Note

The EGL-13 SOX Domain Transcription Factor Affects the Uterine π Cell Lineages in *Caenorhabditis elegans*

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ABSTRACT

We isolated *egl-13* mutants in which the π cells of the *Caenorhabditis elegans* uterus initially appeared to develop normally but then underwent an extra round of cell division. The data suggest that *egl-13* is required for maintenance of the π cell fate.

DURING metazoan development, cells respond to inductive signals by acquiring particular fates and differentiating. One broadly used signaling pathway, defined by the LIN-12/Notch receptors (Kimble and Simpson 1997; Greenwald 1998; Kadesch 2000), involves receptor cleavage. The resulting cytoplasmic moiety translocates to the nucleus and forms a complex with proteins including CSL [an acronym for mammalian CBF-1, Drosophila Su(H), and *Caenorhabditis elegans* LAG-1], leading to transcription of downstream genes.

The *C. elegans* uterine-vulval connection is organized by the uterine anchor cell (AC; Kimble 1981; Newman and Sternberg 1996). The fates of two cells (Z1.ppp and Z4.aaa) are determined when they signal each other during the AC *vs.* ventral uterine (VU) precursor decision (Kimble 1981; Seydoux and Greenwald 1989). The sisters of these two cells (Z1.ppa and Z4.aap) always become VU cells (Kimble and Hirsh 1979). The VU cell fate, specified by *lin-12* activity (Greenwald *et al.* 1983), is to divide twice to produce four VU intermediate precursor cells. Subsequently, the AC induces the three adjacent VU intermediate precursor cells on each side—via LIN-12—to adopt the π (rather than ρ) cell fate (Figure 1; Newman *et al.* 1995). π cells generate the uterine cell types [utse (uterine-seam cell) and uv1 cell] that connect to the vulva (Newman *et al.* 1996).

The AC also induces underlying vulval precursor cells (VPCs) to adopt vulval (1° and 2°) cell fates (Sternberg and Horvitz 1986). In mature hermaphrodites, the 1° cell descendants connect to the utse and uv1 cells (Figure 1; Newman *et al.* 1996; Sharma-Kishore *et al.* 1999). The utse is H-shaped with extensions that connect the uterus to the lateral epidermis (seam) and a thin central process that separates the uterine and vulval lumens. This multinucleate syncytium is formed by fusion of 8 of the 12 π daughters and the AC.

The LIN-11 LIM domain and COG-2 SOX domain transcription factors are expressed in π cells and their daughters (Freyd *et al.* 1990; Hanna-Rose and Han 1999; Newman *et al.* 1999). The *lin-11* gene is also expressed in the vulva, but *cog-2* is not. The π cell lineages are similar to wild type in *lin-11* mutants. By contrast, the differentiation of the utse is abnormal in *lin-11* mutants, the AC fails to fuse with the utse, and a functional uterine-vulval connection is not made (Newman *et al.* 1999). In *cog-2* mutants, the AC also fails to fuse with the utse. π cells were found to divide along the correct axis in *cog-2* mutants, and it was hypothesized that the *cog-2* gene might function specifically in fusion of the AC with the utse (Hanna-Rose and Han 1999).

To identify mutants with defects in *C. elegans* uterine π development, we performed an EMS mutagenesis of the N2 strain (Brenner 1974). F_2 progeny with an egg-laying defective (Egl) phenotype were picked and their uterine anatomies were observed under Nomarski optics. π cells differ from those of the alternative fate ρ cells in that (1) π cells divide along a dorsoventral (rather than longitudinal or transverse) axis; (2) π cells produce two daughters rather than four; and (3) π cell daughters connect to the vulva (Newman *et al.* 1995, 1996). While continuous analysis of cell lineages is necessary to unambiguously determine whether a cell fate transformation has occurred, we used anatomical observation to identify seven mutants that appeared to affect

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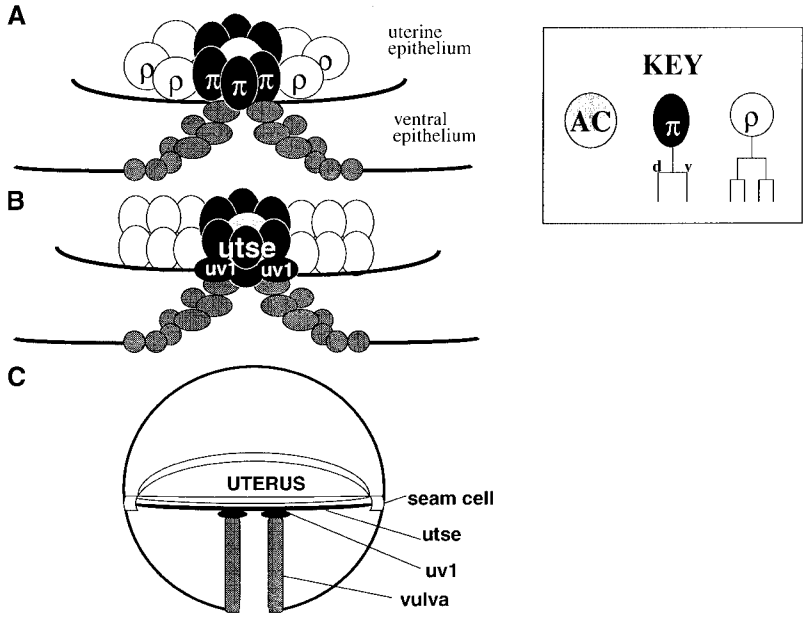

FIGURE 1.—Schematic of the developing and mature uterine-vulval connection. (A) VU intermediate precursor cell stage in a late L3 hermaphrodite. There are six VU intermediate precursor cells per side, surrounding a centrally placed AC (light gray). The intermediate precursor cells closest to the AC have the π cell fate, whereas those that are more distal have the ρ cell fate. Vulval cells of the ventral epithelium are represented by dark gray. (B) One round of cell division later. Each π cell has divided along a d/v axis to produce a larger dorsal daughter and a smaller ventral one. The outer ventral daughters become uv1 cells, while all the remaining π progeny fuse with each other and with the AC to form the utse syncytium. The ρ cell daughters (white) are each the result of an a/p or l/r division. The ρ cell daughters will undergo an additional round of cell division, but the π cell daughters will not. (C) Mature uterine-vulval connection. Schematic of a transverse section through the central region of the hermaphrodite containing the vulva. The central portion of the utse is visible and forms a thin laminar process dorsal to the vulva. The uv1 cells connect to the vulva and to the utse. Adapted from NEWMAN *et al.* (2000), with permission from Elsevier Science.

π cell development on the basis of one or more of the above characteristics. From 20,000 haploid genomes screened, we obtained one allele each of the presenilin gene *sel-12* and the heterochronic gene *lin-29* (NEWMAN *et al.* 2000; CINAR *et al.* 2001). Four mutants defined a distinct complementation group, comprising the *ty3*, *ty7*, *ty8*, and *ty14* alleles. Three-factor crosses demonstrated that *ty3* was located between 2.15 and 6.67 map units to the left of center on the X chromosome. This map position was roughly similar to that of the *egl-13* gene identified in a previous screen for Egl mutants (TRENT *et al.* 1983). We therefore constructed the *egl-13(n483)/ty3 trans*-heterozygote. We found that hermaphrodites of this genotype were Egl, indicating that the alleles failed to complement and that *ty3*, *ty7*, *ty8*, and *ty14* were alleles of the *egl-13* gene.

The *n483*, *ty3*, *ty7*, and *ty8* alleles alter conserved residues of the EGL-13 SOX domain transcription factor: Another genetic screen had identified the *cog-2* gene, which encodes a SOX domain transcription factor (HANNA-ROSE and HAN 1999). *egl-13* and *cog-2* mapped to roughly similar positions. Furthermore, *cog-2* mutants were Egl and had defects in the uterine-vulval connection similar to those observed in the *ty3*, *ty7*, *ty8*, and *ty14* mutants. We therefore performed complementation analysis between *cog-2 (ku194)* and *egl-13 (n483)* and between *cog-2 (ku194)* and *egl-13 (ty3)*. In both cases, the mutations failed to complement. The *cog-2* gene is thus allelic to *egl-13*, which encodes a SOX domain transcription factor.

SOX domain transcription factors, which function broadly in development, have a conserved 79-amino-acid DNA-binding domain known as the SOX box (PEVNY and LOVELL-BADGE 1997; BOWLES *et al.* 2000). DNA sequence analysis of the *n483* allele revealed a predicted protein with leucine substituted for a conserved proline at position 68 of the SOX box (Figure 2). The *ty3* allele is a T to A transversion that converts a tyrosine within the SOX box to a stop codon. The *ty7* allele leads to substitution of Lys for Arg at position 5 of the SOX box, which is conserved among SOX proteins and has been shown in human SRY to make a salt bridge to a phosphate of the DNA backbone (WERNER *et al.* 1995). While an Arg to Lys substitution should not affect the electrostatic interaction, the altered size of the side chain may affect protein conformation and function. Finally, the *ty8* allele deletes approximately the N-terminal half of the SOX box (which is encoded by exons 8 and 9) and introduces a frameshift thereafter (*egl-13* contains 10 exons). Specifically, nucleotides 4327–4623 of the wild-type sequence (starting with the first ATG) are deleted (corresponding to the region from the end of exon 7 through all but the last three nucleotides of exon 8) except for the tetranucleotide sequence TATT. Since the wild-type sequence from nucleotides 4327–4623 contains four copies of TATT, the *ty8* allele could result from two small deletions separated by four nucleotides or from a single deletion with a TATT insertion at the junction. We did not find a mutation in the *ty14* allele.

It was previously argued that *ku194*, which truncates the protein prior to the SOX box, was likely to be a

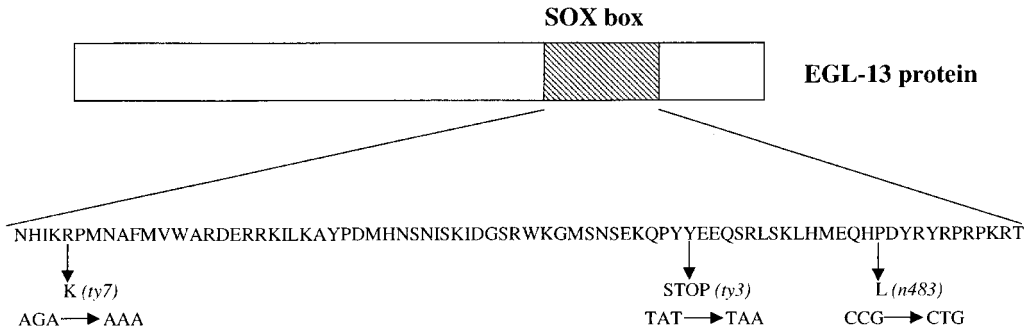

FIGURE 2.—Schematic of the EGL-13 protein. The 79 amino acids of the protein's SOX box (HANNA-ROSE and HAN 1999) are indicated below, as are the amino acid and nucleotide changes of the alleles sequenced in this report. Seven primer pairs were used to PCR amplify the entire *egl-13* genomic region from 130 bp upstream of the predicted start site through the end of the coding sequence (with the exception of the intron between exons 1 and 2) and the PCR products were sequenced. Each sequence was determined from at least two sequencing reactions. Not shown is the *ty8* allele, which deletes approximately the first half of the SOX box and introduces a frameshift thereafter (see text). The SOX box is defined as in BOWLES *et al.* (2000).

molecular null (HANNA-ROSE and HAN 1999). On the basis of the above analysis, the *ty3* and *ty8* alleles are also likely to be null.

The uterine π cell lineages are abnormal in *egl-13* mutants: As discussed above, π cells differ from ρ cells in dividing along a dorsoventral axis, undergoing one less round of cell division, and expressing *egl-13* and *lin-11*. An earlier study found that, in *egl-13* mutants, presumptive π cells were essentially normal in division axis and expression of *egl-13* and *lin-11* (HANNA-ROSE and HAN 1999). Similarly, we did not observe significant alterations in the axis of π cell division in the *n483*, *ty3*, or *ty8* mutants (data not shown). By contrast, there was a striking change in number of divisions. Specifically, we found that many presumptive π daughters divided in each of these three alleles (Table 1). In the previous analysis of *ku194*, the presumptive π cells had not been observed for an additional round of cell division to determine whether their daughters divided. When we observed the uterine cell lineages of *ku194* and *ku241* mutant animals, we found that most π cell daughters divided (Table 1). Thus, in all five *egl-13* alleles whose

TABLE 1

Divisions of presumptive π cell daughters

Strain	Presumptive π cell daughters dividing/total π daughters scored[a] (%)	No. of animals
N2	0	Many
egl-13 (n483)	17/36 (47)	3
egl-13 (ty3)	34/47 (72)	4
egl-13 (ty8)	30/36 (83)	4
egl-13 (ku194)	22/24 (92)	2
egl-13 (ku241)	17/24 (71)	2
lin-12 (n137)[b]	0/140 (0)	5
lin-12 (n137); egl-13 (ty3)[c]	38/39 (97)	3

[a] VU cells undergo two rounds of cell division to produce the VU intermediate precursor cells that then undergo an additional one (π cells) or two (ρ cells) round(s) of cell division (KIMBLE and HIRSH 1979; NEWMAN *et al.* 1995). To determine whether presumptive π cell daughters divided, we observed either the fourth or the third and fourth round(s) of VU cell division under Nomarski optics. In general, animals were followed for at least 1 hr past the last uterine cell division observed. For some animals, the lineages of cells on both the right and left sides were observed, while for others, only one side was followed. In wild-type animals, cells of the dorsal uterine (DU) and VU cell lineages undergo roughly synchronous final rounds of division during the early L4 stage (KIMBLE and HIRSH 1979). The DE4v and DE5v cells of the DU cell lineage are typically the last to divide (see NEWMAN *et al.* 1996 for nomenclature). In general, in *egl-13* mutant animals, the divisions of the presumptive π cell daughters occurred by about the time of the DE4v/DE5v divisions. However, we occasionally observed presumptive π cell daughters divide up to 2 hr past the last DE4v/DE5v division.

[b] Data from NEWMAN *et al.* (1995). All but the most distal VU intermediate precursor cells always adopt the π cell fate in *lin-12 (n137)* mutants (NEWMAN *et al.* 1995), and thus are considered as presumptive π cells in strains containing *lin-12 (n137)*. Since *lin-12 (n137)* mutants have no AC and an extra VU cell, there are eight intermediate precursor cells per side, seven of which always adopt the π cell fate.

[c] Complete genotype: *dpy-19 (e1259) lin-12 (n137); lon-2 (n678) egl-13 (ty3)*. Division of many presumptive π cell daughters was also observed in an additional five animals whose complete cell lineages were not followed.

TABLE 2

Uterine expression of *egl-13::GFP*

Strain			Average no. of fluorescent uterine nuclei[a]		
Allele	Array	Stage	Moderate/bright[b]	Faint[b]	(*n*)
egl-13 (+)	*kuIs29*	Early-mid-L4[c]	6.1	0.3	(19)
egl-13 (+)	*kuIs29*	Mid-L4	6.3	0.75	(16)
egl-13 (+)	*tyIs4*[d]	Early-mid-L4	6.3	—	(31)
egl-13 (+)	*tyIs4*	Mid-L4	6.5	0.5	(22)
egl-13 (ku194)	*tyIs4*	Early-mid-L4	8.2	1.2	(36)
egl-13 (ku194)	*tyIs4*	Mid-L4[e]	10.5	2.7	(31)
egl-13 (ty3)	*tyIs4*	Early-mid-L4	8.4	1.3	(43)
egl-13 (ty3)	*tyIs4*	Mid-L4[e]	9.9	1.7	(17)

n, no. of animals scored.

[a] These values reflect the average no. of fluorescent nuclei present on the lateral side at the closest observable focal plane.

[b] The GFP+ uterine nuclei were scored as moderate to bright fluorescence on the basis of relative intensity. Notably less intensely fluorescent nuclei were scored as faint.

[c] Early-mid-L4 stage animals were identified by progressed vulval morphogenesis, onset of uterine lumen formation, and dorsally reflexed position of distal gonad arms. Mid-L4 stage animals were defined by a more progressed state of vulval development, completely formed uterine lumen, and further dorsally reflexed distal gonad arms in addition to the absence of the later L4 stage formed alae. In *egl-13 (+)* early-mid-L4 stage animals, if the AC nucleus was GFP positive and observable with other GFP+ uterine nuclei, then it was included in the count. However, if the AC was GFP positive yet not biased to either lateral side (*i.e.*, was in the middle plane), then it was excluded from the count. Therefore, the average number of GFP+ uterine cells on one side in *egl-13 (+)* at the early to mid-L4 stage can be between 6.0 and 6.5.

[d] A strain containing the *tyIs4* integrated chromosomal array was generated by injecting an *egl-13::GFP* transcriptional gene fusion (pWH17; Hanna-Rose and Han 1999) into N2 and subjecting transmitting extrachromosomal lines to γ-irradiation; homozygous integrants were then selected. *tyIs4* was mapped to chromosome III.

[e] In mid-L4 stage *egl-13 (+)* animals, an average of 6.5 GFP+ nuclei per side is expected because the AC nucleus is lateral and fluorescent by this stage and has a 50% chance of being on either side. By contrast, in *egl-13* mutant animals, the AC does not express *egl-13::GFP*, so the expected number of fluorescent uterine nuclei is 6.0.

cell lineages were observed, presumptive π cells initially divided along a dorsoventral axis as in the wild type, but then often underwent an additional round of cell division. We conclude that, in *egl-13* mutants, the π cell lineage is initiated but not completed correctly.

Expression of an *egl-13::GFP* reporter construct is abnormal in *egl-13* mutants: As discussed above, an *egl-13::GFP* transcriptional fusion is expressed in the uterine π cells and their daughters. We created additional strains with the *egl-13::GFP* construct (pWH17) integrated into the *C. elegans* genome and utilized *tyIs4* (see Table 2 footnote *d*) as well as the previously characterized *kuIs29* to conduct genetic analyses of mutants involved in π cell development. It was previously observed that the *kuIs29* expression pattern was the same in *egl-13 (ku194)* and *egl-13 (+)* backgrounds during the early L4 stage (Hanna-Rose and Han 1999). Similarly, we found that, during the late L3 through early L4 stages, the number of uterine cells that fluoresced as a result of either the *kuIs29* or the *tyIs4* transgenic array was essentially the same in an *egl-13* mutant or wild-type background (data not shown). This spans the time from when π cell fates are being specified among the VU intermediate precursor population to just prior to the final round of uterine division (when ρ cell daughters normally divide and maintenance-defective π cell daughters would abnormally divide). However, it was also noted that *egl-13* mutant animals sometimes contained extra cells with weak fluorescence during the late L4 stage (Hanna-Rose and Han 1999). To investigate these issues further, we performed close observations of uterine π cell green fluorescent protein (GFP) expression during the early-mid- and mid-L4 stages in wild-type and *egl-13* mutant animals.

When we observed *kuIs29* and *tyIs4* control lines during the early-mid-L4 stage, we saw an average of six to seven fluorescent uterine nuclei per side (Table 2; Figure 3). This is about the range expected for the daughters of three π cells on each side plus or minus the AC nucleus (which may become fluorescent at this early stage and has an equal chance of moving to the left or right in the process of fusing with the utse). An average close to 6.5 GFP+ cells per side persisted through the mid-L4 (Table 2) and late L4 stages, with the uv1 nuclei often showing brighter fluorescence than the utse nuclei as previously reported (Hanna-Rose and Han 1999). By contrast, our observations of the *egl-13 (ku194)* and *egl-13 (ty3)* alleles revealed the presence of roughly two

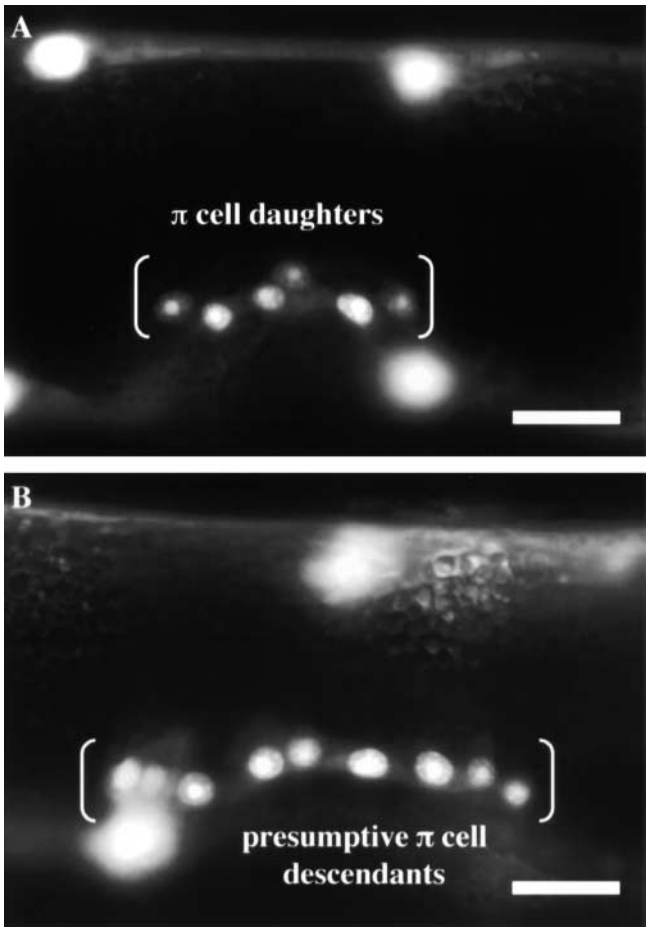

FIGURE 3.—*egl-13::GFP* expression in early-mid-L4 stage animals. (A) Left lateral view of *egl-13 (+)* animal containing the *tyIs4* transgenic array. Six π cell daughter nuclei are visible. (B) Right lateral view of *egl-13 (ty3)* mutant containing *tyIs4*. Nine fluorescent presumptive π lineage nuclei are evident. Brackets demarcate the region containing fluorescent uterine nuclei. Bright fluorescent cells outside brackets are body wall muscles (which also express the gene fusion; HANNA-ROSE and HAN 1999). Animal in B is slightly older than animal in A. Bars, 10 μm.

additional GFP+ uterine nuclei at the early-mid-L4 stage and four additional GFP+ nuclei at the mid-L4 stage (Table 2; Figure 3). Also, significantly fainter yet detectable nuclei were noted in the uterine-vulval and surrounding uterine regions in *egl-13* mutants. We scored these animals at isolated time points, not by continuous observation, and thus cannot definitively conclude that each fluorescent uterine nucleus represents the descendant of a presumptive π cell daughter that divided. However, the presence of additional GFP-positive nuclei is consistent with the cell lineage data presented above, which demonstrates that presumptive π cells often undergo an extra round of cell division in *egl-13* mutants.

It was previously observed that in *egl-13 (+)* animals containing the *kuIs29* array, the AC becomes GFP+ upon fusion with the utse (HANNA-ROSE and HAN 1999). Here, we report that the unfused and often very prominent AC nucleus in *egl-13* mutants consistently fails to express *egl-13::GFP*. This is presumably an additional consequence of the π cell defect revealed by the altered cell lineage.

Presumptive π cells undergo an extra round of division in *egl-13; lin-12(d)* double mutants: In *lin-12* gain-of-function mutants [*lin-12(d)*], excess π cell fates are specified, and most VU intermediate precursor cells divide only once (NEWMAN *et al.* 1995). (Only the most distal cells sometimes undergo a second round of division.) Since *egl-13* and *lin-12(d)* mutants have opposite phenotypes with respect to cell division, we constructed a mutant of genotype *dpy-19 lin-12 (n137); lon-2 egl-13 (ty3)*. We found that of 39 π cell daughters observed, 38 divided (Table 1). Thus the cell lineages of the double mutant are similar to those of *egl-13* alone. This is consistent with the *egl-13* gene's functioning downstream of *lin-12*, which is also suggested by the fact that *lin-12* mutants have an earlier π cell developmental defect than do *egl-13* mutants.

Genetic control of uterine π cell development: We have previously shown that *lin-12* is required for specification of the uterine π cell fate, while the *lin-11* LIM domain transcription factor is required for differentiation of π cell daughters (NEWMAN *et al.* 1995, 1999). In *lin-11* mutants, uterine π cell lineages are essentially wild type, although the π daughters occasionally divide. By contrast, differentiation of the utse is defective; this includes a failure to fuse with the AC. *lin-11* appears to be a direct target of LIN-12 signaling in the π cells (GUPTA and STERNBERG 2002).

We have shown here that *egl-13* mutants disrupt π cell development at an earlier stage than do *lin-11* mutants. Thus, while *lin-11* mutants have only a slight effect on the π cell lineages, *egl-13* mutants have a striking cell lineage defect in which most of the presumptive π cell daughters divide. In addition, the utse does not fuse with the AC in *egl-13* mutants. Thus, *egl-13* appears required for the maintenance of the π cell fate and subsequent differentiation of its daughters. *egl-13* and *lin-11* appear not to be required for each other's expression (HANNA-ROSE and HAN 1999; NEWMAN *et al.* 1999). Further analysis will help to elucidate precisely how these two transcription factors collaborate to mediate proper development of π cells in the *C. elegans* uterus.

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LITERATURE CITED

BOWLES, J., G. SCHEPERS and P. KOOPMAN, 2000 Phylogeny of the SOX family of developmental transcription factors based on sequence and structural indicators. Dev. Biol. **227:** 239–255.

BRENNER, S., 1974 The genetics of *Caenorhabditis elegans*. Genetics **77:** 71–94.

Cinar, H. N., K. L. Sweet, K. E. Hosemann, K. Earley and A. P. Newman, 2001 The SEL-12 presenilin mediates induction of the *Caenorhabditis elegans* uterine π cell fate. Dev. Biol. **237:** 173–182.

Freyd, G., S. K. Kim and H. R. Horvitz, 1990 Novel cysteine-rich motif and homeodomain in the product of the *Caenorhabditis elegans* cell lineage gene *lin-11*. Nature **344:** 876–879.

Greenwald, I., 1998 LIN-12/Notch signaling: lessons from worms and flies. Genes Dev. **12:** 1751–1762.

Greenwald, I. S., P. W. Sternberg and H. R. Horvitz, 1983 The *lin-12* locus specifies cell fates in *Caenorhabditis elegans*. Cell **34:** 435–444.

Gupta, B. P., and P. W. Sternberg, 2002 Tissue-specific regulation of the LIM homeobox gene *lin-11* during development of the *Caenorhabditis elegans* egg-laying system. Dev. Biol. **247:** 102–115.

Hanna-Rose, W., and M. Han, 1999 COG-2, a Sox domain protein necessary for establishing a functional vulval-uterine connection in *Caenorhabditis elegans*. Development **126:** 169–179.

Kadesch, T., 2000 Notch signaling: a dance of proteins changing partners. Exp. Cell Res. **260:** 1–8.

Kimble, J., 1981 Lineage alterations after ablation of cells in the somatic gonad of *Caenorhabditis elegans*. Dev. Biol. **87:** 286–300.

Kimble, J., and D. Hirsh, 1979 Post-embryonic cell lineages of the hermaphrodite and male gonads in *Caenorhabditis elegans*. Dev. Biol. **70:** 396–417.

Kimble, J., and P. Simpson, 1997 The LIN-12/Notch signaling pathway and its regulation. Annu. Rev. Cell Dev. Biol. **13:** 333–361.

Newman, A. P., and P. W. Sternberg, 1996 Coordinated morphogenesis of epithelia during development of the *Caenorhabditis elegans* uterine-vulval connection. Proc. Natl. Acad. Sci. USA **93:** 9329–9333.

Newman, A. P., J. G. White and P. W. Sternberg, 1995 The *C. elegans lin-12* gene mediates induction of ventral uterine specialization by the anchor cell. Development **121:** 263–271.

Newman, A. P., J. G. White and P. W. Sternberg, 1996 Morphogenesis of the *C. elegans* hermaphrodite uterus. Development **122:** 3617–3626.

Newman, A. P., G. Z. Acton, E. Hartwieg, H. R. Horvitz and P. W. Sternberg, 1999 The *lin-11* LIM domain transcription factor is necessary for morphogenesis of *C. elegans* uterine cells. Development **126:** 5319–5326.

Newman, A. P., T. Inoue, M. Wang and P. W. Sternberg, 2000 The *Caenorhabditis elegans* heterochronic gene *lin-29* coordinates the vulval-uterine-epidermal connections. Curr. Biol. **10:** 1479–1488.

Pevny, L. A., and R. Lovell-Badge, 1997 *Sox* genes find their feet. Curr. Opin. Genet. Dev. **7:** 338–344.

Seydoux, G., and I. Greenwald, 1989 Cell autonomy of *lin-12* function in a cell fate decision in *C. elegans*. Cell **57:** 1237–1245.

Sharma-Kishore, R., J. G. White, E. Southgate and B. Podbilewicz, 1999 Formation of the vulva in *Caenorhabditis elegans*: a paradigm for organogenesis. Development **126:** 691–699.

Sternberg, P. W., and H. R. Horvitz, 1986 Pattern formation during vulval development in *Caenorhabditis elegans*. Cell **44:** 761–772.

Trent, C., N. Tsung and H. R. Horvitz, 1983 Egg-laying defective mutants of the nematode *Caenorhabditis elegans*. Genetics **104:** 619–647.

Werner, M. H., J. R. Huth, A. M. Gronenborn and G. M. Clore, 1995 Molecular basis of human 46X, Y sex reversal revealed from the three-dimensional solution structure of the human SRY-DNA complex. Cell **81:** 705–714.

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