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Neurosensory Perception of Environmental Cues Modulates Sperm Motility Critical for Fertilization

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Abstract

Environmental exposures impact gamete function and fertility, but the mechanisms are poorly understood. Here we show that pheromones sensed by ciliated neurons in the *C. elegans* nose alter the lipid microenvironment within the oviduct, thereby affecting sperm motility. In favorable environments, pheromone-responsive sensory neurons secrete a TGF- β ligand called DAF-7, which acts as a neuroendocrine factor that stimulates prostaglandin-endoperoxide synthase (Cox)-independent prostaglandin synthesis in the ovary. Oocytes secrete F class prostaglandins that guide sperm toward them. These prostaglandins are also synthesized in Cox knockout mice, raising the possibility that similar mechanisms exist in other animals. Our data indicate environmental cues perceived by the female nervous system affect sperm function.

Diet and environment have profound, yet largely unexplained effects on fertility in many animals (1, 2). Essential components of the mammalian diet include the polyunsaturated fatty acids (PUFAs), which are oxidized into labile signaling molecules called prostaglandins (Fig. S1). The F class prostaglandins are among the most abundant and

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All data are provided in the main paper and supplementary materials.

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Supplementary Materials

Materials and Methods

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ubiquitous members. Prostaglandins are critical for reproduction (3), but their functions and regulatory mechanisms are incompletely understood. For instance, *in vitro* studies have shown that prostaglandins induce Ca^{2+} influx into human sperm via the CatSper channel (4, 5). The biological role of this mechanism is not clear, largely because monitoring sperm behavior in the female reproductive tract is difficult.

Cox enzymes, the targets of nonsteroidal anti-inflammatory drugs, are thought to be the exclusive enzymatic initiators of prostaglandin synthesis (3). Prostaglandin species are also formed non-enzymatically under conditions of high oxidative stress (6). These latter prostaglandins, which are esterified to phospholipids, lack biological regulation and stereoselective generation. The nematode *C. elegans* generates specific F class prostaglandins, including $\text{PGF1}\alpha$ and $\text{PGF2}\alpha$ stereoisomers independent of Cox (7, 8) (Fig. S1). Prostaglandin metabolism is regulated and has an important function in fertilization (8–10). Hence, *C. elegans* possesses an alternative metabolic pathway for F class prostaglandin synthesis.

Oocytes synthesize numerous F class prostaglandins from PUFA precursors provided in yolk lipoprotein complexes (8, 9). These prostaglandins function collectively to guide sperm to the spermatheca, the fertilization site (Fig. 1A) (7). The worm's transparent epidermis facilitates direct tracking of fluorescently labeled, motile sperm. Over 90% of sperm target the spermatheca (Z3) successfully one hour after mating (Fig. 1B).

Serendipitously, we previously found that mutations in the *daf-7* TGF- β ligand cause sperm targeting defects (8). *daf-7*, which is expressed in ASI sensory neurons (Fig. 1A), functions in a population density sensing mechanism (11–13). DAF-7 signals are transmitted through DAF-1 type I and DAF-4 type II receptors (Fig. 1C) (14). Mutations in *daf-7*, the *daf-1* and *daf-4* receptors, or downstream *daf-8* and *daf-14* R-Smads impair wild-type sperm targeting to the spermatheca (Fig. 1B and Tables S1–S2). Moreover, loss of the antagonistic co-Smad *daf-3* suppresses these defects (Fig. 1B and Table S1). We conclude that the DAF-7/TGF- β pathway is essential for sperm targeting. The mechanism is specific to the DAF-7 pathway, as sperm reach the spermatheca efficiently in other TGF- β pathway mutants (Fig. S2).

During larval development, the DAF-7/TGF- β pathway regulates dauer stage entry (11). In contrast to dauer, the sperm targeting mechanism is not temperature sensitive (Tables S1–S2). At 16°C, TGF- β mutants complete gonad development, generate oocytes that undergo meiotic maturation and ovulation, and are fertile (Fig. S3). Sperm targeting is abnormal and brood size is reduced (15), likely due to sperm loss. To test whether TGF- β functions in adults, we temporally inactivated *daf-1* or *daf-4* receptors using the RNAi feeding method. Down-regulating TGF- β signaling in young adults causes sperm targeting defects (Fig. S4). Therefore, DAF-7/TGF- β promotes sperm targeting independent of earlier roles.

The *daf-7* promoter drives gene expression in ASI neurons (11), which extend cilia through the nose to perceive ascaroside pheromones (13). Ascarosides accumulate as population density rises, triggering reduced *daf-7* expression (12, 13). To test whether *daf-7* functions in ASI for sperm guidance, we expressed *daf-7* cDNA in *daf-7* mutants under the ASI-specific *gpa-4* promoter (16). The *gpa-4p::daf-7* transgene rescues the sperm guidance

defects (Fig. 2A). The ascarosides asc-C6-MK and asc-C9, components of dauer pheromone, repress *daf-7* expression in ASI neurons (12). Synthetic asc-C6-MK and asc-

C9 application to adult hermaphrodites causes sperm targeting defects dependent on the *daf-7* promoter and *daf-3* Co-Smad (Fig. S5). We also observed sperm targeting defects in *daf-19* mutant hermaphrodites lacking sensory cilia, as well as in hermaphrodites exposed to high population density (Fig. S6). Collectively, the data indicate that DAF-7/TGF- β couples ascaroside perception to sperm function.

Sperm do not directly transduce TGF- β signals because wild-type sperm fail to target the spermatheca efficiently in receptor and Smad mutants (Fig. 1B and Tables S1–S2). To determine the cell type that transduces TGF- β signals, we conducted genetic mosaic analysis on the *daf-1* receptor. *daf-1* loss in the germ line or neuronal lineages causes sperm targeting defects, whereas loss in somatic gonadal, muscle, intestinal, or epidermal lineages does not appreciably affect sperm guidance (Fig. S7). Loss of germ line or neuronal *daf-1* expression is not as severe as complete *daf-1* loss, suggesting that both cell types transduce DAF-7 signals in parallel. As an alternative test, we used an RNAi mosaic strategy to restrict receptor inactivation to the adult germ line. Germline *daf-1* or *daf-4* receptor inactivation causes sperm targeting defects, whereas germline *daf-7* ligand inactivation did not (Fig. 2B). These results support the model that DAF-7 functions in part as a neuroendocrine factor that transmits signals directly to oögonia. We also identified a group of head interneurons that perceive DAF-7 signals for sperm guidance (Fig. 2C). These neurons regulate feeding rate and fat metabolism in a DAF-7-dependent manner (17). In contrast to feeding and metabolism, interneuron TGF- β signaling to the gonad appears to be mediated by insulin and steroid ligands (Fig. S8).

Given that DAF-7 receptors function in oögonia, we hypothesized that TGF- β promotes sperm-guiding F class prostaglandin synthesis. Inhibiting oocyte prostaglandin synthesis causes sperm to move with reduced velocity, little directional velocity, and high reversal frequency (7, 8). Similar motility parameters are observed in *daf-7* and *daf-1* mutants (Table S3). To directly test the hypothesis, we used liquid chromatography tandem mass spectrometry (LC-MS/MS) operated in multiple reaction monitoring (MRM) mode to measure prostaglandin levels in wild-type and *daf-1* mutant extracts. Oocytes synthesize a mixture of F class prostaglandins derived from dihomono- γ linolenic (F1 subclass), arachidonic and Ω -3 arachidonic (F2 subclass), and eicosapentaenoic acids (F3 subclass) (7). CePGF1 and CePGF2 are mixtures of co-eluting PGF1 α and PGF2 α stereoisomers, respectively, including the mirror image stereoisomer of PGF2 α called *ent*-PGF2 α (Fig. 3A). *daf-1* mutant extracts contain strongly reduced levels of all F class isomers (Fig. 3B). CePGF1 and CePGF2 are reduced by about 75% in *daf-1* mutants (Fig. 3C), similar to mutants lacking germ cells (7). We conclude that DAF-7/TGF- β signaling promotes oocyte prostaglandin synthesis essential for sperm guidance.

PGF2 α is prevalent in mammalian follicular fluid, although its functions are incompletely understood. Since DAF-7 regulates Cox-independent CePGF2 synthesis, we tested whether mice lacking Cox enzymes synthesize F class prostaglandins. The mouse genome contains two Cox genes called *Cox-1* (*ptgs1*) and *Cox-2* (*ptgs2*). *Cox-1*^{-/-}; *Cox-2*^{-/-} double knockout (dKO) mouse pups appear similar to wild-type pups at birth, but die within 12

hours due to a patent ductus arteriosus (18). To directly test whether Cox-independent prostaglandin synthesis occurs in mice, we extracted lipids from mutant and control pups delivered at term. MRM indicates that 6-keto PGF1 α , a stable metabolite of prostacyclin (see Fig. S9 for prostaglandin structures, retention times, and MRM transitions), is eliminated in dKO mouse extracts (Figs. 4A and S10A). In contrast, PGF2 α is present (Figs. 4A and S11). The PGF2 α stereoisomer 8-*epi*-PGF2 α is increased in the double mutants (Figs. 4A and S11) and an unidentified PGF2 α isomer is unaffected by *Cox* genotype (Fig. S11). More than 50% of total PGF2 α isomer levels remain in *Cox* dKO pups. PGD2 and PGE2 levels were slightly above the detection limit in wild-type pups. The absence of 6-keto PGF1 α , PGD2, and PGE2 isomers in *Cox* knockout extracts indicates that free radical-induced lipid peroxidation, which generates a nonselective mixture of prostaglandin isomers (6), is inconsequential (Fig. S10). These data indicate that Cox enzymes are not essential for mouse F class prostaglandin synthesis.

Our LC-MS/MS method separates most PGF2 α stereoisomers (7, 8, 19) (Fig. S9). The retention times of the three major PGF2 α peaks in *Cox* dKO mouse extracts are remarkably similar to those in *C. elegans* extracts. When mouse and worm extracts are run consecutively, the three PGF2 α isomer retention times are indistinguishable (Fig. 4B). We also found these PGF2 α isomers in brain, stomach, and small intestine tissues from a rare *Cox* dKO adult mouse that survived development (Fig. S12), as well as in wild-type adult mouse and zebrafish tissues (Fig. S13). These results support the idea that Cox-independent prostaglandin synthesis is evolutionarily ancient.

In summary, we delineate a *C. elegans* signaling pathway by which environmental cues are relayed from head sensory neurons to the gonad. As population density rises and food decreases, reduced DAF-7/TGF- β levels in ASI neurons down-regulate R-Smad activity in developing oocytes and head interneurons that modulate feeding rate and fat metabolism. These disrupted neuroendocrine pathways converge on oocytes to inhibit the Cox-independent conversion of PUFAs into F class prostaglandins. Consequently, sperm fail to locate the spermatheca efficiently, reducing the fertilization rate. This mechanism enables *C. elegans* adults to modulate reproductive output in response to a dynamic external environment.

There are two important implications from this study. First, female environmental perception can have strong effects on sperm function. Disrupting the neuroendocrine mechanisms that mediate these effects, through genetic mutation, dietary changes, or environmental exposures, impairs fertility. These mechanisms might be relevant to infertile human couples and internally fertilizing animals with disappearing or changing habitats. Second, an alternative Cox-independent pathway for F class prostaglandin synthesis emerged early in animal evolution. These prostaglandins likely modulate numerous biological processes, perhaps together with Cox-dependent prostaglandins. As prostaglandins are oxidative stress markers and therapeutic targets for many human disorders, the implications are far reaching.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. *C. elegans* sperm guidance and DAF-7/TGF- β pathway

(A) Anatomy of an adult hermaphrodite, showing ASI sensory neurons, nerve ring (NR), and proximal gonad. The oocytes (O), which synthesize F class prostaglandin sperm guidance cues, lie adjacent to the spermatheca (S, outlined in yellow). Males inject sperm into the uterus through the vulva (V). Sperm migrate around fertilized eggs (E) toward the anterior or posterior spermathecae. The uterus is divided into zones Z1, Z2, and Z3 for quantification. Sperm distribution is measured one hour after mating, whereas sperm motility is measured immediately. (B) Fluorescent (TRITC) wild-type sperm distribution in control and selected TGF- β pathway mutants one hour after mating. Average zone distributions $\pm$ SEM are shown. See Tables S1–S3 for complete data. (C) Major DAF-7/TGF- β pathway components.

Figure 2. TGF- β pathway sites of action

(A) Fluorescent (TRITC) wild-type sperm distribution in *daf-7* transgenic mutants. The spermatheca (yellow) is outlined. Average zone distributions $\pm$ SEM are shown. (B) Spermatheca (Z3) targeting following adult germline-restricted TGF- β ligand and receptor inactivation using *rrf-1* mutants (20). (C) Z3 targeting in *daf-1* mutants expressing *daf-1* cDNA under tissue-specific promoters. The *glr-4*, *nmr-2*, and *tdc-1* promoters drive expression in nerve ring interneurons (Fig. 1A). The common target among these promoters is RIM/RIC interneurons (17). Error bars are SEM preceding trial number. **, $P < 0.001$.

Figure 3. Prostaglandins in wild-type and *daf-1* mutant extracts

(A) Planar structures of *C. elegans* prostaglandins CePGF1 and CePGF2. CePGF2 is predominantly *ent*-PGF2 α (7). (B) MRM chromatograms of wild-type and *daf-1* mutant extracts. Worms were grown at 16°C and shifted to 25°C for 24 hours. The F1 class was detected with mass transition m/z 355/311, the F2 class with m/z 353/193, and the F3 class with m/z 351/193 (7, 19). (C) Quantification of CePGF1 and CePGF2 (*, $P < 0.05$ $N=3$). Error bars are SEM. RT, retention time.

Figure 4. Prostaglandins in wild-type and Cox knockout mouse extracts

(A) Quantification of selected prostaglandins. (B) MRM chromatograms showing PGF2 α isomer retention times (RT) in *C. elegans* (red) and Cox dKO (black) extracts. 8-*epi*-PGF2 α elutes at RT= 11.4 and PGF2 α elutes at RT=11.8. The *C. elegans* isomer at RT=12.1 is CePGF2b, a PGF2 α stereoisomer (7). *, $P < 0.05$. **, PGF2 α and *ent*-PGF2 α co-elute, as do 8-*epi*-PGF2 α and 8-*epi*-15(*R*) PGF2 α .