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EGL-17(FGF) expression coordinates the attraction of the migrating sex myoblasts with vulval induction in *C. elegans*

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SUMMARY

During the development of the egg-laying system in *Caenorhabditis elegans* hermaphrodites, central gonadal cells organize the alignment of the vulva with the sex myoblasts, the progenitors of the egg-laying muscles. A fibroblast growth factor [EGL-17(FGF)] and an FGF receptor [EGL-15(FGFR)] are involved in the gonadal signals that guide the migrations of the sex myoblasts. Here we show that EGL-17(FGF) can act as an instructive guidance cue to direct the sex myoblasts to their final destinations. We find that *egl-17* reporter constructs are expressed in the primary vulval cell and that EGL-17(FGF) expression in this cell correlates with the precise positioning of the sex myoblasts. We postulate that EGL-17(FGF) helps to coordinate the development of a functional egg-laying system, linking vulval induction with proper sex myoblast migration.

Key words: EGL-17, FGF, Cell Migration, Vulval Induction, Sex Myoblasts, *Caenorhabditis*

INTRODUCTION

Egg laying in *Caenorhabditis elegans* hermaphrodites requires the coordinated function of multiple tissues including the uterus, the vulva, the egg-laying musculature and a pair of HSN motor neurons that stimulate muscle contraction. The development of these structures is coordinated by a series of cell-cell interactions that are initiated by the gonad (Thomas et al., 1990). Additional interactions among these essential components as well as other associated components help to ensure the integrity of the egg-laying machinery (Li and Chalfie, 1990; Horvitz and Sternberg, 1991; Garriga et al., 1993; Newman and Sternberg, 1996). The ease of observing the development of this system and the dramatic phenotypic consequences that result from perturbing the signaling events involved in the developing egg-laying system have enabled these events to serve as paradigms for the general study of cell-cell interactions in development.

Eggs are laid from the uterus through an opening in the ventral hypodermis known as the vulva. The vulva is formed from the descendants of three of a set of six equipotential blast cells that make up the vulval equivalence group (Sulston and Horvitz, 1977; Sulston and White, 1980). These six cells (P3.p, P4.p, P5.p, P6.p, P7.p and P8.p, also referred to as P(3-8).p) are known as the vulval precursor cells, or the VPCs. In wild type, P(5-7).p form the vulva by acquiring two different vulval cell fates characterized by distinctive numbers of progeny and division planes. P6.p, the cell that lies at the center of the developing vulva, gives rise to 8 daughter nuclei arising from transverse divisions of the P6.p granddaughters (abbreviated TTTT). The cells adjacent to P6.p, P5.p and P7.p, undergo mirror-symmetrical lineages that give rise to 7 daughter nuclei arising from lateral, transverse, or no divisions of their granddaughters (abbreviated LLTN or NTLL). The outer cells, P3.p, P4.p and P8.p, take on non-vulval fates, dividing once and joining the surrounding hypodermal syncytium. Based on the hierarchy of their potential to replace missing cells within the vulval equivalence group, P6.p is said to undergo a 1° lineage, P5.p and P7.p a 2° lineage, and the outer cells a 3° lineage.

The gonad plays a key role in inducing the formation of the vulva while additional signals from adjacent VPCs and the surrounding hypodermal syncytium also contribute to establishing the pattern of VPC cell fates. A single cell in the somatic gonad, the anchor cell, induces nearby VPCs in the ventral hypodermis to undergo 1° and 2° vulval cell fates (Kimble, 1981). In wild type, the anchor cell lies closest, and directly dorsal to, P6.p which acquires the 1° cell fate. Besides inducing vulval cell fates, it appears that both a gradient of this anchor cell inductive cue and a lateral inhibitory signal establish the pattern of 1° and 2° vulval cell fates (reviewed in Kenyon, 1995). These signals overcome an inhibitory signal that appears to emanate from the surrounding hypodermis and maintain the VPCs in non-vulval cell fates. These three signaling mechanisms have been extensively studied and reviewed (Horvitz and Sternberg, 1991; Hill and Sternberg, 1993; Sundaram and Han, 1996) and their interplay helps to establish the highly reproducible pattern of cell fates observed in wild-type hermaphrodites.

The gonad also plays an important role in the proper

positioning of a set of 16 vulval and uterine muscles known as the egg-laying, or sex muscles. In order for eggs to be laid through the vulva, the sex muscles must be positioned properly to allow them to make functional attachments to the uterus, the vulva and the adjacent lateral hypodermis (White, 1988). The precursors to the sex muscles, the sex myoblasts (SMs), are generated in the posterior half of the mid-body region and undergo anterior migrations to final positions that flank the center of the developing gonad (Sulston and Horvitz, 1977). When the gonad is destroyed by laser ablation, the SMs still migrate anteriorly but take up final positions within a broad, centrally dispersed range (Thomas et al., 1990). Thus, the gonad is responsible for the precise positioning of the SMs. This precise positioning appears to result from a gonad-dependent attractive signaling mechanism since the SMs can migrate into novel territory in response to an altered positioning of the gonad (Thomas et al., 1990). The simplest model that can account for these data is that the gonad emits the attractive signal responsible for guiding and properly positioning the SMs during their migrations. In the absence of the gonad, a gonad-independent mechanism is responsible for the anterior migrations of the SMs to centrally dispersed positions (Thomas et al., 1990).

Because of the redundancy of mechanisms that drive the SMs anteriorly, mutations that compromise either only the gonad-dependent or the gonad-independent mechanism do not dramatically affect the positioning of the SMs (Sundaram et al., 1996; Chen et al., 1997). By contrast, mutations in the genes *egl-15* and *egl-17* cause the SMs to be severely posteriorly displaced (Stern and Horvitz, 1991). *egl-15* encodes a fibroblast growth factor receptor (FGFR; DeVore et al., 1995) and *egl-17* encodes a fibroblast growth factor (FGF; Burdine et al., 1997). In these mutants, instead of a gonad-dependent attraction, the gonad is responsible for the severe posterior displacement of the SMs by what appears to be a repulsive mechanism (Stern and Horvitz, 1991). Thus, mutations affecting this FGF signaling system, unlike gonad ablation, do not abolish the interaction between the SMs and the gonad, but rather change its nature from an attraction to a repulsion. Since these *egl-17* mutations eliminate EGL-17, another signal besides EGL-17 (FGF) must be capable of influencing SM migration in a gonad-dependent manner (Burdine et al., 1997).

We have proposed two models to explain how mutations in this FGF signaling system can change a gonad-dependent attraction to a gonad-dependent repulsion. In the first model, EGL-17 is the gonad-dependent attractive cue and the additional signal is an underlying repulsive cue. By this model, mutations in *egl-15* and *egl-17* compromise the attraction, revealing the underlying gonad-dependent repulsion. Alternatively, the additional signal could be the gonad-dependent attractive cue that precisely positions the SMs. By this model, EGL-17, acting through EGL-15, would permit the SMs to interpret this signal as an attractive cue rather than a repulsive one.

The distinguishing feature of these models is that EGL-17 either acts instructively, guiding the SMs to the source of its expression, or permissively, allowing the SMs to be attracted to the source of a different gonad-dependent instructive cue. The sites of expression of EGL-17 may, thus, distinguish which of these two general models is correct. Here we use GFP and *lacZ* reporter constructs as an indication of where *egl-17* might be expressed. We find these reporter constructs to be expressed in 1˚ and 2˚ vulval lineages at different stages of development. In addition, we present evidence indicating that EGL-17 expressed in the VPCs can act as an attractive cue that guides the precise positioning of the SMs. Thus, EGL-17 can help to coordinate two essential components of the egg-laying system by aligning the sex muscles with the vulva.

MATERIALS AND METHODS

Plasmid construction and germline transformation

To generate reporter constructs where the *egl-17* promoter drives expression of GFP (NH#293) or β-galactosidase (NH#291), coding sequences for these reporters were obtained from pPD95.67 (kindly provided by A. Fire, J. Ahnn, G. Seydoux and S. Xu) and pPD21.28 (Fire et al., 1990), respectively. The *egl-17* promoter region is derived from NH#75 (Burdine et al., 1997) and contains 3.9 kb of sequence upstream of the initiating codon. These constructs are predicted to code for proteins that contain the first 11 amino acids of EGL-17 fused at the endogenous *Sal*I site to the coding region for the respective reporters. To create a full-length EGL-17 protein tagged with GFP at its C terminus (NH#375), the coding region for the reporter was obtained from pPD95.85 (kindly provided by A. Fire, J. Ahnn, G. Seydoux and S. Xu) and placed in frame at an *Age*I site generated at the EGL-17 stop codon. This construct maintains the *egl-17* 5′ and 3′ untranslated and non-coding regions.

The reporter genes in the two promoter constructs contained nuclear localization sequences and were found in both the nucleus and cytoplasm. Expression of the full-length *egl-17::gfp* construct was observed in a punctate pattern surrounding the head neuron and the vulval region, consistent with the protein being processed via the secretory pathway as predicted by the presence of a potential signal sequence in EGL-17 (Burdine et al., 1997).

The *egl-15::GFP* reporter construct (NH#332) was generated by the same strategy used to generate NH#375 using pPD95.67 as the source of the GFP coding region. NH#332 encodes a full-length EGL-15 protein tagged with GFP at its C terminus.

Transgenic lines were obtained using both pRF4 [*rol-6(su1006dm)*] and pMH86 [*dpy-20(+)*] as co-transformation markers following standard procedures (Mello et al., 1991; Burdine et al., 1997).

Characterization of reporter construct expression

β-galactosidase expression was detected as described (Hill and Sternberg, 1992) in transgenic animals grown at 25˚C. For characterization of GFP expression, worms were mounted on 5% agar pads in 3 μl of 1 mM levamisole. Fluorescence was observed on an Axioplan Universal Microscope with filter set Green H546 (Carl Zeiss) using an OptiQuip 1500 with a Ushio 100W Hg bulb as the UV source. GFP expression was characterized in animals that had been allowed to develop for at least 12 hours at 25˚C as the higher temperature appears to greatly increase GFP fluorescence in these transgenic animals. For both lineage determination and scoring GFP expression in unablated animals, strains were grown continuously at 25˚C prior to scoring with the exception of *ayIs4; dpy-20(e1282ts); lin-15(n309)* animals, which were grown at 20˚C and

transferred to 25˚C as eggs or L1s. In *ayIs4; dpy-20(e1282ts)* animals, GFP expression is similar in stocks grown at 25˚C as in animals grown at 20˚C and shifted to 25˚C by the end of the L1 stage.

Early vulval *egl-17::GFP* expression was scored in animals in which the VPCs were enlarged or had divided (L2 to early L4 stages). Animals in which invagination of VPC descendants had initiated were excluded from this group. Late vulval expression was scored in mid to late L4 stage animals where the invagination of the vulva appeared complete and the anchor cell nucleus had disappeared. In animals in which vulval induction did not occur, animals were staged by the size of the gonad and the extent of the gonad arm migrations.

Mapping of the spontaneous integrant *ayIs4[egl-17::GFP]*

ayIs4[egl-17::GFP dpy-20(+)] was mapped on the basis of its ability to confer a non-Dpy phenotype in a *dpy-20(e1282ts)* background and was found to show linkage with the chromosomal marker *unc-73(e936) I*. The integration site of *ayIs4* was more finely determined by two- and three-factor mapping with respect to *lin-17(n677)* and *bli-3(e767)*: from *bli-3(e767) + lin-17(n677)/+ ayIs4 +; dpy-20(e1282ts)/+* heterozygotes, 1/41 Bli, 1/7 Bli non-Lin and 4/8 Lin non-Bli showed GFP expression from *ayIs4*. These data map *ayIs4* approximately 1 map unit to the right of *bli-3*.

Nomarski scoring and laser ablations

SM positions were scored as described (Thomas et al., 1990) unless otherwise noted. The cell fates of the VPC granddaughters were scored as previously described (Sternberg and Horvitz, 1986). Laser ablations of cell nuclei were performed as described (Avery and Horvitz, 1987). *ayIs4* animals in which either P6.p or all of the VPCs were ablated were picked to a separate plate as early L1 larvae, grown at room temperature for 13 hours, mounted for laser ablation of the VPCs and grown after the ablation at 25˚C for approximately 12 hours prior to scoring GFP expression and SM positioning. *ayIs4; kuIs14* animals in which Z1, Z4 and the VPCs were ablated were treated as described above except that Z1 and Z4 were ablated in the early L1 larvae.

RESULTS

As a first step towards defining the sites of *egl-17* expression, a number of reporter constructs were made and introduced by germline transformation into *C. elegans* hermaphrodites. Two fusions were created at the initiating methionine of EGL-17 such that the *egl-17* promoter drives expression of either the green fluorescent protein (GFP) or β-galactosidase. An additional construct was created that fuses GFP to the carboxyterminus of full-length EGL-17. All three constructs resulted in similar sites of reporter expression independent of the co-transformation marker used to create the transgenic animals (Fig. 1 and data not shown). Furthermore, the full-length *egl-17::GFP* construct is capable of rescuing both the egg-laying and SM positioning defects of *egl-17(n1377)* hermaphrodites (data not shown). Thus, this construct must be expressed in a functionally relevant location. Based on the functional complementation and the reproducibility of the expression pattern using multiple reporter genes, portions of *egl-17* and co-transformation markers, the sites of expression observed for these reporter constructs are likely to reflect real sites of *egl-17* expression.

We identified a spontaneously integrated transgenic array carrying the *egl-17::GFP* promoter fusion construct. This array, termed *ayIs4*, conferred an expression pattern identical to that seen in the other non-integrated transgenic lines and was used throughout the remainder of this study. The complete genotype for *ayIs4*-bearing strains is *ayIs4[egl-17::GFP dpy-20(+)] I; dpy-20(e1282ts) IV* but will be referred to in the text either as *egl-17::GFP* or *ayIs4* for clarity.

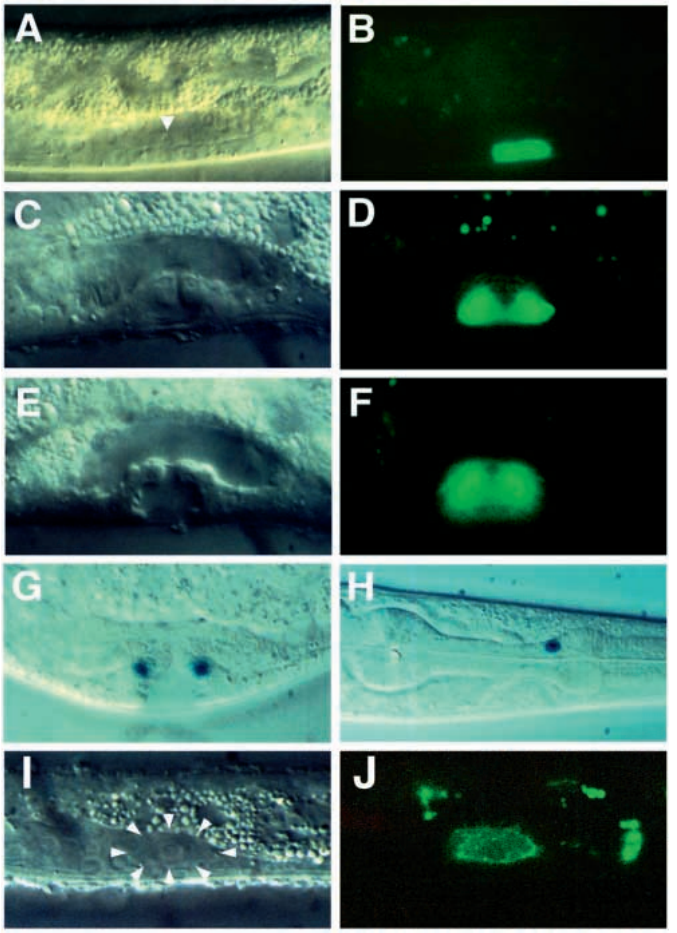

Fig. 1. Pattern of *egl-17* reporter construct expression. (A,B) Nomarski and GFP views of a mid-L3 *ayIs4* animal in which P6.p has divided once. The arrowhead in A denotes the position of the anchor cell. (C,D) Nomarski and GFP views of an L4 *ayIs4* animal showing GFP expression in the T lineages of P5.p and P7.p. (E,F) Nomarski and GFP views of the same *ayIs4* animal in C and D in a different plane of focus that shows GFP expression in the N descendants of P5.p and P7.p. (G) Expression of β-galactosidase from the *egl-17::lacZ* reporter construct in the N descendants of P5.p and P7.p of an L4 stage animal. The expression pattern is similar to the expression seen at this stage with the *egl-17::GFP* reporter constructs (F). (H) Expression of the *egl-17::lacZ* reporter construct in the M4 neuron in the anterior bulb of the pharynx. Similar expression in M4 was seen with *egl-17::GFP* reporter constructs. (I,J) Nomarski and GFP views of a transgenic animal containing the *egl-15::GFP* reporter construct. Arrowheads denote the outline of the undivided SM.

Table 1. Temporal expression of *egl-17::GFP* in the VPCs and their descendants*

Developmental stage†			Vulval precursor cell‡						
Stage	Cells	Time (hours)	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	n‖
L2-L3	Pn.p	22-27	–	–	–	52%	–	–	23
L3	Pn.p	27-30	–	–	–	87%	–	–	15
mid L3	Pn.px	~30	–	–	–	100%	–	–	9
L3-L4	Pn.pxx	~32	–	–	–	100%	–	–	14
early L4	Pn.pxxx	~34	–	–	–	100%	–	–	19
mid L4	Pn.pxxx	~40	–	–	100%§	4%¶	100%	–	27
late L4	Pn.pxxx	~50	–	–	100%	–	100%	–	16

*GFP expression was observed in *ayIs4[egl-17::GFP dpy-20(+)]; dpy-20(e1282ts)* animals raised at 25˚C.

†The developmental stage and time were deduced based on the size of the gonad and the number of divisions the most advanced Pn.p cell had completed. The stages and times were extrapolated from Sulston and Horvitz (1977).

‡Dashes represent 0% expression.

§GFP expression is observed in the N and T descendants of P5.p and P7.p.

¶One animal out of 27 observed had very faint GFP expression in the descendants of P6.p along with strong expression in the P5.p and P7.p descendants.

‖Similar expression patterns were observed in a large number of additional *ayIs4* animals in which the developmental stage was not rigorously determined.

Characterization of expression from *ayIs4[egl-17::GFP]*

GFP expression in *ayIs4[egl-17::GFP]* hermaphrodites is first observed in two large unidentified cells in the head at the 3-fold stage of embryogenesis. After hatching, expression in these cells fades and is then observed in the M4 pharyngeal neuron where it remains throughout postembryonic development (Fig. 1). Expression in M4 was unaffected by any of the genetic and laser microsurgery manipulations reported here, and thus serves as a good indication that these manipulations do not have general effects on the expression of the reporter construct.

Weak GFP expression can occasionally be observed in the ventral hypodermis in late first stage larvae (L1). This weak expression disappears by the L1 lethargus and was not characterized further. Strong GFP expression is observed in the VPCs and their descendants in two phases starting in late L2 and persisting through adulthood (Table 1; Fig. 1). During early vulval development (late L2-L3), expression is observed in P6.p and its descendants, fading in the early L4. In the mid L4, GFP expression is seen in the N and T descendants of P5.p and P7.p where it remains throughout adulthood.

Vulval expression of *egl-17::GFP* is dependent upon vulval induction

In wild type, the gonad is required to induce P6.p to a 1˚ and P5.p and P7.p to 2˚ vulval cell fates (Sulston and White, 1980; Kimble, 1981; Sternberg and Horvitz, 1986). This inductive event requires the action of a RAS-MAP kinase signaling pathway that is activated by the stimulation of the LET-23 receptor tyrosine kinase (Sundaram and Han, 1996). To determine whether expression of *egl-17::GFP* in the VPCs is dependent on vulval induction, we observed GFP expression in gonad-ablated animals and in animals in which the vulval inductive pathway was compromised by a *let-23* mutation. Both early and late *egl-17::GFP* expression is abolished in gonad-ablated *ayIs4* animals (Table 2). Similarly, a *let-23* mutation that compromises induction of the VPCs and leads to a highly penetrant vulvaless phenotype (Aroian and Sternberg, 1991) also dramatically reduces *egl-17::GFP* expression in the VPCs (Table 2). These data indicate that both early and late *egl-17::GFP* expression in the vulva is dependent upon vulval induction by the gonad.

egl-17::GFP is expressed in vulval cells early in 1˚ cell lineages and late in 2˚ cell lineages

Induction-dependent expression of *egl-17::GFP* early in P6.p and later in P5.p and P7.p descendants suggests that this expression is dependent upon the particular vulval cell fates rather than the specific Pn.p cells in which expression is seen in a wild-type background. To determine whether *egl-17::GFP* expression is restricted to these specific cells or whether it can act as a marker for 1˚ and 2˚ vulval cell fates, we tested whether other cells that acquire 1˚ and 2˚ vulval cell fates can show the correct temporal pattern of *egl-17::GFP* expression.

To test whether early vulval expression is a characteristic of a 1˚ cell fate and is not dependent upon some intrinsic property of P6.p, we took advantage of two known situations in which other cells in the vulval equivalence group acquire the 1˚ cell fate. In the first case, *ayIs4* was placed in a *dig-1* background. In *dig-1* mutants, the gonad is anteriorly displaced, often resulting in VPCs anterior to P6.p acquiring the 1˚ cell fate (Thomas et al., 1990). In *ayIs4; dig-1* animals, early expression was observed in P4.p and P5.p at approximately the same frequency at which 1˚ cell fates are induced in these VPCs (Table 2; Thomas et al., 1990). In 18/21 cases, *egl-17::GFP* expression was seen in the VPC directly ventral to the anchor cell, consistent with the VPC closest to the anchor cell acquiring the 1˚ cell fate (Table 2). In the second case, we took advantage of the ability of P5.p or P7.p to acquire a 1˚ cell fate when P6.p is destroyed by laser microsurgery (Sulston and White, 1980). When P6.p was removed by laser ablation in *ayIs4* animals, early *egl-17::GFP* expression was observed in P5.p or P7.p. In 32/33 cases, this expression correlated with the proximity of the Pn.p cell to the anchor cell (Table 2). Taken together, these experiments indicate that the GFP expression seen in P6.p is not an intrinsic property of P6.p but instead reflects induction to the 1˚ cell fate.

To test whether late vulval expression is characteristic of a 2˚ cell fate and not dependent upon some intrinsic property of P5.p and P7.p, we observed *egl-17::GFP* expression in *lin-12(n137gf)* mutants where all VPCs acquire a 2˚ cell fate (Greenwald et al., 1983). In *ayIs4; lin-12(n137gf)* animals, early *egl-17::GFP* expression was not observed in any of the VPCs, but late *egl-17::GFP* expression was seen in the descendants of essentially all VPC cells (Table 2). Conversely, *lin-11* mutations affect the execution of 2˚ cell fates, altering

Table 2. Effect of ablations or mutations on vulval *egl-17::GFP* expression

Early expression		Vulval precursor cell*						
Genotype	Ablated cells	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	*n*
ayIs4	–	–	–	–	100%	–	–	30
	Z1/Z4	–	–	–	–	–	–	30
	P6.p†	–	–	94%	x	21%	–	33
ayIs4; dig-1(n1321)‡	–	–	29%	67%	10%	–	–	21
ayIs4; let-23(sy1)	–	–	–	7%	13%	–	–	30
ayIs4; lin-12(n137gf)	–	–	–	–	–	–	–	30
ayIs4; lin-15(n309)	–	17%	63%	13%	93%	3%	77%	30
	Z1/Z4	23%	43%	27%	80%	10%	87%	30
ayIs4; let-60(sy130gf)	–	–	–	–	100%	3%	–	30
	Z1/Z4	–	–	–	18%	–	–	33

Late expression			Ectopic pseudovulval sites			
Genotype	Ablated cells	Normal site§	1	2	≥3	*n*
ayIs4	–	100%	n.a.	n.a.	n.a.	32
	Z1/Z4	–	n.a.	n.a.	n.a.	5
ayIs4; let-23(sy1)	–	33%	n.a	n.a	n.a	30
ayIs4; lin-12(n137gf)	–	100%	–	–	100%	30
ayIs4; lin-15(n309)	–	97%	48%	26%	6%	31
ayIs4; let-60(sy130gf)	–	100%	47%	13%	–	30

*Dashes represent 0% expression.

†In 5 out of 33 animals, GFP expression was observed in both P5.p and P7.p. In 4 of those cases, the anchor cell lay between the two expressing cells. In the remaining case the anchor cell was dorsal to P7.p. The propensity of P5.p to express *egl-17::GFP* in this background may result from a higher intrinsic competence of P5.p to assume a 1˚ cell fate or to the spatial distribution of the P(5-7).p cells prior to induction. The anchor cell is often seen anterior of P6.p in the late L2, and cellular debris that remains after ablation of P6.p might block the ability of P7.p to move in towards the anchor cell.

‡Only animals with ventral gonads are included. 2/5 animals with dorsal gonads had expression in the VPCs.

§The normal site of expression refers to expression observed at the vulva which was distinguished from ectopic pseudovulvae by its morphology and position relative to the gonad.

the asymmetric LLTN lineage to a symmetric LLLL lineage (Ferguson et al., 1987). Since late vulval *egl-17::GFP* expression is observed in the N and T descendants of P5.p and P7.p, strong *lin-11* mutations should abolish this expression. Consistent with this prediction, in *ayIs4 lin-11(n389)* animals, early vulval expression is normal, but late vulval expression is severely reduced or abolished (*n*=30). The pattern of *egl-17::GFP* expression observed in these strains is consistent with late expression reflecting the acquisition of a 2˚ vulval cell fate.

As a final confirmation that the early and late vulval *egl-17::GFP* expression can act as markers for 1˚ and 2˚ vulval cell fates, respectively, we tested whether expression patterns correlated with 1˚ and 2˚ vulval cell fates in two mutants where most of the VPCs acquire vulval cell fates. Loss-of-function mutations in *lin-15* and gain-of-function mutations in *let-60 ras* cause additional VPCs to adopt 1˚ and 2˚ cell fates due to hyperactivation of the RAS-MAP kinase pathway (Ferguson et al., 1987; Horvitz and Sternberg, 1991). GFP expression patterns and vulval lineages were determined for the multivulval mutants *ayIs4; lin-15(n309)* and *ayIs4; let-60(sy130gf)*. Consistent with the presence of extra 1˚ cell fates, early *egl-17::GFP* expression is seen both in P6.p as well as additional VPCs in most *lin-15(n309)* animals (28/30) and a few *let-60(sy130gf)* animals (1/30) (Table 2). These strains also show additional late vulval *egl-17::GFP* expression, consistent with the presence of extra 2˚ cell fates (Table 2). The patterns of GFP expression that we observe in these animals are consistent with their lineages and also resemble the inferred cell fates based solely on lineage data (Table 3; Ferguson et al., 1987).

Thus, in a wild-type background *egl-17::GFP* is expressed during vulval development early in P6.p, which adopts the 1˚ cell fate, and late in P5.p and P7.p, which express 2˚ cell fates. Like these vulval cell fates, this expression is dependent upon vulval induction. Under conditions in which P6.p does not

Table 3. Lineage analysis

	Vulval precursor cell					
Genotype	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p
ayIs4	SS	SS	*LLTN*	**TTTT**	*NTLL*	SS
ayIs4; let-60(sy130gf)	LLOL	SS	OOLN	**OOOO**	NOLL	SS
	OOLL	LLON	*LLLN*	**TTTO**	*NLLL*	LOOO
	LLOO	SOO	*LLLN*	**OTTT**	*NOLL*	OOTL
ayIs4; lin-15(n309)	LLLO	LLOT	LLLL	**OTTO**	*NTLL*	**LLLO**
	OOLL	**LOOL**	*LLLN*	**LTTO**	*NLLL*	**LLOL**

Terminal divisions were scored as described (Sternberg and Horvitz, 1986). Individual cells that did not detach from the cuticle are underlined. Lineages where early GFP expression was observed are denoted in bold. Lineages where late GFP expression was observed are denoted in italics.

adopt the 1˚ cell fate, early *egl-17::GFP* expression is not observed in P6.p. Conversely, under conditions when cells other than P6.p can adopt the 1˚ cell fate, early *egl-17::GFP* expression is seen in these other cells, consistent with their induction to a 1˚ cell fate. Thus, early *egl-17::GFP* expression behaves as an early, convenient specific marker for the 1˚ cell fate. Similar arguments demonstrate that late *egl-17::GFP* expression behaves as a convenient, specific marker for 2˚ cell fates.

egl-17 expression in the VPCs is not necessary for proper SM migration

At the time when the SMs are migrating, the only source of *egl-17::GFP* observed in the mid-body region is that in P6.p. The SMs complete their migrations by the time P6.p divides and align with P6.p and its descendants. This places expression of *egl-17* at the right place and time to guide the SMs to their precise final positions. Furthermore, expression of *egl-17::GFP* is gonad-dependent, making *egl-17* a likely candidate for the gonad-dependent attractive cue.

If P6.p is the sole source of *egl-17* that can affect SM migration, then removal of this source should affect the SMs equivalently to loss of *egl-17* function. Since *egl-17::GFP* expression is not restricted to P6.p per se but rather to VPCs that have been induced to the 1˚ cell fate, to remove *egl-17* expression in the VPCs we needed to eliminate the potential of all of the VPCs to express the 1˚ cell fate. This was accomplished in two different ways: (1) cells of the vulval equivalence group, P(3-8).p, were removed by laser ablation; and (2) a *lin-39* mutation was used to homeotically transform P(3-8).p to non-vulval equivalence group cell fates, thus prohibiting them from expressing a 1˚ cell fate (Clark et al., 1993; Wang et al., 1993). In both cases, expression of *egl-17::GFP* was abolished from P6.p and was not observed elsewhere in the mid-body region of these animals (data not shown). Despite eliminating this source of *egl-17*, the SMs migrated normally to final positions flanking the anchor cell (Fig. 2). This is in stark contrast to the severe posterior displacement of the SMs observed in *egl-17* null mutants (Burdine et al., 1997). Therefore, P6.p cannot be the only source of *egl-17* that can affect SM migration. As described below, the expression of EGL-17 in M4 cannot account for the precise positioning of the SMs observed in VPC-ablated animals. Thus, this additional source of EGL-17 is not detectable with our reporter constructs.

Expression of *egl-17* in P6.p is sufficient for the proper migrations of the SMs

While expression of *egl-17* in P6.p is not required for normal SM migration guidance, we wished to determine whether it could play a role in SM migration. To test this, we needed to remove the gonad by laser ablation to eliminate the strong gonad-dependent attraction and express *egl-17* in P6.p in a gonad-independent manner. Expression of *egl-17* in P6.p appears to be dependent upon the induction of P6.p to a 1˚ vulval cell fate. Vulval induction requires the action of a *C. elegans* Ras pathway, and hyperactive alleles of *let-60 ras* can allow vulval cell fates in the absence of the gonad (Beitel et al., 1990; Sundaram and Han, 1995; Sundaram et al., 1996). Thus, gain-of-function *let-60 ras* mutations may lead to expression of *egl-17* in the absence of the gonad, allowing us to test how this expression in the vulval precursor cells affects SM migration guidance.

Various alterations of the *let-60 ras* gene can result in its hyperactivation in *C. elegans*. These include a set of endogenous chromosomal mutants (including *let-60(sy130gf)*) all of which bear a mutation that changes the glycine at codon 13 to glutamic acid (G13E; Beitel et al., 1990). In addition, integrated transgenic arrays with multiple copies of either the wild-type *let-60 ras* gene (*kuIs12*) or the *let-60(G13E)* construct (*kuIs14*) also result in *let-60 ras* hyperactivation (Sundaram and Han, 1995; Sundaram et al., 1996).

ayIs4 was crossed into these hyperactive *let-60 ras* backgrounds to see whether they could affect early *egl-17* expression in the vulval precursor cells. Early expression of *egl-17::GFP* in the VPCs is dependent upon the manner of *let-60 ras* hyperactivation both in the presence and absence of the gonad. In the presence of the gonad, *egl-17::GFP* is expressed in P6.p or in the cell lying closest to the anchor cell in all animals observed (Table 4). Additional VPCs also express *egl-17::GFP* in 1/30 *let-60(sy130gf)* animals, 7/30 *kuIs12[let-*

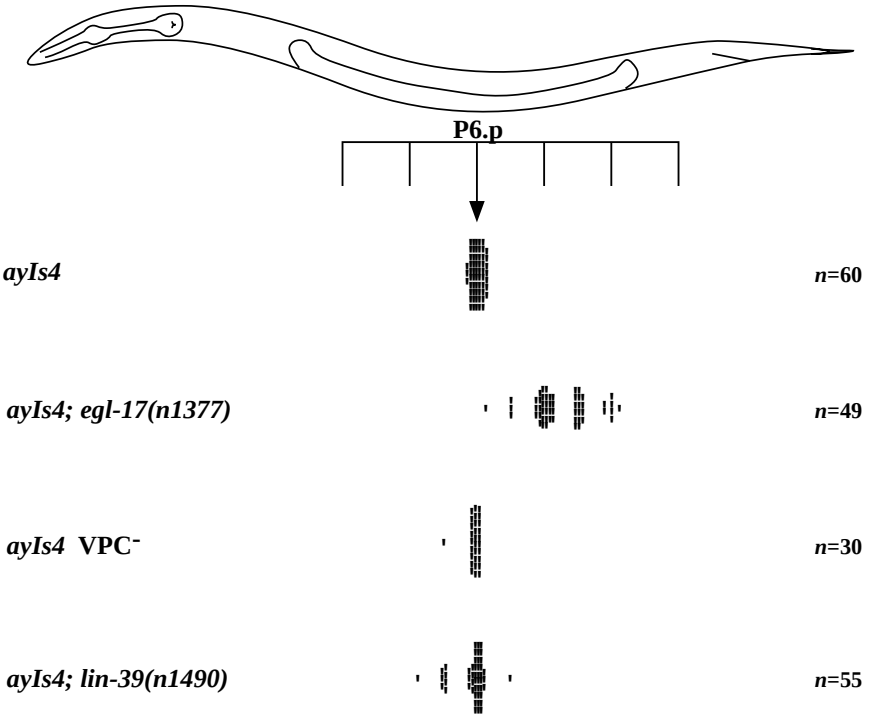

Fig. 2. P6.p is not the sole source of EGL-17 that can affect SM migration. Each hash mark represents the position of a single SM (scored with respect to the animal diagrammed above). SM positions in *ayIs4* and *ayIs4; egl-17(n1377)* were scored as described with respect to the positions of the Pn.p cells (Thomas et al., 1990). SM positions in *ayIs4; lin-39(n1490)* and *ayIs4* VPC- were scored with respect to the anchor cell and correlated to Pn.p position.

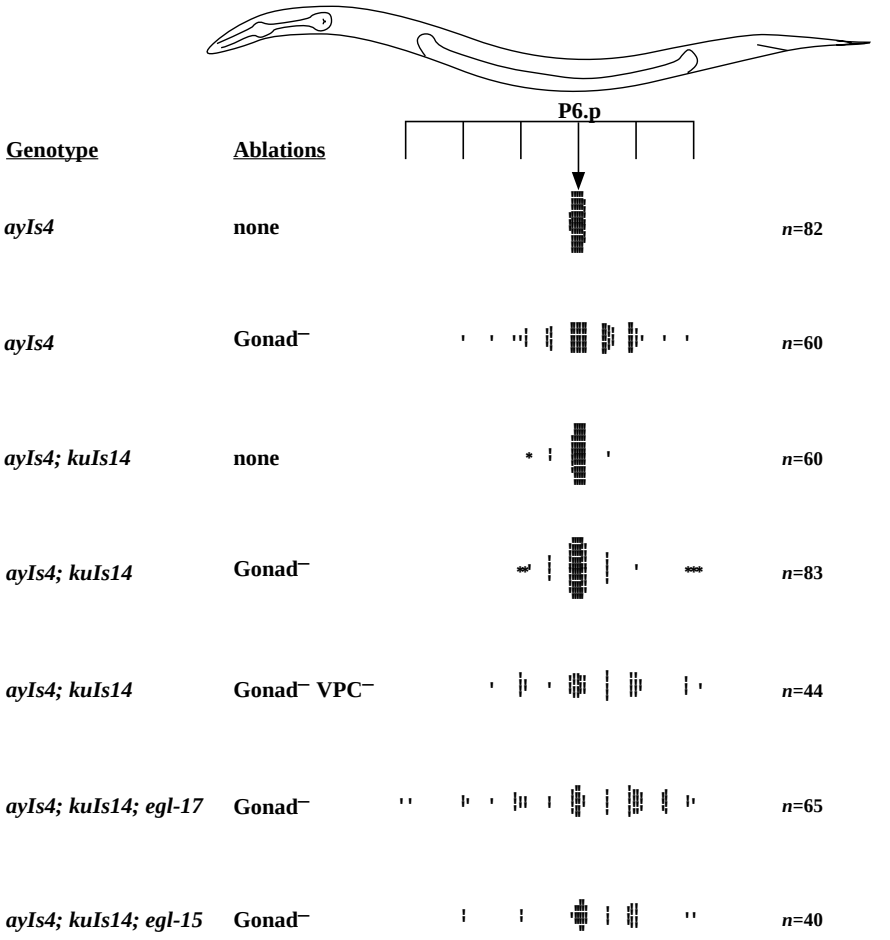

Fig. 3. Gonad-independent precise SM positioning in *kuIs14* animals is due to EGL-17 expression in the VPCs. SM positions were scored as described in the legend to Fig. 2, with the exception of *ayIs4; kuIs14* animals in which the gonad and VPCs were ablated. In these animals, SM positions were scored with respect to the body wall muscles and correlated to Pn.p position. SM distributions in gonad-ablated *kuIs14* animals were found to be indistinguishable whether scored with respect to the body wall muscles or Pn.p cell nuclei. Asterisks denote SMs that were not positioned over P6.p, but were positioned over GFP expressing cells. The alleles used were *egl-17(n1377)* and *egl-15(n1458)*.

60(+)] animals and 12/31 *kuIs14[let-60(G13E)]* animals. By contrast to wild type, in the various hyperactivated *let-60 ras* backgrounds *egl-17::GFP* is expressed early in some of the VPCs even in the absence of the gonad (Table 4). Gonad-independent expression of *egl-17::GFP* is infrequent in *let-60(sy130gf)* (7/33) and *kuIs12[let-60(+)]* (6/30) animals, but is often observed in *kuIs14[let-60(G13E)]* animals (43/50). Interestingly, *egl-17::GFP* is expressed predominantly in P6.p even in the absence of the gonad.

The gonad-independent early expression of *egl-17::GFP* in these hyperactivated *let-60 ras* backgrounds was used to address how EGL-17 expression in the VPCs affects SM migration guidance. Ablation of the gonad resulted in centrally dispersed SMs in both *let-60(sy130gf)* and *kuIs12[let-60(+)]* backgrounds, similar to what is observed after gonad ablation in wild type (data not shown). By contrast, gonad ablation in a *kuIs14[let-60(G13E)]* background resulted in predominantly precisely positioned SMs (Fig. 3) as has been previously reported (Sundaram et al., 1996). The gonad-independent precise SM positioning in a *kuIs14[let-60(G13E)]* background correlates with the high degree of gonad-independent *egl-17::GFP* expression in P6.p, suggesting that early *egl-17* expression in the VPCs might account for the observed precise positioning of the SMs. Further support for this hypothesis comes from the finding that, in 5/16 cases where SMs were found over cells other than P6.p, the SMs were found to align precisely with GFP+ cells (asterisks in Fig. 3). The centrally dispersed SMs observed in *let-60(sy130gf)* and *kuIs12[let-60(+)]* backgrounds may reflect the infrequent expression of *egl-17::GFP* observed in these animals.

To test whether expression of EGL-17 in P6.p could account for the precise positioning of the SMs in gonad-ablated

Table 4. Early *egl-17::GFP* expression in gain-of-function *let-60* mutant backgrounds

		Vulval precursor cell						
Genotype	Ablated cells	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	*n*
ayIs4; let-60(sy130gf)	–	–	–	–	100%	3%	–	30
	Z1/Z4	–	–	–	18%	–	–	33
ayIs4; kuIs12	–	–	13%	10%	100%	3%	3%	30
	Z1/Z4	–	–	–	20%	–	–	30
ayIs4; kuIs14	–	–	17%	20%	100%	10%	20%	30
	Z1/Z4	–	2%	4%	80%	2%	14%	50

kuIs14[let-60(G13E)] animals, we tested whether this effect was dependent upon both *egl-17* and the VPCs in which we saw expression of the *egl-17::GFP* reporter construct. When the VPCs as well as the somatic gonad primordium were ablated in *kuIs14[let-60(G13E)]* animals, the gonad-independent precise positioning of the SMs was abolished (Fig. 3). Thus, the effect is dependent upon the VPCs. When the somatic gonad primordium was ablated in *kuIs14[let-60(G13E)]; egl-17(n1377)* animals where *egl-17* is eliminated by the null allele *n1377* (Burdine et al., 1997), the gonad-independent precise positioning of the SMs also was abolished (Fig. 3). Hypomorphic mutations in *egl-15*, the gene encoding the putative receptor for EGL-17, also compromise the gonad-independent precise positioning of the SMs caused by *kuIs14[let-60(G13E)]* (Fig. 3). Thus, the effect is dependent upon the VPCs in which we see expression of the *egl-17::GFP* reporter construct, and upon both EGL-17(FGF) and its putative receptor EGL-15(FGFR). On the basis of these results, we conclude that expression of *egl-17* in P6.p in the absence of the gonad can act to attract and precisely position the SMs. We postulate that EGL-17 acts directly on the SMs since the expression of *egl-15::GFP* reporter constructs can be observed in the migrating SMs (Fig. 1).

The tendency of SMs to align with GFP+ VPCs when the SMs were not positioned dorsal to P6.p suggested that VPCs that acquire the 1˚ cell fate can attract the SMs to ectopic positions. To test this hypothesis more rigorously, we took advantage of our finding that multiple VPCs can express *egl-17::GFP* in *lin-15(n309)* mutants in the absence of the gonad (see Table 2). To remove multiple potential sources of EGL-17, both the gonad and five of the six VPCs were ablated (Fig. 4). When P4.p was the sole remaining VPC, the SMs migrated past their normal positions to align with P4.p. When P8.p was the sole remaining VPC, the SMs terminated their migrations posterior to their normal positions to align with P8.p. These data indicate that EGL-17 expression in novel locations can guide the SMs to ectopic positions. The slight spread in SM positions in gonad-ablated *lin-15(n309)* animals in which the VPCs were left intact might be due to multiple competing sources of EGL-17. The more precise positioning of the SMs in intact *lin-15(n309)* animals is likely due to the cumulative effect of expression of EGL-17 in the gonad and P6.p outcompeting any additional sources.

DISCUSSION

egl-17::GFP can act as a marker for vulval cell fates

While assessing the role of FGF signaling in SM migration guidance, we have observed that the main sites of postembryonic expression of a number of *egl-17* reporter constructs are in the M4 pharyngeal neuron and the vulval precursor cells (VPCs). The expression observed in the VPCs can be separated into two distinct temporal phases: the early phase consists of expression in P6.p between shortly after induction is thought to occur (Kimble, 1981) and the end of the third larval stage; the later phase consists of expression in the N and T descendants of P5.p and P7.p starting in the mid-fourth larval stage.

The vulval sites of expression make *egl-17::GFP* an excellent marker for the analysis of vulval cell fate determination. P6.p normally acquires a 1˚ cell fate and the early phase of *egl-17::GFP* expression displays all known characteristics of 1˚ cells to make this an accurate reporter of induction towards a 1˚ cell fate. Like 1˚ cells, early *egl-17::GFP* expression is gonad-dependent and is observed in situations where cells other than P6.p can acquire the 1˚ cell fate, such as when P6.p is destroyed by laser ablation or in *dig-1* animals where the gonad position is altered. In addition, in *lin-15* animals that have extra VPCs that take on 1˚ cell fates, additional VPCs can display early expression of *egl-17::GFP* and undergo 1˚-like cell lineages. Taken together, these data demonstrate that *egl-17::GFP* expression correlates with VPC induction to the 1˚ cell fate.

P5.p and P7.p are normally induced to acquire 2˚ cell fates, and late *egl-17::GFP* expression in the N and T descendants of these cells appears to reflect the acquisition of the 2˚ cell fate. Similar to the requirements for 2˚ cell fates, late *egl-17::GFP* expression is abolished by gonad ablation and reduced both in mutants in which vulval induction is compromised and in *lin-11* mutants where 2˚ lineages are not properly executed. Furthermore, late expression is observed in the descendants of additional VPCs in backgrounds where extra 2˚ cell fates are observed (*lin-12gf, lin-15, let-60gf*).

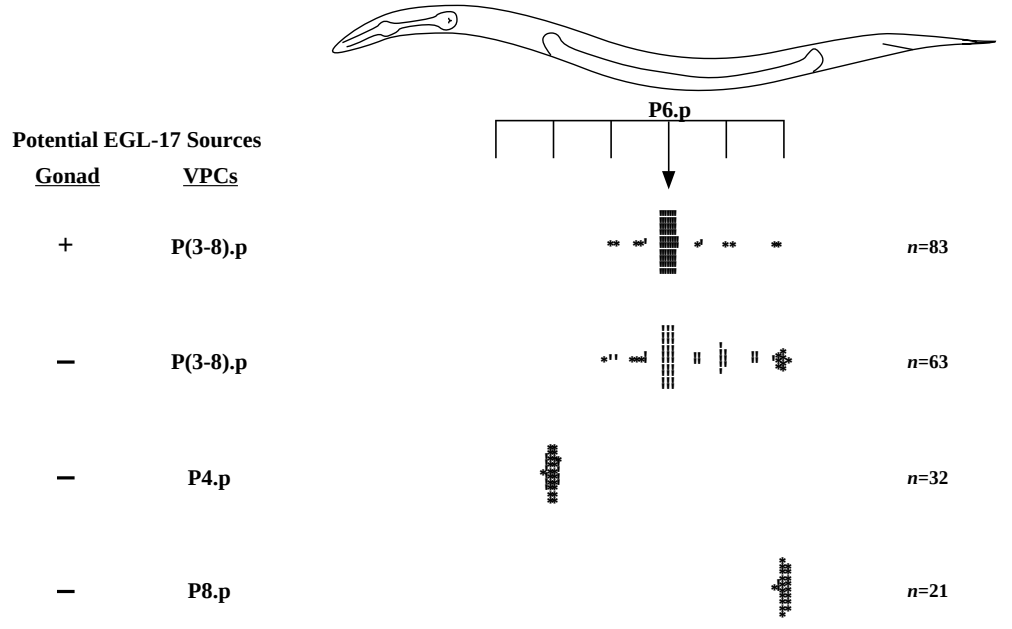

Fig. 4. SMs can be guided to ectopic positions by isolated induced VPCs. All data are shown for animals of genotype *ayIs4; lin-15(n309)*. SM positions were scored with respect to the Pn.p cells and are indicated as described in the previous figures. Isolated Pn.p cells did not appear to move significantly from the positions in which they are normally found presumably due to the absence of the gonad.

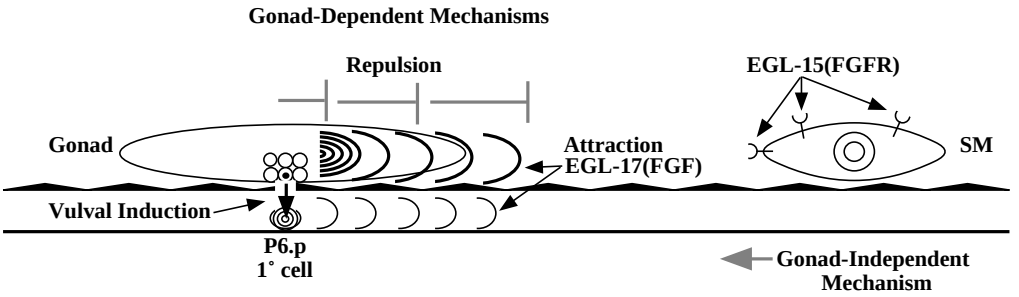

Fig. 5. A model for wild-type SM migration guidance mechanisms. There are two sources of the gonad-dependent attraction: the gonad and P6.p when it is induced by the gonad to acquire a 1˚ cell fate. EGL-17 appears to act as an attractant from P6.p; EGL-17 has not yet been observed to be expressed in the gonad. *egl-15::GFP* reporter construct expression has been observed in the migrating SMs (Fig. 1 and R. D. B., C. S. B. and M. J. S., unpublished observations). A gonad-dependent repulsion is inferred from the behavior of the SMs in *egl-15* or *egl-17* mutants (Stern and Horvitz, 1991). In the absence of all gonad-dependent influences on SM migration, the gonad-independent mechanism propels the SMs anteriorly (Thomas et al., 1990).

Thus, *ayIs4[egl-17::GFP]* is a convenient marker that can be scored in live animals for both 1˚ and 2˚ vulval cell fates by temporally distinct patterns of expression. Early *egl-17::GFP* expression is the first 1˚ cell-fate-specific marker known and also reflects the earliest indication of induction to a 1˚ cell fate. In cases where late expression of *egl-17::GFP* might reflect either a delay in 1˚ cell fate determination or bona fide 2˚ cell fate determination, another 2˚ cell fate reporter gene, *lin-11::lacZ* (Freyd, 1991; Struhl et al., 1993), can be used to help distinguish between these two possibilities.

In the wild type, it appears that multiple mechanisms cooperate to reinforce the cell fate determinations of the VPCs, inexorably propelling the initial proclivities of these cells towards the acquisition of those initial specific cell fates and making these events invariant (Kenyon, 1995). In this background, early *egl-17::GFP* expression reflects commitment to a 1˚ cell fate while late expression reflects the acquisition of a 2˚ cell fate. Under circumstances where the cooperation among the mechanisms is compromised, the execution of cell fates is less uniformly driven. In these situations, as is seen in the variability of the lineages themselves, the degree of *egl-17::GFP* expression is also more variable, reflecting the intermediate status of their vulval cell fates. Thus, in situations where vulval cell fate determination is ambiguous, *egl-17::GFP* expression may reflect an early indication of 1˚ cell fate specification but does not necessarily reflect a state of commitment to the execution of that fate.

Mutations in a number of genes can cause excessive vulval differentiation, and *ayIs4* can help in understanding the mechanisms by which VPCs acquire their fates by aiding our ability to infer cell fate determination in these mutants. One example of this can be seen in the analysis of the developmental potential of the VPCs. In gonad-ablated animals, the anchor cell can no longer bias which of the VPCs will acquire the 1˚ cell fate when these cells are activated by mutational perturbation. However, VPC activation by *kuIs14* appears to stimulate 1˚ cell fates primarily in P6.p, suggesting that cells within the vulval equivalence group are not completely equivalent in developmental potential. Some of the developmental bias among the VPCs is thought to be set up by the action of the Hox cluster genes (Clandinin et al., 1997). While these underlying regulatory mechanisms do not play a primary role in establishing VPC cell fate determination in *C. elegans*, our knowledge of them can provide a basis for understanding how the developmental mechanisms used in other nematodes might arise from changes in the relative importance of the various regulatory mechanisms found in *C. elegans* (Sommer, 1997).

Functional significance of sites of *egl-17::GFP* expression

Similar patterns of expression were observed for a number of *egl-17* reporter constructs using both different types of reporter genes as well as different co-transformation markers. In addition, a full-length *egl-17::GFP* fusion construct is capable of rescuing the SM migration defect caused by *egl-17* mutations. These results suggest that the sites where *egl-17::GFP* expression is observed may reflect the endogenous sites of *egl-17* expression and have led us to address the functional significance of these sites of expression.

Null alleles of *egl-17* appear only to confer SM migration defects and do not appear to affect vulval lineages or subsequent vulval development (Burdine et al., 1997). Thus, while *egl-17::GFP* is a good marker for vulval cell lineages, *egl-17* is not essential for normal vulval development. However, the early phase of expression in the VPCs correlates with the time of SM migration. Furthermore, the SMs terminate their migrations flanking the anchor cell and aligning with P6.p. Thus, the early phase of EGL-17 expression in P6.p places EGL-17 at the right place and time to influence SM migration.

It is interesting to note that vulval *egl-17::GFP* expression occurs at sites between which the vm1 and vm2 vulval muscles attach (White, 1988). Furthermore, we have observed strong *egl-15::GFP* expression in the vm1 muscles and in the progenitors to the vm2 muscles (C. S. B. and M. J. S., unpublished observations). Thus, EGL-17(FGF) and its putative receptor, EGL-15, appear to be expressed in places that could aid in guiding the attachments of the vulval muscles. Since the vulval muscles appear capable of making functional attachments in *egl-17* mutant animals in which the SMs migrate close enough to the center of the animal, a role for EGL-17 in sex muscle attachment may be too subtle to be readily observed or may be obscured by redundant mechanisms.

EGL-17 can act as the gonad-dependent attractant for SM migration

While the gonad is normally required to attract and precisely position the SMs (Thomas et al., 1990), precise positioning of the SMs has also been observed in the absence of the gonad in animals containing a transgenic array carrying multiple copies

of a hyperactivated *let-60 ras* gene (*kuIs14[let-60(G13E)]*; Sundaram et al., 1996). We have observed gonad-independent *egl-17::GFP* expression predominantly in P6.p in this background but do not observe similar levels of gonad-independent *egl-17::GFP* expression in other hyperactivated *let-60 ras* backgrounds where gonad-independent precise SM positioning is not observed.

Since EGL-17(FGF) is required for the gonad-dependent attraction in a wild-type background (Stern and Horvitz, 1991), these data suggest a model in which EGL-17(FGF) is the gonad-dependent attractant. According to this model, in a *kuIs14[let-60(G13E)]* background, gonad-independent EGL-17 expression in P6.p can attract the SMs to their precise final positions even when the gonad is destroyed by laser ablation. This model is supported by the finding that the VPCs, the putative source of EGL-17(FGF) in this background, are required for the precise positioning of the SMs. This finding also indicates that the effect of *kuIs14* on gonad-independent SM positioning is not due to other effects of *kuIs14* such as the activation of *ras* within the migrating SMs. This model is further supported by the finding that gonad-independent precise SM positioning in *kuIs14* backgrounds depends both upon EGL-17(FGF), the proposed attractant, and its putative receptor, EGL-15(FGFR). Additional support for EGL-17 acting as an attractant comes from the finding that SM positions often correlate with the site of *egl-17::GFP* expression even when it occurs in VPCs other than P6.p. This is true both in a *kuIs14* background as well as in a *lin-15(n309)* background. A similar role for *branchless* (FGF) has been proposed for guiding tracheal branching in *Drosophila* (Sutherland et al., 1996).

EGL-17 expression in the VPCs is not the only source of EGL-17 that can affect SM migration

Although EGL-17 expression in the VPCs is gonad-dependent and appears to act as a chemoattractant in SM migration guidance, this site of expression does not account for the entire gonad-dependent attraction observed in wild-type animals. Animals that fail to express *egl-17::GFP* in the VPCs but have intact gonads still have precisely positioned SMs. This is observed when the VPCs are removed by laser ablation and in *lin-39* mutants where the VPCs are homeotically transformed and thus unable to express EGL-17. Thus, removal of the source of observable *egl-17::GFP* does not abolish the gonad-dependent attraction and does not mimic the complete loss-of-function *egl-17* phenotype. These results demonstrate that the VPCs are not the sole source of either the attractant or EGL-17 that can affect SM migration. In support of this, SMs are often found correctly positioned in vulvaless mutants where the VPCs are not induced and presumably do not express EGL-17 (Li and Chalfie, 1990; M. J. S, unpublished observations). Additionally, in *dig-1* animals with dorsal gonads, the SMs will often follow the gonad and not align with the induced VPCs (Thomas et al., 1990), further demonstrating the existence of an additional source that can influence SM migration.

egl-17::GFP expression is observed in the M4 pharyngeal neuron during the time when the SMs are migrating, but this expression cannot account for the P6.p-independent source of EGL-17. First, gonad ablation does not affect M4 expression but does abolish precise positioning of the SMs. Second, the precise positioning of SMs in the *kuIs14* background is abolished when the VPCs are ablated, but the expression in M4 is unaltered. Thus, expression of EGL-17 in M4 cannot precisely position the SMs. Since EGL-17 appears to act as an attractant for SM migration, we postulate that the additional source of both attractant and EGL-17 are the same and are not observable using currently available reporter constructs. Since the additional source of the attractant is gonad-dependent, the simplest model is that this source will be within the gonad itself. Our inability to observe this source with our reporter constructs may be due to the absence of required enhancer sequences or may reflect expression levels below the limits of detection for GFP and β-galactosidase in those cells.

A gonad-dependent repulsion can affect SM migration

Mutations that affect EGL-17(FGF) or its putative receptor EGL-15(FGFR) do not merely abolish the gonad-dependent attraction, but instead change the attraction to a repulsion (Stern and Horvitz, 1991). Since mutations in *egl-17* eliminate its function (Burdine et al., 1997), an additional signal must be able to affect SM migration in the absence of *egl-17*. Our results indicate that EGL-17 can act as an attractant for the SMs, suggesting that the additional signal affecting SM migration is an underlying repulsion. This repulsion is only revealed when the attractive mechanism is compromised. Like the attraction, the repulsion has been shown to be gonad-dependent as it is abolished by ablation of the gonad in *egl-15* or *egl-17* backgrounds (Stern and Horvitz, 1991). Intriguingly, the gonadal cells required for the repulsion in these mutants are the same cells that are required for the attraction in wild-type animals (Thomas et al., 1990; Stern and Horvitz, 1991). Mutations that compromise the repulsion are not currently available, but would help to define the normal roles of the repulsion and determine how it is regulated and how the attraction dominates in a wild-type background.

A model for SM migration

Our results suggest a model in which a hierarchy of localization signals are used to guide the migrations of the SMs to their precise final positions (Fig. 5). A gonad-independent mechanism drives the SMs anteriorly to within a broad range of positions that span the center of the animal. This rough positioning is then honed by an attractant that emanates from the gonad and precisely positions the SMs to flank the center of the gonad. Finally, the anchor cell at the center of the gonad induces P6.p to acquire a 1° cell fate and express EGL-17 which reinforces the gonadal attraction of the SMs to their precise final positions. The distinct roles of these three guidance mechanisms might suggest an order for their action. In fact, a temporal analysis of SM migration has begun to reveal overlapping but distinct time periods during which these mechanisms appear to act (C. S. B. and M. J. S., unpublished data). In the absence of the attraction by EGL-17(FGF), another signal emanates from the gonad that repels the SMs. The normal role of this repellant signal is not currently known.

Although EGL-17 has not been observed to be expressed in the gonad, several lines of evidence are consistent with it being the gonadal attractant. First, our data indicate that EGL-17(FGF) can attract the SMs to their precise final positions. Second, mutations in *egl-17* abolish the gonad-dependent attraction. Third, *egl-17* must be expressed from additional cells that we do not detect with our reporter constructs. Mosaic

analysis of *egl-17* could help determine whether EGL-17(FGF) is required to be expressed in the gonad and thereby simultaneously resolve whether the gonad itself emits an attractive cue as well as whether that cue is EGL-17.

Whether or not the gonad emits EGL-17 as a chemoattractant for the SMs, our results show that the expression of EGL-17 in P6.p intertwines the development of two of the egg-laying components. This mechanism that links SM migration guidance to vulval induction further aids the integrity of the egg-laying machinery by helping to ensure that the muscles that are required to open the vulva during egg laying are generated in positions at which they can make functional attachments.

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