals (Fig. 1c; Table 1, compare lines 1 and 2; and data not shown). These results are consistent with the hypothesis that oocytes are the source of a diffusible sperm attractant.

Two common precursors of signalling molecules in mammals are cholesterol and PUFAs. We examined whether either of these lipids were required for directional sperm motility. *C. elegans* cannot synthesize sterols *de novo* and must receive them exogenously[11]. MitoTracker-labelled males grown on cholesterol-containing medium were mated to cholesterol-depleted adult hermaphrodites. Average sperm velocity, vectorial velocity and reversal frequency were not significantly different from those values obtained using cholesterol-containing hermaphrodites (Table 1, compare lines 1 and 3; $P > 0.10$). *C. elegans* synthesizes PUFAs from dietary precursors through the activation of lipid biosynthetic enzymes encoded by the *fat* genes[12]. To determine whether PUFAs are required for sperm targeting in hermaphrodites, we examined wild-type sperm movement in *fat-2(wa17)* mutants. *fat-2* encodes a Δ12-desaturase required to generate 18- and 20-carbon polyunsaturated fats[12]. The *wa17* strain is capable of carrying out a small amount of Δ12-desaturase activity, approximately 5% of the wild-type activity. Wild-type sperm in *fat-2(wa17)* mutants had reduced velocity and vectorial velocity and a higher reversal frequency than sperm in wild-type

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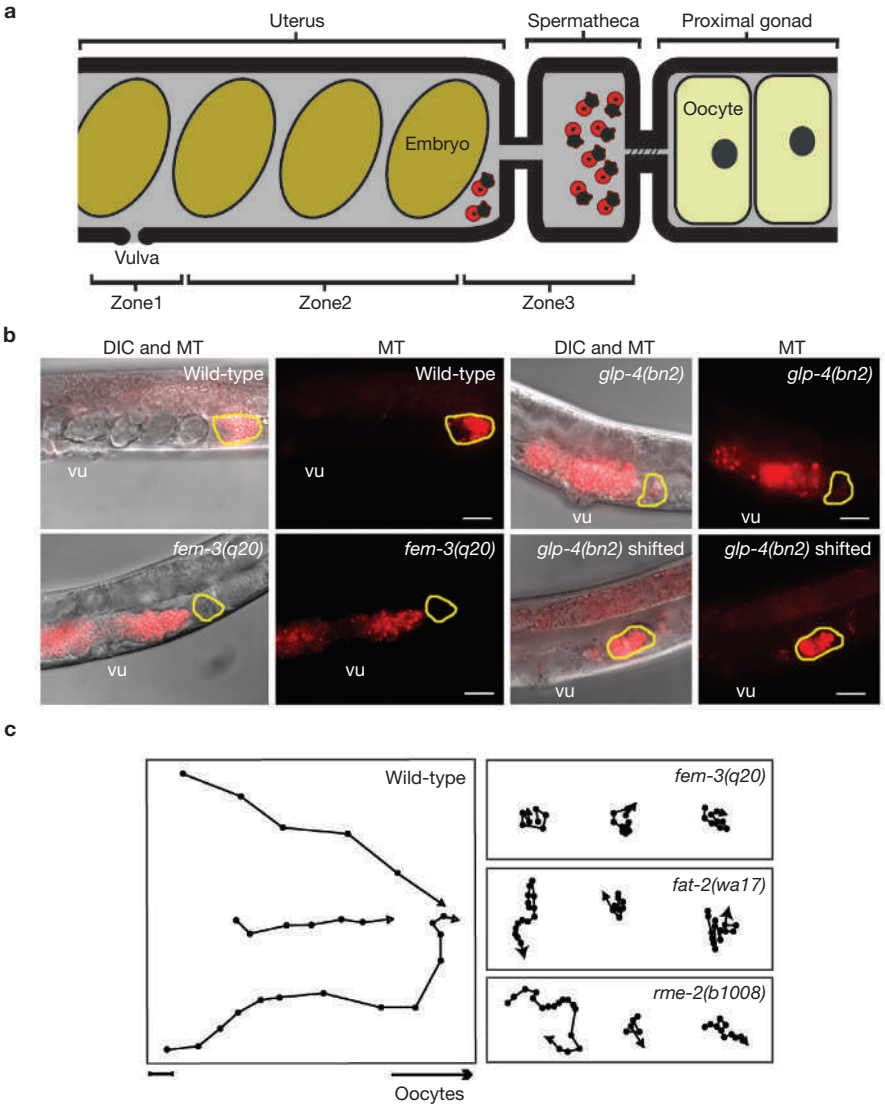

Figure 1 Sperm migration in the hermaphrodite reproductive tract. (**a**) Schematic representation of the hermaphrodite reproductive tract. Anterior (not shown) and posterior gonad arms are connected by a common uterus. Within the spermatheca, sperm (red) are separated from oocytes by a valve-like constriction. During oocyte maturation and ovulation, this constriction dilates and the maturing oocyte enters the spermatheca. Self-derived sperm develop in the proximal gonad and enter the spermatheca during the first ovulation. Male-derived sperm are injected through the vulva into the uterus. (**b**) Oocytes are required for sperm (red) targeting to the spermatheca (yellow outline). MitoTracker (MT)-labelled males were mated to wild-type and mutant hermaphrodites overnight (18 h) before imaging. *glp-4(bn2)* shifted animals were grown at the restrictive temperature, mated overnight, then shifted to the permissive temperature for 24 h. (**c**) Traces of wild-type sperm paths within the uteri (zone 2) of wild-type and mutant hermaphrodites. Traces were generated from time-lapse video microscopy immediately after mating. Dots indicate positions at 30 s intervals. Vu, vulva. Scale bars represent in 20 μm in **b** and 5 μm in **c**.

hermaphrodites (Table 1, compare lines 1 and 4; $P < 0.001$; Fig. 1c; and see Supplementary Information, Movie 2). These sperm motility values were similar to those of *glp-4(bn2)* and *fem-3(q20)* mutants (Fig. 1c and Table 1, compare lines 4 and 2). *fat-2(wa17)* hermaphrodites had reduced reproductive efficiency, due in part to loss of self-derived sperm from the reproductive tract during egg laying (Table 1, compare lines 1 and 4, and see Supplementary Information, Fig. S1). These results support the hypothesis that PUFAs are precursors of signals that control directional sperm motility.

To determine the requirements for specific PUFA classes, sperm motility was examined in other *fat* mutants. *fat-3* mutants lack Δ6 desaturase activity and consequently fail to produce most C20 PUFAs, such as arachidonic acid and eicosapentaenoic acid[12]. When *fat-3(wa22)* hermaphrodites were mated with wild-type males, defects in sperm motility were observed (Table 1, compare lines 1 and 5). *fat-3(wa22)* hermaphrodites have reduced fertilized egg production compared with wild type[13], due in part to sperm loss from the reproductive tract (Table 1, compare lines 1 and 5, and see Supplementary Information, Fig. S1). The sperm motility defects of *fat-3* mutants were less severe than those of *fat-2* mutants, suggesting that C20 PUFAs are not absolutely essential for directional movement. Sperm motility defects were not observed in *fat-1* mutants, which fail to produce omega-3 (n-3) PUFAs, or in *fat-4* mutants, which fail to produce significant amounts of arachidonic acid and eicosapentaenoic acid, but produce other classes of C18 and C20 PUFAs[12]. These results indicate that flexibility or redundancy exists in the PUFA classes required for regulating sperm motility.

Table 1 Directional sperm motility data

Description	Average velocity (μm min^{-1})	Average vectorial velocity (μm min^{-1})	Reversal frequency (rev per h)	N	Fertilized eggs (20 °C)
1. Wild type	8.07 ± 0.67	4.38 ± 0.85	1.14	74	308 ± 25
2. *glp-4(bn2)*	3.43 ± 0.25	–0.23 ± 0.25	16.17	79	NA
3. Cholesterol-depleted	7.41 ± 0.42	4.16 ± 1.10	1.11	26	ND
4. *fat-2(wa17)*	3.19 ± 0.20	0.36 ± 0.28	6.83	65	87 ± 17[a]
5. *fat-3(wa22)*	3.59 ± 0.32	2.20 ± 0.44	1.86	40	144 ± 11
6. *fat-2(wa17)* + 18:2n6	5.46 ± 0.53	3.96 ± 0.58	1.20	40	220 ± 60[b]
7. *fat-2(wa17)* + 20:4n6	6.27 ± 0.46	3.33 ± 0.60	1.29	60	229 ± 36[b]
8. *fat-2(wa17)* + 20:5n3	6.54 ± 0.47	3.79 ± 0.57	2.70	50	182 ± 42[b]
9. Wild-type 24 h starved	3.91 ± 0.41	–0.33 ± 0.50	8.81	42	NA
10. *rme-2(b1008)*	3.68 ± 0.28	0.45 ± 0.32	9.46	75	72 ± 16

The average velocity, average vectorial velocity toward the spermatheca and average reversal frequency was determined for wild-type sperm within the uterus (Zone 2) of wild-type, mutant and experimentally manipulated hermaphrodites. Values ± s.e.m. are shown. Directional motility defects are observed for lines 2, 4, 9 and 10 after normalization for average velocity. Values ± s.d. are shown for egg production. NA, not applicable; ND, not determined. [a]Only those *fat-2(wa17)* hermaphrodites surviving long enough to run out of sperm were used. [b]The minimum concentration of PUFAs necessary to rescue the directional motility defects, but not total egg production, was used.

To determine whether addition of exogenous PUFAs to *fat-2(wa17)* hermaphrodites can rescue the directional movement defects of wild-type sperm, the hermaphrodite culture plates were supplemented with free fatty acids. Addition of linoleic acid (18:2n-6), arachidonic acid (20:4n-6), or eicosapentaenoic acid (20:5n-3) to *fat-2(wa17)* hermaphrodites rescued the directional sperm motility defects (Table 1, compare lines 1 and 4 with 6–8; P <0.001). Rescue was observed when *fat-2(wa17)* hermaphrodites at the L4 and young adult stages were added to supplemented plates, suggesting that PUFAs function in adults. The *fat-2* mutant worms accumulated the exogenous PUFAs in their membranes to the extent that ~2% of their total fatty acids were derived from dietary supplementation. This concentration, which is much less than the PUFA concentration in wild-type hermaphrodites[12], was the minimum amount necessary for complete rescue. Rescue with such low PUFA concentrations is consistent with a signalling role. These results demonstrate that the PUFA synthesis deficiency in *fat-2(wa17)* hermaphrodites is responsible for the sperm motility defects.

Hermaphrodites mobilize intestinal fat stores on starvation for 8 h[14]. If PUFAs act as sperm attractant precursors, then starvation may alter PUFA metabolism or transport, thereby disrupting the sperm targeting mechanism. When fed MitoTracker-labelled males were mated to adult hermaphrodites starved for 24 h, sperm moved with reduced velocity and no directional velocity within the uterus (Table 1, compare lines 1 and 9; P <0.001). This may represent an adaptive strategy to limit outcross progeny when food is scarce. Starvation for 16 h caused severe defects in directional sperm motility, suggesting that the putative sperm attractant has a short half-life *in vivo*, a common property of PUFA-derived signals in mammals[15]. We concluded that sperm motility in the hermaphrodite reproductive tract is dependent on dietary intake.

The *fat* genes are expressed in the adult intestine[13,16]. *fat-2* mRNAs were not detected in the germ line using DNA microarray analysis or *in situ* hybridization[17,18]. To eliminate the possibility that *fat-2* functions in oocytes, its site of action was examined using an RNAi mosaic strategy. *rrf-1(pk1417)* mutants are sensitive to RNAi in the female germ line, but refractory to RNAi in the soma[19]. Sperm from wild-type males exhibited directional motility defects in *fat-2* RNAi hermaphrodites, but not in *fat-2* RNAi *rrf-1(pk1417)* mutants or controls (Table 2, compare lines 1-4; P <0.01). We concluded that *fat-2* does not function in oocytes to regulate directional sperm motility.

We reasoned that PUFAs must be transported to oocytes to function as precursors of signalling molecules. To investigate how this may occur, hermaphrodites were fed Bodipy-FA — a fluorescent probe equivalent to a C18 fatty acid. Bodipy-FA was incorporated into yolk at the site of yolk synthesis (the intestine) and then transported to oocytes (Fig. 2a). We hypothesized that PUFAs are transported by the same mechanism. Previous studies have shown that yolk endocytosis into oocytes is mediated by RME-2, an LDL receptor specifically expressed in oocytes[3]. Oocytes in *rme-2(b1008)*-null hermaphrodites fail to endocytose yolk, resulting in a drastic reduction in brood size[3]. This reduction is due, in part, to sperm loss from the reproductive tract (see Supplementary Information, Fig. S1). To examine sperm motility in the absence of *rme-2* function, MitoTracker-labelled males were mated to *rme-2(b1008)* and *rme-2* RNAi hermaphrodites. Compared with the wild type, severe sperm movement defects were observed in *rme-2(b1008)* hermaphrodites (Fig. 1c), including reduced velocity and vectorial velocity, and a higher reversal frequency (Table 1, compare lines 1 and 10; P <0.001; and see Supplementary Information, Movie 3). Even 1 h after mating, few sperm were observed in the spermatheca (see Supplementary Information, Movie 4). RNAi mosaic analysis using *rrf-1(pk1417)* mutants indicated that *rme-2* is required in the female germ line (Table 2, compare lines 1, 2, 5 and 6; P <0.001). These results support the hypothesis that yolk provides oocytes with a signalling precursor required for sperm recruitment.

Yolk is a macromolecular complex consisting of lipids and lipid-binding proteins called vitellogenins[20]. To determine the fate of yolk fatty acids following endocytosis into oocytes, we examined deconvolved serial sections of dissected gonads from wild-type and *rme-2(b1008)* hermaphrodites that were fed Bodipy-FAs. In the wild type, Bodipy-FAs were distributed throughout the membranes of oocytes and their precursors (Fig. 2a), with most outside of vitellogenin-containing yolk endosomes[3,21] (see Supplementary Information, Fig. S2a). In contrast, considerably less Bodipy-FAs were present in membranes of *rme-2(b1008)* gonads (Fig. 2a). Differences between vitellogenin and Bodipy-FA localization in wild-type gonads suggest that yolk fats are released from yolk complexes during or following endocytosis (Fig. 2a and see Supplementary Information, Fig. S2a). We concluded that *rme-2* mediates the transport of exogenous fatty acids from yolk to oocyte membranes. Alternative modes of fat transport independent of RME-2 must also exist.

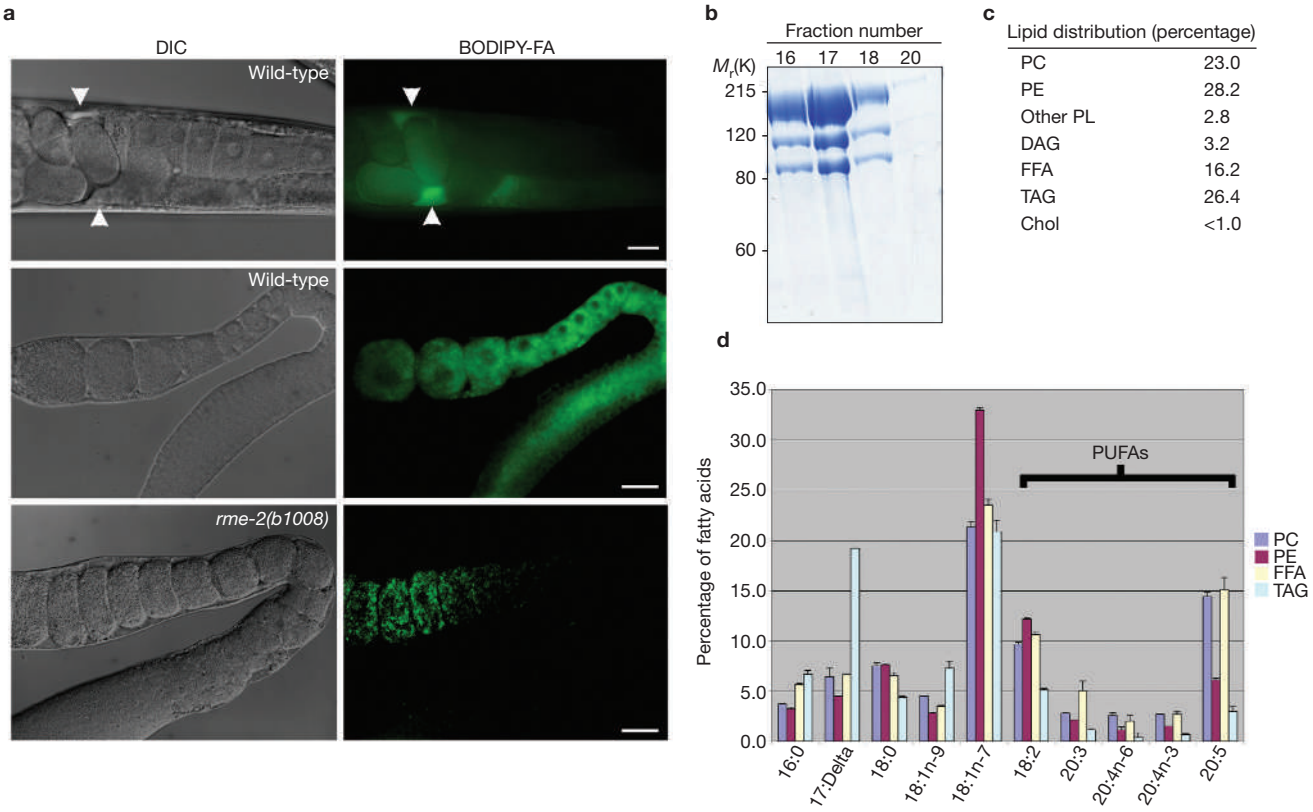

Figure 2 Fatty acid transport and yolk analysis. (**a**) Distribution of Bodipy-FAs (green) in wild-type and *rme-2(b1008)* hermaphrodites. Top panels show Bodipy-FAs present in yolk (arrowheads). Middle and bottom panels are deconvolved mid-focal plane images of dissected gonads. In *rme-2(b1008)* gonads, most Bodipy-FAs are concentrated in what seems to be an endosomal compartment in oocytes. (**b**) SDS–PAGE analysis of sucrose gradient fractions containing yolk complexes. The bands with relative molecular masses (M_r) of approximately 170K, 115K and 88K are the vitellogenins. (**c**) Percentage weight distribution of yolk lipid classes. (**d**) Percentage weight distribution of fatty acid types in phosphatidylcholine (PC), phosphatidylethanolamine (PE), free fatty acid (FFA) and triacylglycerol (TAG) lipid classes. The error bars represent s.d.. Fatty acid structures are abbreviated as in 20:4n-6, which has 20 carbons and four double bonds, the last occurring at the n-6 position. PL, phospholipids; DAG, diacylglycerol; Chol, cholesterol. The scale bars in **a** represent 20 μm.

The results presented thus far support the hypothesis that yolk provides oocytes with a source of PUFAs for generating signalling molecules. If this model is correct, then yolk complexes should contain significant quantities of PUFAs before endocytosis into oocytes. To test this hypothesis, we purified yolk complexes from *rme-2(b1008)* hermaphrodites. Fractions containing yolk were of high purity, as judged by the absence of non-vitellogenin protein bands on SDS–PAGE gels (Fig. 2b). Thin layer and gas chromatography indicated that the predominant lipid classes associated with yolk complexes are phosphatidylcholine, phosphatidylethanolamine and triacylglycerol (Fig. 2c). A significant fraction of lipids present as free fatty acids was also detected (Fig. 2c). Gas chromatography analysis demonstrated that yolk complexes have significant quantities of n-3 and n-6 PUFAs, including linoleic acid, arachidonic acid and eicosapentaenoic acid (Fig. 2d). PUFAs were present as free fatty acids, as well as in the polar lipids phosphatidylcholine and phosphatidylethanolamine (Fig. 2d). A smaller fraction of PUFAs was found in triacylglycerols, the major form used for energy storage (Fig. 2d). We concluded that yolk contains significant quantities of n-3 and n-6 PUFAs.

To determine whether the addition of exogenous PUFAs to oocytes could promote sperm recruitment in *fat-2(wa17)* mutants, microinjection experiments were performed and sperm accumulation at the spermatheca was examined over time (see Supplementary Information, Fig. S2). Microinjecting arachidonic acid or purified PUFA-containing yolk complexes into the reproductive tract of *fat-2(wa17)* hermaphrodites rescued the sperm targeting defect (see Supplementary Information, Fig. S2). Furthermore, time-lapse video microscopy showed that sperm in these experiments moved with greater velocity compared with sperm in *fat-2(wa17)* controls. Oocytes are the only cell type in the reproductive tract capable of yolk endocytosis[3] (see Supplementary Information, Fig. S2a). Collectively, our results strongly support a model in which PUFAs act in oocytes to control directional sperm motility.

We reasoned that genes encoding proteins involved in synthesizing PUFA-derived signals should be required in oocytes to control sperm recruitment. To generate a list of candidates for screening, DNA microarray and *in situ* hybridization datasets were scanned for genes whose mRNAs are expressed in oocytes[17,18]. These genes were cross-referenced with bioinformatic data to identify those with predicted roles in lipid metabolism and transport. We selected 35 genes for a pilot RNAi screen (see Supplementary Information, Table S1) – a small fraction of the total number. RNAi hermaphrodites were tested for premature depletion of self-derived sperm compared with control hermaphrodites and for defects in the directional movement of sperm from non-RNAi males. Systematic analysis of the 35 candidates resulted in six genes that fit both criteria (Table 3). We suspected that redundancy may limit the effectiveness of the screen, given that oocytes express multiple members of

Table 2 Gene site-of-action analysis in hermaphrodites

Description	Average velocity (μm min^{-1})	Average vectorial velocity (μm min^{-1})	Reversal frequency (rev per h)	N
1. Wild type	8.07 ± 0.67	4.38 ± 0.85	1.14	74
2. *rrf-1(pk1417)*	6.96 ± 0.60	3.88 ± 0.68	1.34	53
3. *fat-2* RNAi	5.38 ± 0.48	1.52 ± 0.69	4.27	34
4. *fat-2* RNAi *rrf-1(pk1417)*	6.35 ± 0.61	4.14 ± 0.95	1.27	23
5. *rme-2* RNAi	3.75 ± 0.38	0.67 ± 0.44	8.26	35
6. *rme-2* RNAi *rrf-1(pk1417)*	4.11 ± 0.49	0.46 ± 0.76	13.30	27
7. H02I12.8 RNAi	6.67 ± 0.63	1.21 ± 1.08	6.19	39
8. H02I12.8 RNAi *rrf-1(pk1417)*	5.80 ± 0.51	1.60 ± 0.54	6.49	31
9. F21G4.2/C10C6.5 RNAi	6.28 ± 1.03	1.13 ± 0.69	5.80	24
10. F21G4.2/C10C6.5 RNAi *rrf-1(pk1417)*	5.37 ± 0.45	0.67 ± 0.65	7.72	29

RNAi of the indicated genes was performed on L4 stage hermaphrodites. Sperm motility values are from non-RNAi wild-type males. *rrf-1(pk1417)* mutants are sensitive to RNAi in the female germ line, but refractory to RNAi in the soma[19]. Values ± s.e.m. are shown.

Table 3 Summary of pilot RNAi screen results

Gene	Description
H02I12.8	Class 4 cytochrome P450 predicted to hydroxylate PUFAs[a]
K08F4.7	Related to glutathione-requiring Prostaglandin D synthase[a]
C01F6.1	Copine family of calcium-dependent phospholipid binding proteins
T28F3.1	Copine family of calcium-dependent phospholipid binding proteins
R107.7	Glutathione *S*-transferase, pi class
F27C8.6	Related to arylacetamide deacetylase, a putative microsomal lipase
F21G4.2[b]	Member of the ATP-binding cassette transporter family, subfamily C[a]
C10C6.5[b]	Member of the ATP-binding cassette transporter family, subfamily G

A total of 35 genes whose mRNAs are expressed in oocytes were screened (see Supplementary Information, Table S1). For the genes listed in this table, RNAi of L4 stage hermaphrodites causes premature depletion of self-derived sperm and directional motility defects of sperm from non-RNAi males. [a]Gene class implicated in mammalian eicosanoid synthesis or transport. [b]RNAi of both genes is necessary to see an effect.

several gene classes. Consistent with this idea, double RNAi knockdown of C10C6.5 and F21G4.2, which encode ATP-binding Cassette (ABC) transporters, resulted in sperm movement defects when RNAi of either gene alone did not. Other genes classes have not yet been checked for redundancy. Therefore, a total of eight genes were identified in the pilot screen (Table 3).

The cytochrome P450 H02I12.8 and ABC transporters C10C6.5 and F21G4.2 were chosen for quantitative analysis. Sperm from non-RNAi males had reduced vectorial velocities and higher reversal frequencies in RNAi hermaphrodites compared with wild-type hermaphrodites (Table 2, compare lines 1, 7 and 9; P <0.01). To determine whether these genes function in the female germ line, we again used *rrf-1(pk1417)* hermaphrodites. The directional sperm motility defects in RNAi hermaphrodites and RNAi *rrf-1(pk1417)* hermaphrodites are similar and significantly different from controls (Table 2, compare lines 1, 2 and 7–10; P <0.02). These results support the hypothesis that the cytochrome P450 H02I12.8 and ABC transporters F21G4.2 and C10C6.5 function in oocytes to control directional sperm movement.

We propose the following model for sperm recruitment to the spermatheca: FAT-2 converts monounsaturated fatty acids into PUFAs in the intestine, the site of fat metabolism and yolk synthesis; PUFAs are incorporated into yolk complexes in two major forms, esterified to phospholipids and unesterified; yolk is released into the pseudocoelom and flows to the gonad; the RME-2 LDL receptor mediates yolk endocytosis and fatty acid transport, providing oocytes with a source of PUFAs for synthesizing signalling molecules; modified PUFAs released into the reproductive tract promote sperm migration to the spermatheca, resulting in fertilization. The spermatheca may have an active role in the metabolism or transport of the signals.

In mammals, arachidonic acid and other C20 PUFAs are converted into signalling molecules called eicosanoids[15,22]. During immune responses, prostaglandin D_2 and leukotriene B_4 are rapidly generated at sites of inflammation and are required for eosinophil and T cell recruitment, probably by acting as chemoattractants[23–25]. Therefore, in *C. elegans* and mammalian cells, PUFAs are likely to act as precursors of signals that control local recruitment of motile cells to target tissues (see Supplementary Information, Table S2 and Discussion). Whether PUFAs function in sperm recruitment within the mammalian reproductive tract remains an open question[26]. □

METHODS

Strains and RNA interference. *C. elegans* variety Bristol, strain *N2* was the wild-type strain used. Males were generated from *N2* crosses or by using the *fog-2(q71)* strain, which segregates 'female' and male progeny. The following strains were also used: SS104 [*glp-4(bn2)I*], JK816 [*fem-3(q20)IV*], JK2321 [*mog-5(q449) unc-4(e120)/mIn1[dpy-10(e128)]II*], JK1466 [*gld-1(q485)/dpy-5(e61) unc-13(e51) I*], BX26 [*fat-2(wa17)IV*], BX30 [*fat-3(wa22)IV*], BX24 [*fat-1(wa9) IV*], BX17 [*fat-4(wa14)IV*], PD8488 [*rrf-1(pk1417)I*] and DH1390 [*rme-2(b1008)IV*]. RNAi was performed at 25 °C on either L1 or L4 larva by the feeding method[27].

MitoTracker staining and mating. MitoTracker Red CMXRos (Invitrogen, Carlsbad, CA), a fluorescent dye that specifically stains mitochondria, was used to label male sperm by modification of a previously described method[4]. MitoTracker does not affect sperm motility or fertilization. Anesthetized hermaphrodites were mated to labelled males for 30 minutes and mounted for microscopy, unless stated otherwise (see Supplementary Information, Methods, for more details.)

Microscopy and sperm movement analysis. Sperm movement was analysed from traces generated from time-lapse videos of freshly mated hermaphrodites. Motility values were determined using sperm traces from zone 2 within the uterus (Fig. 1a). Vectorial velocity toward the spermatheca was measured by creating a straight line through the uterus from the vulva to the spermatheca. The distance travelled along this line from the beginning of a sperm trace to the end was divided by time. A reversal was defined as occurring when the angle generated from a sperm trace during three consecutive time-lapse frames is less than 90 degrees. A two sample *t*-test was used to test for significance.

Fatty acid supplementation, cholesterol extraction and starvation. For Bodipy-FA experiments (Invitrogen), 200 μM solutions dissolved in DMSO were dropped onto seeded plates and allowed to dry. L4 stage hermaphrodites were added to the plates and kept in the dark for 24–48 h at 20 °C. For dietary PUFA supplementation experiments, PUFA stocks were prepared by diluting fatty acid salts (Nu-Chek Prep, Elysian, MN) to 100 mM in ddH_2O as previously described[13,16]. For cholesterol depletion experiments, worms were grown on plates containing ether-extracted peptone and agarose without cholesterol addition as previously described[28]. For starvation experiments, adult hermaphrodites were washed several times in M9 buffer and transferred to unseeded plates.

Yolk complex purification. Yolk complexes were purified from *rme-2(b1008)* hermaphrodites, which accumulate yolk in the pseudocoelom, using a procedure modified from a previous study[20].

Lipid analysis. Lipids were extracted from frozen yolk pellets with (1:1) chloroform:methanol at –20 °C overnight. The extract was washed with 0.2 M H_3PO_4, 1 M KCL. Lipids were recovered in the chloroform phase, dried under nitrogen and redissolved in chloroform. Yolk lipids and authentic standards were separated by thin layer chromatography using a two-step development scheme (see Supplementary Information, Methods, for more details). Fatty acid methyl esters were prepared with 2.5% methanolic H_2SO_4 for analysis by gas chromatography as previously described[12].

Microinjection. Arachidonic acid (20:4n-6, 500 μM), purified PUFA-containing yolk complexes (250 μg ml^{-1} protein), or PBS buffer was microinjected through the vulva into the *fat-2(wa17)* hermaphrodite reproductive tract using a Zeiss Axiovert 200 microscope, hydraulic fine type micromanipulator and Narishige IM-30 microinjector. Fluid disperses throughout the uterus, spermatheca and proximal gonad. This method causes much less internal damage than microinjection directly into the proximal gonad[29]. Microinjected animals were allowed to recover for 2 h before mating with MitoTracker-labelled males.

Note: Supplementary Information is available on the Nature Cell Biology website.

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COMPETING FINANCIAL INTERESTS

The authors declare that they have no competing financial interests.

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