Distinct roles of transcription factors EGL-46 and DAF-19 in specifying the functionality of a polycystin-expressing sensory neuron necessary for C. elegans male vulva location behavior

Hui Yu¹, René F. Prétôt², Thomas R. Bürglin³ and Paul W. Sternberg¹,*

¹HHMI and Division of Biology, California Institute of Technology, Pasadena, CA 91125, USA
²Division of Cell Biology, Biozentrum, University of Basel, Klingelbergstr. 50/70, CH-4056 Basel, Switzerland
³Department of Biosciences at Novum, Karolinska Institutet, Alfred Nobels alle 7, Södertörns Högskola, SE-141 89 Huddinge, Sweden

*Author for correspondence (e-mail: pws@caltech.edu)

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Summary

Caenorhabditis elegans polycystins LOV-1 and PKD-2 are expressed in the male-specific HOB neuron, and are necessary for sensation of the hermaphrodite vulva during mating. We demonstrate that male vulva location behavior and expression of lov-1 and pdk-2 in the ciliated sensory neuron HOB require the activities of transcription factor EGL-46 and to some extent also EGL-44. This EGL-46-regulated program is specific to HOB and is distinct from a general ciliogenic pathway functioning in all ciliated neurons. The ciliogenic pathway regulator DAF-19 affects downstream components of the HOB-specific program indirectly and is independent of EGL-46 activity. The sensory function of HOB requires the combined action of these two distinct regulatory pathways.

Key words: Transcriptional regulation, Cell specification, Zinc finger proteins, TEF, RFX factors, Polycystins

Introduction

Because of its simple nervous system with invariant cell lineage and position, C. elegans provides an excellent model to study how diverse neuronal subtypes are specified (Sulston and Horvitz, 1977; Sulston, 1983). The anatomy and interconnectivity of all 118 hermaphrodite neuron types are known (White, 1986), as are the molecular details of many neuronal subtypes (Chalfie, 1995). The C. elegans male has 79 additional neurons, falling into 37 classes (Sulston et al., 1980).

Most of those male-specific neurons are located in the tail region and contribute to specific motor output during mating behavior (Sulston and White, 1980; Loer and Kenyon, 1993; Liu and Sternberg, 1995; Garcia et al., 2001; Garcia and Sternberg, 2003).

During mating, the C. elegans male scans for the vulva by touching the hermaphrodite with the ventral side of his tail and backing along her body. If the vulva is not found, he turns at the hermaphrodite head or tail and scans the other side (Liu and Sternberg, 1995). The male hook sensillum is a copulatory structure that is located just anterior to the cloaca and mediates vulval location behavior (Liu and Sternberg, 1995). Intact wild-type males usually stop at their first or second vulval encounter. When the hook sensillum is ablated, operated males circle the hermaphrodite multiple times and fail to stop at the vulva (Liu and Sternberg, 1995). This defect is referred to as the Lov (location of the vulva defective) phenotype (Barr and Sternberg, 1999). The hook sensillum consists of five cells, including a structural cell and two ciliated sensory neurons HOA and HOB (Sulston et al., 1980). The two hook neurons have large nuclei and send dendrites into the hook structure; however, their anatomy can be distinguished by cell morphology and synaptic contacts (Sulston et al., 1980). Ablation of either HOA or HOB results in alov phenotype, indicating that HOA and HOB have non-redundant functions (Liu and Sternberg, 1995).

The C. elegans homologues of human autosomal dominant polycystic kidney disease genes PKD1 (lov-1) and PKD2 (pdk-2) are expressed in the HOB hook neuron (Barr and Sternberg, 1999; Barr et al., 2001; Kaletta et al., 2003). Human PKD genes, which encode divergent members of the TRP family of cation channels, possibly act in signal transduction important for renal epithelial differentiation, as mutations in PKD1 and PKD2 are associated with pathogenic renal cyst formation (reviewed by Wu, 2001). In C. elegans, lov-1 and pdk-2 mutations disrupt vulva location behavior, consistent with a defect in HOB sensory function (Barr and Sternberg, 1999; Barr et al., 2001). Although LOV-1 and PKD-2 are localized in sensory cilia and cell bodies, the ultrastructure of cilia and dendrites appears normal in lov-1 and pdk-2 mutants (Barr et al., 2001).

Another class of genes required for vulva location affects the formation of ciliated endings in sensory neurons. This class includes che-3, daf-10, osm-5 and osm-6 (Barr and Sternberg, 1999). che-3, osm-5 and osm-6 are required for most or all sensory cilia (Lewis and Hodgkin, 1977; Perkins et al., 1986), while daf-10 only functions in a subset of ciliated sensory neurons (Albert et al., 1981). The hermaphrodite expression of osm-5, a homolog of the mouse autosomal recessive polycystic
kidney disease (ARPKD) (Haycraft et al., 2001; Qin et al., 2001), and osm-6 has been shown to be regulated by a RFX transcription factor DAF-19, which plays a critical role in general sensory cilium differentiation (Swoboda et al., 2000; Haycraft et al., 2001).

We report the isolation of an allele of egl-46, a putative zinc-finger transcription factor, in a screen for loci required for fate specification of C. elegans hook neuron HOB. egl-46 was previously characterized as a gene when mutated affecting the development of two mechanosensory neurons (FLP cells) (Wu et al., 2001), as well as having defects in the hermaphrodite HSN egg-laying motoneurons (Desai et al., 1988; Desai and Horvitz, 1989). We demonstrate that EGL-46 and the transcription enhancer factor (TEF) homolog EGL-44 are expressed in the HOB hook neuron and are required for expression of genes encoding polycystins LOV-1 and PKD-2, homeodomain protein CEH-26, and neuropeptide-like protein NLP-8. egl-44 and egl-46 mutants are defective in vulva location behavior during mating, suggesting compromised normal HOB function. This HOB-specific pathway is distinct from the DAF-19-regulated general cilia formation pathway in sensory neurons. We found that daf-19 acts independently of egl-44 and egl-46 to affect expression of downstream genes in the HOB-specific program, indicating that general and cell-specific regulatory factors work in concert to establish cell-specific features crucial for HOB neuronal function in sensory behavior.

Materials and methods

Strains

Nematodes were cultured at 20°C as described (Brenner, 1974). All strains used contain him-5(e1490) V to obtain males, except for egl-46(n127), in which case we used him-5(e1490) IV (Hodgkin et al., 1979). The following alleles were used in this study: daf-19(m86) II (Perkins et al., 1986); egl-46(n1080) II, egl-46(n1127) V (Desai and Horvitz, 1989); phe-1(e12123ts) III (Schnabel and Schnabel, 1990); unc-119(ed4) X (Ferguson and Horvitz, 1989); III (Schnabel and Schnabel, 1990); pha-1(e2123ts) egl-44(n1080) at 40 ng/µl using pmyo-2::gfp plasmid pPD118.33 (5.5 ng/µl) as co-transformation marker (Mello et al., 1991). Three stable lines were obtained from individual F1 progeny that expressed pmyo-2::gfp in pharynx. Injection of cosmid K11G9 restored the ceh-26::gfp expression in HOB in 76/81 males from three independent transgenic lines. Injection of another cosmid in the same interval, F44C4, which contains a different predicted zinc-finger transcription factor, showed no rescue of HOB expression of ceh-26::gfp in fourteen stable transgenic lines (n = 172). Those transgenic lines had a non-sex-specific ectopic expression of ceh-26::gfp in a neuron anterior to HOB, most likely PVT. It is not clear that this ectopic expression is due to injected F44C4 cosmid or interaction between pmyo-2::gfp plasmid and F44C4 cosmid. Cosmids were obtained from the Sanger Institute (Cambridge, UK).

To test for complementation, PS3568 ceh-26::gfp; egl-46(n127) him-5(e1490) males were crossed to MT2316 egl-46(n1127) hermaphrodites. F1 hermaphrodites with Ceh-26::GFP expression were cross progeny, and were examined for an Egl phenotype. F1 males were analyzed by HOB expression of ceh-26::gfp. All 79 sy628(1127) heterozygous males examined lacked ceh-26::gfp expression in HOB, and heterozygous hermaphrodites were Egl. Thus, sy628 and n1127 fail to complement.

PCR and sequencing

A 2318 bp genomic DNA fragment containing the entire egl-46 coding region was PCR amplified from sy628 mutant DNA using the pair of primers 5'-CTGCCCTTCTTGTGAAGGTTCTT-3' and 5'-AATTCATCAGGAATTTGGAAA-3'. The PCR products from six independent PCR reactions were separately purified using QiAquick PCR purification kit and were pooled together for direct sequencing. Two nested primers, 5'-TTGTTACACACGCGTAACCC-3' to the 5' end of the gene and 5'-CCGGGGGAAATGGAAGGAAGT-3' to the 3' end, and two internal primers, the reverse primer 5'-CCTTATGTGCGGATCTCTTT-3' at 109-131 bp of the intron 2 and the forward primer 5'-GCTTACGAGCAGAGGAAGAAGAC-3' at 274-297 bp of the same intron, were used for sequencing. This sequencing therefore did not cover the 189 bp gap in the intron 2 between reverse and forward primers. The PCR primers and two outside sequencing primers were picked by an oligo design program in the C. elegans genome project at the Sanger Institute (www.sanger.ac.uk/Projects/C_elegans/). The two internal sequencing primers were obtained using Macvector software (Oxford Molecular Group). The G-to-A lesion site at nucleotide 165 of the first exon was observed in both strands.

Transgenics

The N-terminal cfp::egl-46 translational fusion plasmid TU#627 and yfp::egl-44 fusion plasmid TU#628 were kindly provided by Ji Wu and Martin Chalfie. Plasmid DNAs of TU#627 and TU#628 were carrying the HOB marker ceh-26::gfp with EMS using standard protocols (Rosenbluth et al., 1983). In particular, we picked males descended from each single hermaphrodite daughter of mutagenized parents and examined them under a conventional epi-fluorescence microscope for GFP expression. Three-factor mapping of sy628 on linkage group V used alleles of unc-46, dpy-11, unc-68 and unc-42: unc-46 (16/16 recombinants) dpy-11 (0/16 recombinants) sy628; dpy-11 (0/44) sy628 (44/44) unc-42; dpy-11 (4/10) sy628 (6/10) unc-68. During the mapping experiments, the presence of sy628 mutation was determined by loss of ceh-26::gfp expression in HOB.

The -0.6 map unit interval between dpy-11 and unc-68 was covered by 17 cosmids, including 97 identified genes or predicted coding sequences (www.wormbase.org, version WS74). The sy628 hermaphrodites had a mild egg-laying defective (Egl) phenotype. A previously identified gene associated with an Egl phenotype, egl-46, is located in the middle of that interval. Cosmid K11G9, which contains the entire egl-46 locus, was injected into the strain PS3568 ceh-26::gfp; egl-46(n127) him-5(e1490) at 40 ng/µl using pmyo-2::gfp plasmid pPD118.33 (5.5 ng/µl) as co-transformation marker (Mello et al., 1991). Three stable lines were obtained from individual F1 progeny that expressed pmyo-2::gfp in pharynx. Injection of cosmid K11G9 restored the ceh-26::gfp expression in HOB in 76/81 males from three independent transgenic lines. Injection of another cosmid in the same interval, F44C4, which contains a different predicted zinc-finger transcription factor, showed no rescue of HOB expression of ceh-26::gfp in fourteen stable transgenic lines (n = 172). Those transgenic lines had a non-sex-specific ectopic expression of ceh-26::gfp in a neuron anterior to HOB, most likely PVT. It is not clear that this ectopic expression is due to injected F44C4 cosmid or interaction between pmyo-2::gfp plasmid and F44C4 cosmid. Cosmids were obtained from the Sanger Institute (Cambridge, UK).

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Mating behavior of mutant or control males was observed with Mating assay (Vulva location behavior) marker had similar expression patterns in the male tail. CF and YFP plasmids but with lines was characterized. Transgenic animals generated with the same obtained for each construct and the male expression pattern in those expressed as the co-injection marker. Transgenic animals were recognized by rescue of the Unc phenotype of unc-119 (Maduro and Pilgrim, 1995). Three independent lines were obtained for each construct and the male expression pattern in those lines was characterized. Transgenic animals generated with the same CFP and YFP plasmids but with myo-2::gfp as a transformation marker had similar expression patterns in the male tail.

### Table 1. HOB gene expression in wild type, egl-46 and egl-44 males

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Marker gene</th>
<th>GFP expression in the HOB neuron</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>Wild type</td>
<td>ceh-26::gfp*</td>
<td>100%</td>
</tr>
<tr>
<td>egl-46(sy628)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>egl-46(n1127)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>egl-44(n1080)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>lov-1::gfp†</td>
<td>93%</td>
</tr>
<tr>
<td>egl-46(sy628)</td>
<td></td>
<td>1%</td>
</tr>
<tr>
<td>egl-44(n1080)</td>
<td></td>
<td>21%</td>
</tr>
<tr>
<td>Wild type</td>
<td>pkd-2::gfp(n1s128)*</td>
<td>100%</td>
</tr>
<tr>
<td>egl-46(sy628)</td>
<td></td>
<td>4%</td>
</tr>
<tr>
<td>Wild type</td>
<td>pkd-2::gfp(n1s133)*</td>
<td>100%</td>
</tr>
<tr>
<td>egl-46(sy628)</td>
<td></td>
<td>8%</td>
</tr>
<tr>
<td>egl-46(n1127)</td>
<td></td>
<td>2%</td>
</tr>
<tr>
<td>egl-44(n1080)</td>
<td></td>
<td>87%</td>
</tr>
<tr>
<td>Wild type</td>
<td>nlp-8::gfp§</td>
<td>96%</td>
</tr>
<tr>
<td>egl-46(sy628)</td>
<td></td>
<td>96%</td>
</tr>
<tr>
<td>egl-44(n1080)</td>
<td></td>
<td>96%</td>
</tr>
<tr>
<td>Wild type</td>
<td>osm-6::gfp*</td>
<td>17%</td>
</tr>
<tr>
<td>egl-46(sy628)</td>
<td></td>
<td>100%</td>
</tr>
<tr>
<td>egl-44(n1080)</td>
<td></td>
<td>100%</td>
</tr>
<tr>
<td>Wild type</td>
<td>osm-5::gfp†</td>
<td>96%</td>
</tr>
<tr>
<td>egl-46(sy628)</td>
<td></td>
<td>100%</td>
</tr>
<tr>
<td>egl-44(n1080)</td>
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<tr>
<td>Wild type</td>
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<td>100%</td>
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<tr>
<td>egl-44(n1080)</td>
<td></td>
<td>94%</td>
</tr>
<tr>
<td>Wild type</td>
<td></td>
<td>98%</td>
</tr>
</tbody>
</table>

*Integrated transgenes
†Extrachromosomal arrays; a few animals lack expression in HOB because of mosaicism.

Results

Mutations of egl-46 affect gene expression in the hook neuron and disrupt vulva location behavior in male mating

The male tail is remodeled during the L4 stage, undergoing a series of changes in cell shape and position (Sulston et al., 1980). By the late L4 stage, most of the cells that function in adults reach their final locations, and initiate morphological changes to form the adult tail structures. At this stage, a homeodomain-containing putative transcription factor ceh-26 (Bürglin, 1994) begins to be expressed and persists through the adulthood in the HOB hook neuron (Fig. 1A1,A2). Therefore, the presence of CEH-26::GFP indicates a differentiated neuronal fate of HOB. Non-sex-specific expression of ceh-26::gfp is mostly in nuclei of the head (R. F. P. and T. R. B., unpublished). To identify genes involved in HOB fate specification, we performed a screen for mutants with altered expression of ceh-26::gfp in the HOB cell. This pattern allows for a rapid visual inspection of GFP fluorescence under a compound microscope in the male tail.

One of the mutants recovered from this screen, sy628, failed to express ceh-26::gfp in the HOB neuron of homozygous males with complete penetrance (Fig. 1A3; Table 1). No effect on non-sex-specific ceh-26::gfp expression (e.g., the head nuclei) was observed in sy628 animals, suggesting that the sy628 mutation does not cause a general defect in expression of GFP transgenes or of ceh-26 (data not shown). Anatomical examination of sy628 males at the third and the fourth larval stages showed that P10.ppap, the presumptive HOB neuron in wild-type animals, was present and occupied its normal position in sy628 mutants. In addition, the hook structure and overall tail morphology appeared normal under Nomarski

Microscopy

GFP expression was analyzed by conventional fluorescence microscopy (Zeiss Axioskop) using a Chroma Technology High Q GFP long-pass filter set (450 nm excitation, 505 nm emission). CFP and YFP were visualized using a Chroma Technology GFP filter set ‘31044v2’ (exciter D436/20, emitter D480/40, beamsplitter 455dclp) and an YFP set ‘41029’ (exciter HQ 500/20, emitter HQ520lp, beamsplitter Q515lp).
Initial observations indicated that sy628 males had a decreased mating efficiency. Analysis of their mating behavior determined that sy628 males were deficient in vulval location (the Lov phenotype), but had no obvious defect in other steps of mating, such as response, turning, spicule insertion, or sperm transfer. About 97% of wild-type control males stopped at the vulva during the first two vulva encounters (88% vulva location efficiency), as opposed to only 39% of sy628 males (Fig. 2A). On average, sy628 males required more than five encounters to find the vulva, with an overall vulva location efficiency of 36%.

We mapped sy628 to linkage group V between dpy-11 and unc-68, and identified it as an allele of egl-46 (see Materials and methods). egl-46 encodes a putative C2H2-type zinc-finger transcription factor homologous to human and mouse IA1 protein, mouse MLT1 protein and Drosophila Nerfin 1 protein (Wu et al., 2001). The lesion in sy628 mutants was a G-to-A transition at position 165 of the first exon (161-TCTGGAAACCAACGC-175), which changes a tryptophan codon UGG to an UGA opal stop codon. This residue is located at position 55 out of 286 of the inferred EGL-46 protein, before the putative glutamine-rich transcriptional activation domain (residues 61 to 75) and other conserved domains (Wu et al., 2001). This early stop is not necessarily a null allele.

We confirmed the male phenotypes of the egl-46 mutant using a different allele, n1127, which alters the splicing donor of intron 2, located before the region encoding the three zinc fingers of EGL-46 protein (Wu et al., 2001). n1127 and sy628 failed to complement (see Materials and methods). Desai and Horvitz (Desai and Horvitz, 1989) found that n1127 has a decreased male mating efficiency (~50%). We observed that n1127 males had a Lov phenotype similar to sy628 mutants (Fig. 2B). The vulva location efficiency of n1127 males was 39% (n=17), compared with 94% (n=16) for the control males.

**Fig. 1.** HOB gene expression in wild-type and mutant males. Left lateral views (anterior leftwards, ventral downwards). Scale bar: 20 μm. (A1,A2) Expression of ceh-26::gfp in the HOB neuron of a wild-type adult male. Absence of fluorescence in an egl-46(sy628) mutant (A3), an egl-44(n1080) mutant (A4) and a daf-19(m86) mutant (A5). (B1,B2) HOB and ray expression of pkd-2::gfp was observed in a wild-type adult male. (B3) An egl-46(sy628) male with ray but not HOB expression. (B4) An egl-44(n1080) male with expression in both HOB and ray cells. (B5) No visible expression in both HOB and rays of a daf-19 mutant. (C1,C2) Normal osm-6::gfp expression in HOA and HOB at the L4 stage. Expression was not affected in egl-46(sy628) (C3) and egl-44(n1080) mutants (C4). (C5) No expression was observed in HOA and HOB cells of a daf-19(m86) mutant male. Cell positions of HOB in A3,A4,A5,B3,B5, and HOA and HOB in C5 were located by overlaying with the Nomarski pictures of the same animal. Hook structure autofluorescence is indicated by small arrows. The original osm-6::gfp strain has a ncl-1(–) background. ncl-1(–) was still present in the him-5 strain of osm-6::gfp integrant (C1,C2) and an egl-46(sy628) mutant background (C3), but was crossed out in egl-44(n1080) (C4) and daf-19(m86) mutants (C5). egl-46(sy628) mutant also had a dpy-11 mutation in the background (C3). No effect on osm-6::gfp expression was detected for ncl-1(–) and dpy-11 mutations.
There was a marked decrease of ceh-26::gfp expression in n1127 HOB neurons (Table 1). Only two out of 118 n1127 homozygous males examined retained a faint GFP expression in HOB. No altered expression of ceh-26::gfp was detected in cells other than HOB in n1127 mutants.

**Fig. 2.** Vulval location behavior. The x-axis represents the number of vulva encounters measured until a tested male stopped at the hermaphrodite vulva. The y-axis represents the distribution of males in the tested group that located the vulva at each vulva encounter. (A) egl-46(+) versus egl-46(sy628). Both strains have ceh-26::gfp III; him-5(e1490) V in the background. (B) egl-46(+) versus egl-46(n1127). Strains in B contain him-8(e1489) IV. (C) egl-44(+) vs. egl-44(n1080). Animals in C are all with him-5(e1490) V. In each assay, similar number of wild-type control males and mutant males were examined at same time using the same microscope.

The dependence of ceh-26::gfp expression on EGL-46 activity suggested that the defective HOB sensory behavior caused by an egl-46 mutation could result from loss of HOB-specific gene expression. The C. elegans polycystin genes lov-1 and pkd-2 are expressed in HOB and are required for vulva location (Barr and Sternberg, 1999) (Table 1; Fig. 1B1,B2). To test whether egl-46 regulates these two genes, we used GFP transgenes to visualize their expression in an egl-46(sy628) mutant background. sy628 mutants lacked expression of lov-1::gfp in the HOB neuron (Table 1): only one out of 92 animals examined had detectable expression. The expression of pdk-2::gfp in HOB was also greatly reduced by the sy628 and n1127 mutations of egl-46 (Table 1; Fig. 1B3). A neuropeptide-like protein-encoding gene, nlp-8, is also expressed in HOB as well as in the non-sex-specific neuron PVT in the tail (Nathoo et al., 2001). The PVT expression of nlp-8::gfp was not affected by egl-46 mutations; however the HOB expression of nlp-8::gfp was absent in about half of egl-46(sy628) males and was decreased in the remainder (Table 1). Therefore, EGL-46 activity is necessary for the HOB expression of all three genes, (Fig. 3A-D), consistent with the timing of HOB differentiation, and a potential role in the maintenance of HOB function. No detectable expression was seen in the HOA hook neuron. The egl-46 mating defect is reminiscent of ablation of a hook neuron (Liu and Sternberg, 1995). Based on expression of egl-46 gene in a single hook neuron, we infer that the Lov phenotype of egl-46 mutant males is probably due to impaired HOB function.

The hermaphrodite expression pattern of egl-46 has been described by Wu et al. (Wu et al., 2001). Using an egl-46::cfp construct, we analyzed its expression in males and found a similar pattern for non-sex-specific expression (such as the FLP cells, ventral cord neurons and PVD). Both HOA and HOB are born from a single precursor cell (P10.p) at the late L3 stage, and they differentiate into their neuronal fates during the L4 stage. egl-46::cfp was expressed in the HOB neuron beginning at the L4 stage and continuing throughout adulthood.
and the lack of lov-1 and pkd-2 expression could account for the mating defect of egl-46 mutants.

We also observed male-specific egl-46:::cfp expression in ciliated ray neurons. The C. elegans male has nine pairs of rays (ray 1-9 for both the left and right sides), each associated with an A-type neuron and a B-type neuron (RnA and RnB, n=1-9) (Sulston et al., 1980). egl-46:::cfp was observed in one of the two ray neurons for each ray (Fig. 3D,E); this neuron is an A-type neuron and a B-type neuron (RnA and RnB, n=1-9) (Sulston et al., 1980). (C,D) An L4 male with faint YFP expression in the PCB, PCC and PCh cells of the left postcloacal sensilla, in addition to cells from ray lineage in the background. (E,F) Bright expression in hypodermal R1.p, R2.p, R3.p, R4.p, and R5.p at the left side (arrowheads).

Fig. 4. egl-44:::yfp expression in the male tail. Left lateral view. Scale bars: 20 μm. (A,B) Different levels of YFP expression in PVX, PVY, HOA and HOB (arrows). In this particular animal, HOA has extremely faint YFP fluorescence (in most cases, YFP expression is undetectable in the HOA hook neuron; data not shown). (C,D) An L4 male with faint YFP expression in the PCB, PCC and PCh cells of the left postcloacal sensilla, in addition to cells from ray lineage in the background. (E,F) Bright expression in hypodermal R1.p, R2.p, R3.p, R4.p, and R5.p at the left side (arrowheads).

egl-44 exhibits a similar Lov defect for male mutants and may regulate gene expression in HOB

Wu et al. (Wu et al., 2001) reported that egl-46 acts with egl-44 to specify subtypes of mechanosensory neurons, and for HSN development in hermaphrodites (Desai and Horvitz, 1989). egl-44 encodes a transcription enhancer factor of the TEA domain class (Bürghlin, 1991) and is orthologous to the mammalian TEF factors (Wu et al., 2001). We therefore examined the behavior of egl-44(n1080) males, and found that this egl-44 mutation reduced vulva location behavior (Fig. 2C). Similar to egl-46 mutants, egl-44 mutant males passed the vulva frequently and it took an egl-44(n1080) male about five encounters on average to locate the vulva. Specifically, egl-44 mutant males had an overall 43% vulva location efficiency, while control males (wild-type for the egl-44 locus) had an 88% vulva location efficiency.

We determined the male tail expression pattern of the egl-44 gene with the yfp construct described by Wu et al. (Wu et al., 2001). Expression of egl-44 overlapped with but was not identical to that of egl-46. At the L4 stage, the four neurons PVX, PVY, HOA and HOB are positioned in a signature anterior-to-posterior row at the middle left side (Sulston et al., 1980). egl-44:::yfp fluorescence was obvious in HOB, PVX and PVY, with HOB usually the brightest, but was barely visible in HOA (Fig. 4A,B). As stated above, egl-46:::cfp was only present in HOA. A few neurons anterior to PVX (e.g. PVV) had faint egl-44:::yfp expression, as did several cells from the B and Y lineage, including PCB, PCC and PCh (Fig. 4C,D). These cells did not express egl-46:::cfp. In addition, almost all the descendants of the ray precursor cells (Rn) expressed egl-44:::yfp, including the ray neurons (RnA and RnB) and the ray structure cells (Rnst), all of which are derived from the anterior daughter Rn.a, as well as posterior daughter Rn.p hypodermal cells (Fig. 4E,F; data not shown). EGL-46 showed a more limited expression in the ray lineage. In adults, egl-44:::yfp was still expressed in HOB, RnA, RnB and Rnst cells. Hypodermal Rn.p cells no longer displayed bright YFP expression in adults, possibly because of their fusion with the tail hypodermal syncytium. Owing to dramatic changes in cell shapes and positions during the extensive male tail remodeling at the L4-adult transition, the faint egl-44:::yfp expression in PCB, PCC and PCh was hard to trace in adults. Overall, egl-44:::yfp was expressed more extensively in the male tail than was egl-46. However, a mutation in egl-44 did not result in broader defects in male mating behavior than did an egl-46 mutation.

Based on its behavioral phenotype and its expression in HOB, egl-44 might regulate HOB fate specification, similar to egl-46. We therefore examined HOB-specific gene expression in an egl-44(n1080) mutant background, and found that egl-44 mutants displayed a significant decrease in HOB-specific expression of ceh-26::gfp. 50% (49/99) of egl-44(n1080) males lacked ceh-26::gfp in HOB, while the remaining 50% (50/99) had weak HOB expression (Fig. 1A; Table 1). Reduction of lov-1::gfp expression in HOB by an egl-44 mutation was striking, but only a small effect on pkd-2 and nlp-8 expression was observed (Table 1; Fig. 1B). The lesion in egl-44(n1080) allele is a missense mutation. It is possible that the residual EGL-44 activity in n1080 mutants led to an incomplete reduction of HOB gene expression. egl-44 has six differently spliced isoforms (www.wormbase.org, version WS74). The n1040 mutation affects four of them. Currently, we have no information about which isoform might be dominate in the HOB neuron. Expression of the ‘c’ form egl-44 CDNA under control of the 3.1kb egl-46 promoter gave an ambiguous result, with only about 10% restoration of ceh-26::gfp expression in
HOB in each of three transgenic lines (data not shown). egl-44 mutants were not defective in ray B neuron expression of lov-1 and pdk-2.

Even though the egl-46 mutations caused a more severe defect in HOB gene expression than did an egl-44 mutation, the Lov phenotypes are similar in male mutants. One possibility is that incomplete decrease of gene expression in the HOB neuron by the egl-44 mutation could reduce the HOB function enough to display a comparable Lov phenotype; however, we cannot rule out the possibility that EGL-44 and EGL-46 might have some distinct targets in HOB. In addition, the faint EGL-44 expression in the HOA hook neuron, as well as in the PCB and PCC neurons of the postcloacal sensilla, might also contribute to the vulva location activity (Liu and Sternberg, 1995). The Lov phenotype is not synergistic in the egl-44; egl-46 double mutant, and there was no observable difference in the efficiency of vulva location compared with single mutants (data not shown). By contrast, C. elegans males with HOB ablated have a 0% vulva location efficiency (Liu and Sternberg, 1995). Both egl-44 and egl-46 mutants had an incomplete loss of nlp-8::gfp expression, but no further elimination of nlp-8::gfp expression was seen in an egl-44; egl-46 double mutant background (Table 1). This lack of enhancement for the Lov phenotype and a defect in nlp-8 expression indicates that egl-44 and egl-46 act at least partially in a common pathway for HOB specification. The egl-44; egl-46 double mutant males seemed less active than each of the single mutants and took longer to initiate mating behavior, which might be due to insufficient function of the ray neurons in the double mutant.

**egl-44 and egl-46 do not regulate each other’s expression in the HOB neuron**

In the non-sex-specific FLP cells, wild-type egl-44 is required for normal egl-46 expression (Wu et al., 2001). To determine whether egl-44 and egl-46 regulate each other’s expression in the HOB neuron, we introduced an extrachromosomal egl-46::cfp array into an egl-44 mutant, and an egl-44::yfp array into an egl-46 mutant. The timing and relative brightness of egl-46::cfp expression in HOB was not affected in an egl-44(n1080) mutant background compared with a wild-type background, but CFP expression in FLP neurons was reduced. Similarly, no change in the HOB expression of egl-44::yfp was observed in egl-46(sy628) males. We infer that there is no interdependence of egl-44 and egl-46 expression in HOB.

**The daf-19 general cilium formation pathway is required for cell-specific features of HOB**

Genes that are expressed in HOB and mutate to a Lov phenotype can be grouped into two separate pathways (Barr and Sternberg, 1999) (this work). osm-5 and osm-6 belong to a general ciliogenic pathway common to all ciliated neurons, including HOA and HOB (Collet et al., 1998; Qin et al., 2001). The other genes discussed above, including egl-44, egl-46, lov-1 and pdk-2, define a program specific for HOB differentiation. We thus asked if there are any interactions between these two pathways; i.e., whether regulators in the cell-specific pathway, egl-44 and egl-46, affect the HOB expression of the general cilium structure genes (osm-5 and osm-6), and whether ciliogenesis might be a prerequisite for execution of an HOB-specific program.

In wild-type males, OSM-5::GFP and OSM-6::GFP are expressed in the cell bodies and dendrites of HOA and HOB at the late L4 stage; their then expression decreases, which is coincident with the formation of ciliated sensory endings in these two neurons (Collet et al., 1998; Qin et al., 2001). Using an integrated osm-6::gfp line (mn1s17) and an extrachromosomal array carrying osm-5::gfp, we found that the HOB expression of these two GFPs at the L4 stage in egl-44(n1080) and egl-46(sy628) mutants was comparable with wild-type (Table 1; Fig. 1C1-C4). osm-5::gfp expression in HOA and HOB was also not affected by egl-46(n1127) (Table 1). In these egl-44 and egl-46 mutant males, the HOB dendritic process, visualized by osm-5::gfp or osm-6::gfp, was extended correctly into the male hook. Neither egl-44(n1080) nor egl-46(sy628) mutants had dye-filling defects (data not shown). We conclude that mutation of either egl-44 or egl-46 impedes neither gross cell morphology nor the ultimate neuronal outgrowth and wiring of HOB.

Qin et al. (Qin et al., 2001) showed that an osm-5 mutation affects subcellular localization of LOV-1 and PKD-2, but not their expression. We found that ceh-26::gfp expression was not affected in osm-5(p813) animals. Therefore, it is unlikely that establishment of the HOB-specific program depends on the activities of downstream structure genes (such as OSM-5) in the ciliogenic pathway. The RFX transcription factor DAF-19 is a key upstream regulator of general ciliogenesis (Swoboda et al., 2000; Haycraft et al., 2001). In the male tail, we observed exclusively nuclear-localized GFP expression of daf-19 in
male-specific ciliated sensory neurons, including the two hook neurons (Fig. 5A,B) and the 36 ray neurons. The fluorescence in HOA was usually fainter than in HOB. We observed no difference in the HOB expression of daf-19::gfp in egl-44 or egl-46 mutants compared with wild type (Fig. 5C,D). We then analyzed egl-44::yfp and egl-46::cyp in daf-19(m86) mutant males, and found that the timing and relative brightness of expression in HOB was similar to daf-19(+ ) animals. We infer that, during HOB differentiation, egl-44 and egl-46 are expressed independently of a general cilium formation pathway governed by daf-19.

We next examined the expression of three HOB-specific genes (ceh-26, pkd-2 and nlp-8) in daf-19 mutants. Swoboda et al. (Swoboda et al., 2000) have shown that daf-19 is required for general cilium formation, but not for cell-specific properties. Surprisingly, daf-19(m86) mutants lacked ceh-26 HOB expression (n=87) (Fig. 1A5). Non-sex-specific, non-sex-specific expression of ceh-26::gfp in some head neurons was also substantially reduced by the daf-19 mutation. All male-specific expression of pkd-2 was diminished in the daf-19 mutant background, including the four ciliated CEM neurons in the head, and the HOB and B-type ray neurons in the tail (n=97) (Fig. 1B5). Only the faint non-sex-specific pkd-2::gfp expression in a few neurons posterior to the nerve ring was retained in daf-19 mutant animals. Similarly, expression of nlp-8::gfp in daf-19(m86) males was only observed in the non-sex-specific PVT neuron and was totally absent in the HOB neuron (n=91). Therefore, complete execution of the HOB-specific program requires DAF-19 activity.

DAF-19 has been proposed to act on the X-box motifs in the cis-regulatory regions of downstream target genes to regulate their transcription (Swoboda et al., 2000). So far, 5' regions of demonstrated DAF-19 target genes all harbor the X boxes in close proximity to the coding region (the typical spacing is within less than 200 nucleotides upstream). As expected from this hypothesis, expression of X-box-containing osm-6::gfp in the hook and ray neurons was not detected in daf-19 mutants (n=68) (Fig. 1C5). A single X-box sequence is located at about 1.3 kb upstream of the ATG start codon of egl-46. This relatively upstream X box in egl-46 promoter was apparently not a functional target site, as egl-46::cyp expression was not altered in daf-19 mutants. We found no matches to C. elegans X-box consensus sequences in the 5' regions, introns and immediate 3' regions of ceh-26, lov-1, pkd-2 and nlp-8. Regulation of ceh-26, pkd-2 and nlp-8 by daf-19 is thus likely to be indirect and mediated by some unknown factor(s), which is probably cell-type specific.

**Discussion**

**Specification of the HOB neuron**

We have found that egl-46 is necessary for vulva location behavior, and for gene expression during HOB differentiation. HOB is a ciliated neuron required for C. elegans males to sense the vulva during mating (Sulston et al., 1980; Liu and Sternberg, 1995; Barr and Sternberg, 1999; Barr et al., 2001). The regulatory relationships among egl-46, another transcription factor, egl-44, HOB-specific genes and a ciliogenic pathway support a model involving coordinate contributions of general and cell-specific factors to specify a functional HOB sensory neuron (Fig. 6).

![Fig. 6. Distinct pathways involved in HOB gene regulation.](image)

Transcriptional regulation by egl-44 and egl-46 directs a cell-specific pathway necessary for HOB function in vulva location behavior. In the general ciliogenic pathway, the RFX-transcription factor DAF-19 controls expression of cilium structural genes to provide functional compartment common for all ciliated sensory neurons. DAF-19 has an additional influence on HOB neuronal function by affecting expression of downstream genes in the HOB-specific pathway through some unknown factor(s), indicated by Y. Genes in the shadowed box are the ones in which the Lov phenotype were analyzed in mutants (Barr and Sternberg, 1999) (this work). There are no existing mutants for ceh-26 and nlp-8.

To fulfill its sensory function, HOB must build specific structures and express appropriate molecules to receive and transduce signals. In our model, the general cilium formation pathway governed by daf-19 programs HOB to have sensory cilia, and egl-46, partly with egl-44, regulates expression of genes in HOB involved in signal transduction cascades. These two pathways are distinct. Formation of the cilium structures is not necessary for HOB-specific gene expression, and regulators in the cell-specific pathway, egl-44 and egl-46, showed no obvious effect on the HOB expression of the cilium structure genes osm-5 and osm-6. However, these two pathways do interact: not only are they both necessary for HOB function; but the ciliogenic pathway regulator daf-19 has an effect on downstream components of the HOB-specific program without affecting egl-44 or egl-46 expression.

Previous studies suggested that daf-19 is only required for genes functioning in common aspects of cilium formation (Swoboda et al., 2000). We provide the first evidence that daf-19 is required for the expression of some cell-type-specific factors. We propose that daf-19 acts through some unknown factor(s) [which could be an X-box containing gene(s)] to modify HOB-specific gene expression. We observed stronger daf-19::gfp expression in HOB than in HOA, but whether it is associated with additional daf-19 regulation of HOB-specific gene expression is not known. This daf-19 regulation is not limited to the HOB neuron as daf-19 also affects pkd-2 expression in the ray neurons and CEM neurons, indicating some general features are common in this subtype of ciliated sensory neurons. Coupled regulation of general neuronal features and cell-specific identities by multiple transcriptional factors has been found in several different organisms, such as specification of the C. elegans AIY interneuron (Altun-Gultekin et al., 2001), C. elegans olfactory neurons (Troenmel et al., 1997) and vertebrate motoneurons (Novitch et al., 2001;
Zhou and Anderson, 2002), and thus might be a general aspect of the logic of neuronal cell type specification.

Both male hook neurons, HOA and HOB, play a role in vulva location behavior. They both detect the presence of a hermaphrodite vulva, and then produce a distinctive output. This output causes the male to stop at the vulva and to proceed to the next step of mating (Liu and Sternberg, 1995) (M. M. Barr and P.W.S., unpublished). One possible explanation for the functional non-redundancy of HOA and HOB is that they possess different sensory specificity, and hence respond to different cues from the vulva. Another possibility is HOA and HOB might receive the same cues at different times. egl-44 is broadly expressed in many cells of the male tail, but its expression is almost undetectable in HOA. None of the other genes, including egl-46 and its downstream targets in the HOB-specific program described here, is expressed in HOA. The unequal expression of those genes in the two hook neurons provides molecular evidence supporting distinct roles for HOA and HOB in mating.

**EGL-46 and EGL-44 regulation in HOB sensory function**

egl-46 mutations result in an extra cell division in the terminal differentiation of the *C. elegans* Q neuroblast lineage (Desai and Horvitz, 1989). Loss of either egl-44 or egl-46 function does not cause a cell division defect or a failure in establishment of primary ciliated neural fate during HOB specification. This was determined by anatomical examination and by expression of the cilium structure genes, osm-5 and osm-6. In the non-sex-specific FLP cells, it has been shown that egl-44 and egl-46 act as transcriptional repressors (Wu et al., 2001). They promote the correct subtype of mechanosensory neurons by suppressing expression of genes dedicated to another subtype. Possible positive roles in gene transcription are implicated for egl-44 and egl-46 in the HSN neurons, but no target has been identified (Desai and Horvitz, 1989; Wu et al., 2001). Our data suggest a positive effect of egl-44 and egl-46 on the expression of downstream HOB-specific genes. However, we have not ruled out that EGL-44 and EGL-46 activate gene expression in HOB by repression of a repressor of HOB-specific genes.

We propose that the sensory abilities of the HOB neuron are established by individual cell-specific components regulated by egl-44 and egl-46. One of these components, ceh-26, is the *C. elegans* ortholog of *Drosophila prospero* (*pros*) gene (Bürglin, 1994). *pros* is involved in the initiation of differentiation in specific neurons following asymmetric cell division (Hirata et al., 1995; Broadus et al., 1998; Manning and Doe, 1999). However, expression of ceh-26 in HOB is not coupled with cell division. Instead, it is expressed at a much later stage, after basic features of cell fate have been established. Similar to HOB, ray B neurons express both egl-44 and egl-46, but unlike HOB, these neurons do not express ceh-26::gfp. Therefore, we think that co-expression of egl-44 and egl-46 is not sufficient to activate ceh-26::gfp in HOB and additional co-factors are also required. The other downstream components, lov-1, pkd-2 and nlp-8, encode proteins that are probably involved in HOB sensory input and output. LOV-1 and PKD-2 accumulate in the sensory cilia and have been proposed to act in a complex; a working model is that LOV-1 is a sensory receptor and PKD-2 is a channel protein (Barr et al., 2001; Koulen et al., 2002). Neuropeptide-like protein NLP-8 might act as a neurotransmitter or neuromodulator released by HOB to mediate the response to the stimuli from the hermaphrodite vulva.

Potential mechanosensory and chemosensory interactions between the male and the hermaphrodite during mating is implied by the vulva location behavior itself, as well as by the requirement of functional ciliated sensory endings in the two hook neurons. Whether HOB is a mechanical sensor or a chemical sensor or both, as is the case for the polymodal ASH neuron (Kaplan and Horvitz, 1993), is not known. Because egl-44 and egl-46 distinguish between mechanosensory neuron subtypes during FLP fate specification, it is possible that these two genes regulate downstream targets that confer mechanosensory ability to the HOB neuron. If so, as members of TRP protein gene family, lov-1 and pkd-2 might be such targets. Known examples of TRP proteins that play a role in mechanotransduction include a *C. elegans* TRP protein OSM-9 and the *Drosophila* TRP-like NOMPC protein (Colbert et al., 1997; Walker et al., 2000). Both of these TRP proteins are expressed in mechanosensory neurons and are involved in mechanosensory response.

**Transcriptional regulation of polycystins and polycystic kidney disease**

Human PKD1 and PKD2 were identified as two loci responsible for the autosomal dominant polycystic kidney disease (ADPKD), a genetic disorder that causes renal failure at various ages of adulthood (reviewed by Gabow, 1993; Wu, 2001). Relatively little is known about the regulation of these PKD genes and possible alterations during the disease process. In this work, we showed that expression of *C. elegans* PKD gene homologs, *lov-1* and *pkd-2*, is affected by transcription factors egl-44 and egl-46. The mammalian TEF proteins, homologous to egl-44, have been implicated in multiple developmental processes (Chen et al., 1994; Jacquemin et al., 1996). Specific expression in kidney was reported for multiple members of TEF proteins (Jacquemin et al., 1996; Kaneko et al., 1997; Jacquemin et al., 1998). *C. elegans* EGL-46 belongs to a novel zinc-finger protein subfamily. Identified close mammalian homologs of egl-46 includes insulinoma associated (IA) proteins, implicated in islet differentiation of the pancreas, and murine MLT 1 protein, silenced in the liver tumors (Goto et al., 1992; Tateno et al., 2001), but their possible roles in the kidney have not been investigated. Progressive cyst formation in ADPKD is not restricted to kidney: involvement of the liver and the pancreas occurs, indicating that those organs suffer similar pathogenesis during progression of the disease (Gabow, 1993; Chauveau et al., 2000). The demonstrated gene regulation network in HOB might reveal important insights into the regulation of human polycystin gene expression.

The dependence of ciliogenesis for the function of PKD-2 may be even more relevant to renal development in mammals. In *C. elegans*, the ARPKD homolog *osm-5* is a direct target of the RFX factor DAF-19 (Haycraft et al., 2001), making the requirement of DAF-19 activity for *pkd-2* expression particularly interesting with regard to the link between ADPKD and ARPKD. Mammalian polycystins and the cilia of the kidney cells might participate in a common signaling pathway crucial for renal differentiation and function. This
hypothesis implies that RFX factor(s) might play a role in the renal development.

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