Development 122, 2823-2833 (1996)
Printed in Great Britain © The Company of Biologists Limited 1996
DEV5020

A Ras-mediated signal transduction pathway is involved in the control of sex myoblast migration in *Caenorhabditis elegans*

Meera Sundaram*, John Yochem and Min Han

Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Boulder, CO 80309-0347, USA

*Author for correspondence (e-mail: sundaram@beagle.colorado.edu)

SUMMARY

Sex myoblast migration in the *Caenorhabditis elegans* hermaphrodite represents a simple, genetically amenable model system for studying how cell migration is regulated during development. Two separable components of sex myoblast guidance have been described: a gonad-independent mechanism sufficient for the initial anterior migration to the mid-body region, and a gonad-dependent mechanism required for precise final positioning (J. H. Thomas, M. J. Stern and H. R. Horvitz (1990) *Cell* 62, 1041-1052). Here, we demonstrate a role for a Ras-mediated signal transduction pathway in controlling sex myoblast migration. Loss-of-function mutations in *let-60 ras, ksr-1, lin-45 raf, let-537/mek-2* or *sur-1/mpk-1* cause defects in sex myoblast final positions that resemble those seen in gonad-ablated animals, while constitutively active *let-60 ras(G13E)* transgenes allow fairly precise positioning to occur in the absence of the gonad. A mosaic analysis demonstrated that *let-60 ras* is required within the sex myoblasts to control proper positioning. Our results suggest that gonadal signals normally stimulate *let-60 ras* activity in the sex myoblasts, thereby making them competent to sense or respond to positional cues that determine the precise endpoint of migration. *let-60 ras* may have additional roles in sex myoblast guidance as well. Finally, we have also investigated genetic interactions between *let-60 ras* and other genes important for sex myoblast migration, including *egl-15*, which encodes a fibroblast growth factor receptor tyrosine kinase (D. L. DeVore, H. R. Horvitz and M. J. Stern (1995) *Cell* 83, 611-623). Since mutations reducing Ras pathway activity cause a different phenotype than those reducing *egl-15* activity and since constitutive Ras activity only partially suppresses the migration defects of *egl-15* mutants, we argue that *let-60 ras* and *egl-15* do not act together in a single linear pathway.

Key words: migration, Ras, *Caenorhabditis elegans*, sex myoblast, *let-60, ksr-1*, signal transduction

INTRODUCTION

Sex myoblast (SM) migration in the *Caenorhabditis elegans* hermaphrodite provides a simple genetic model system to study the control of cell migration during development. The two SMs are born during the L1 larval stage in the posterior region of the animal, one on the left side and one on the right. The SMs then migrate approximately 65 μm anteriorly during the L2 stage to occupy very precise final positions flanking the gonad in the mid-body region, near the site of the future vulva (Fig. 1A). During the late L3 stage, the SMs undergo a series of cell divisions to produce sixteen sex muscles that function in the adult to contract the uterus and open the vulva during egg laying (Sulston and Horvitz, 1977; Trent et al., 1983). Strict regulation of SM final positions ensures that the sex muscles will be generated in the appropriate place to make their necessary connections to the hypodermis, uterus, vulva and innervating neurons. In mutants where SM final positions are abnormal, the displaced SMs still divide to produce the wild-type number of sex muscles, but these sex muscles are often not able to attach to their targets (Stern and Horvitz, 1991). Such mutants are therefore egg-laying defective (Egl).

Several types of experiments have established that the somatic gonad is one source of signals regulating SM migration. Thomas et al. (1990) observed that, in *dig-1* mutants, where the gonad is located more anteriorly than normal and is sometimes displaced dorsally as well, the SMs alter their migrations accordingly so as to flank the center of the displaced gonad. Therefore, the gonad provides an attractive cue capable of controlling the extent and direction of SM migrations. The same researchers found that when the somatic gonad is ablated in wild-type animals prior to the time of SM migration, the SMs still migrate anteriorly but adopt final positions spanning a broader anterior-posterior range than normal (Fig. 1B). Therefore, non-gonadal cues are sufficient to guide the SMs to the appropriate general region, but gonadal signals are required to specify the precise endpoint of the migration. Finally, analysis of *egl-15* and *egl-17* mutants has suggested that the gonad can also be a source of repulsive cues (Stern and Horvitz, 1991). In these mutants, the SMs stop at strikingly abnormal posterior positions; however, the SMs will migrate further anteriorly if the gonad has been ablated. One possible interpretation of the *egl-15* and *egl-17* mutant phenotypes is that these mutations eliminate some attractive guidance system(s) and thereby reveal an underlying repulsive influence of the gonad (Stern and Horvitz, 1991). Taken together, the

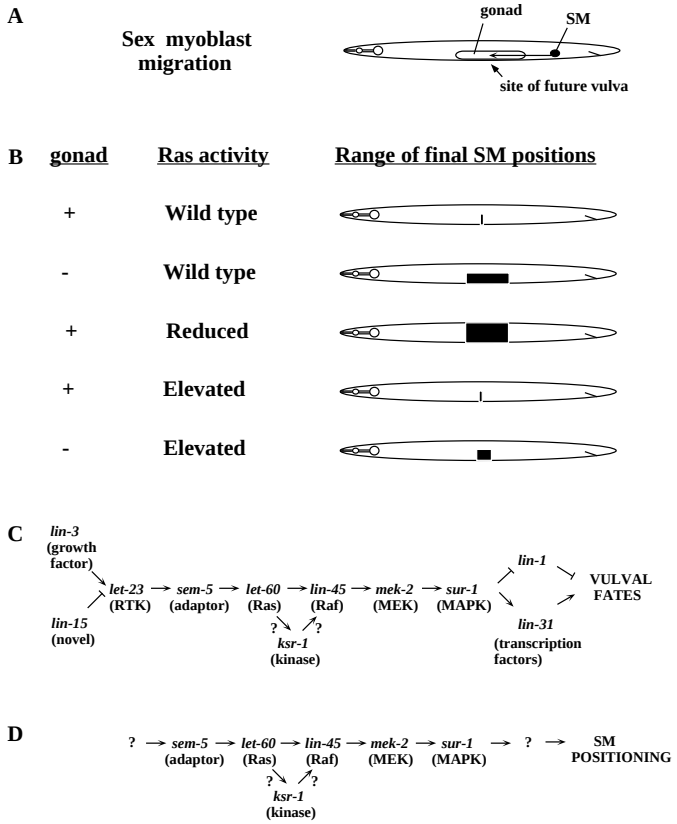


Fig. 1. Sex myoblast migration and the Ras pathway. (A) SM migration in the wild-type *C. elegans* hermaphrodite. Anterior is left, ventral is down. Only the left SM is shown here; another SM is located in an equivalent position on the right side of the animal. The SMs arise in the posterior body region during the L1 stage, and then migrate to the mid-body during L2, as indicated by the arrow. (B) Summary of data showing that *let-60 ras* activity is both necessary and sufficient for the gonad-dependent regulation of SM positioning. The black box indicates the approximate range of final SM positions relative to the body of the worm. +, gonad present. –, gonad ablated. reduced, animals bearing loss-of-function mutations in *let-60 ras*. elevated, transgenic animals bearing integrated arrays of *let-60(G13E)* DNA (see text). (C) The Ras pathway controlling vulval induction. A growth factor-like signal (LIN-3) from the gonadal anchor cell stimulates the LET-23 RTK in the underlying vulval precursor cells (VPCs), triggering a cascade involving a Grb2-like adaptor protein (SEM-5; Clark et al., 1992a), a Ras protein (LET-60; Han and Sternberg, 1990), a Raf (LIN-45; Han et al., 1993), a MEK (LET-537/MEK-2; Church et al., 1995; Kornfeld et al., 1995a; Wu et al., 1995) and a MAPK (SUR-1/MPK-1; Lackner et al., 1994; Wu and Han, 1994). Loss-of-function alleles of *let-60 ras* and other Ras pathway genes cause a Vulvaless phenotype similar to that seen after elimination of the signaling anchor cell, while constitutively active *let-60 ras* alleles cause a Multivulva phenotype in which extra VPCs adopt vulval fates in a signal-independent manner (reviewed by Kayne and Sternberg, 1995; Sundaram and Han, 1996). Because Ras-mediated signaling is also involved in many other processes in *C. elegans*, severe loss-of-function alleles of the above genes also cause other pleiotropic defects including lethality, sterility and male tail abnormalities (reviewed by Sternberg, 1993). *lin-15* encodes two novel proteins that function to negatively regulate the Ras pathway (Clark et al., 1994; Huang et al., 1994), *ksr-1* encodes a putative kinase that appears to regulate positively *lin-45 raf* and/or be regulated positively by *let-60 ras* (Kornfeld et al., 1995b; Sundaram and Han, 1995; M. S. and M. H., unpublished data), and *lin-1* and *lin-31* encode putative transcription factors that may be regulated by the Ras pathway (Beitel et al., 1995; Miller et al., 1993). (D) The Ras pathway controlling SM positioning. Mutations in *sem-5* (Clark et al., 1992a), *let-60 ras, ksr-1, lin-45 raf, mek-2* and *sur-1/mpk-1* all cause similar defects in SM positioning suggesting that these genes act together during this process as they do during vulval induction. The ligand and receptor acting upstream of the Ras pathway are unknown, although EGL-15 FGFR is one candidate receptor (DeVore et al., 1995; see text). Downstream targets of this cascade in the SMs are also unknown.

above observations suggest that precise SM positioning in wild type may rely on a balance between attractive and repulsive cues.

egl-15 has recently been shown to encode a protein related to the fibroblast growth factor receptor tyrosine kinase (FGFR) (DeVore et al., 1995). In vertebrate systems, the FGFR is thought to act at least in part by stimulating a common signaling cascade involving the small GTPase Ras and the downstream kinases Raf, MAPK/ERK kinase (MEK) and Mitogen-Activated Protein Kinase/Extracellular-signal Regulated Kinase (MAPK/ERK) (Whitman and Melton, 1992; Wood et al., 1992; MacNicol et al., 1993; Umbhauer et al., 1995). Experiments with a *Drosophila* FGFR are consistent with such a model. The *Drosophila breathless* gene encodes an FGFR required for the migrations of tracheal cells (Glazer and Shilo, 1991; Klambt et al., 1992), and activated *breathless*, *Dras1* or *Draf1* constructs can partially rescue the tracheal migration defects of *breathless* mutants (Reichman-Fried et al., 1994). However, the normal roles of *Dras1* and *Draf1* in tracheal migration remain unclear since the loss-of-function phenotypes of these genes have not been reported for the trachea.

We have been studying a Ras→Raf→MAPK signaling cascade that controls vulval induction and other processes in *C. elegans* (Fig. 1C), and we and others have recently described a new gene, *ksr-1*, that encodes a novel protein kinase that influences signaling through this cascade (Kornfeld et al., 1995b; Sundaram and Han, 1995). The phenotypes and genetic interactions associated with *ksr-1* mutations suggest that these mutations generally reduce the effective level of Ras pathway activity. Interestingly, we found that *ksr-1* mutants have defects in SM positioning (Sundaram and Han, 1995). Some mutations in *sem-5*, which encodes a GRB2-like adaptor protein that functions during vulval induction, also cause defects in SM positioning (Clark et al., 1992a). These observations, in conjunction with the finding that *egl-15* encodes an FGFR (DeVore et al., 1995), prompted us to explore the possibility that the Ras pathway is involved in SM migration.

MATERIALS AND METHODS

Strains

Bristol var. N2 was the parent for all strains. Methods for the handling, culture and genetic manipulation of animals were as described previously (Brenner, 1974). Experiments were performed at 20°C unless otherwise noted. Mutations used were: *dig-1(n1321) III* (Thomas et al., 1990), *dpy-20(e1282) IV* (Hosono et al., 1982), *egl-15(n484) X* (Trent et al., 1983; Stern and Horvitz, 1991), *egl-17(n1377) X* (M.

Stern, personal communication), *him-5(e1490) V* (Hodgkin et al., 1979), *let-23(sy1) II* and *let-23(sy97) II* (Aroian and Sternberg, 1991), *let-60(n1046gf) IV* (Ferguson and Horvitz, 1985), *let-60(s1124) IV* and *let-60(s1155) IV* (Clark et al., 1988), *let-60(sy101 sy127) IV* (Han et al., 1990); *let-537/mek-2(ku114) I* (Wu et al., 1995), *let-537/mek-2(ku159) I* (D. Sieburth and M. H., unpublished data), *let-537/mek-2(h294) I* (McKim, 1990), *lin-1(ar147) IV* (Tuck and Greenwald, 1995), *lin-3(n1058) IV* (Ferguson and Horvitz, 1985), *lin-15(n765) X* (Ferguson and Horvitz, 1985); *lin-31(n301) II* (Miller et al., 1993), *lin-45(ku112) IV* (D. Green and M. H., unpublished data; Sundaram and Han, 1995), *lin-45(sy96)* (Han et al., 1993), *lon-2(e678) X* (Brenner, 1974), *ncl-1(e1865) III* (Hedgecock and Herman, 1995), *sem-5(n1779) X* (Clark et al., 1992a), *sur-1/mpk-1(ku1)* (Wu and Han, 1994), *sur-1/mpk-1(oz140)* (Church et al., 1995), *ksr-1(ku68) X* and *ksr-1(ku83) X* (Sundaram and Han, 1995), *unc-22(s7) IV* (Moerman and Baillie, 1979), *unc-31(e169) IV* and *unc-36(e251) III* (Brenner, 1974). *DnT1* is a derivative of the translocation *nT1(IV; V)* (Ferguson and Horvitz, 1985) containing recessive lethal and dominant Unc markers.

Homozygous strains bearing multiple mutations were constructed by standard genetic methods. As controls for the various marker mutations used, we have shown that animals mutant for *dpy-20*, *him-5*, *lon-2* or *unc-22* have wild-type sex myoblast positioning (n>30 for each).

Scoring sex myoblast positions

Mid L3 stage hermaphrodites were mounted for observation by Nomarski microscopy as described (Sulston and Horvitz, 1977). Sex myoblast (SM) final positions were scored in animals in which the SMs and vulval precursor cells (VPCs) were enlarged prior to dividing, or in which the SMs and/or VPCs had already divided once. To aid us in quantitating SM migration defects, we arbitrarily defined compartments along the anterior-posterior axis of the animal based on the positions of the VPCs at mid-L3, with compartment boundaries located midway between each VPC. The position of the anchor cell (usually over P6.p) was noted. Animals having abnormal AC positioning relative to the VPCs were discarded, except in the case of *let-60(sy101 sy127)* mosaic animals (Fig. 3 legend). Individual SM positions were assessed relative to the VPCs, and each SM was assigned to the corresponding compartment defined by the nearest VPC. SMs positioned precisely at the compartment boundary were assigned to the outer compartment. All SMs located posteriorly of P8.p were assigned to the P8.p compartment [SMs in *egl-15* or *egl-17* mutants may be located near P9.p or P10.p (Stern and Horvitz, 1991)]. If the SM and/or VPC had divided, the position of that cell was taken to be midway between the two daughter cells. We made every attempt to score both SMs in each animal, but this was not always possible (particularly in the less healthy mutants).

Laser ablations

The somatic gonad precursors Z1 and Z4 were ablated prior to their divisions in L1 larvae using a laser microbeam as described previously (Avery and Horvitz, 1987). Ablations were confirmed 24 hours later by checking that the gonad remnant contained only a few germ cell nuclei.

Mosaic analysis

Mosaic animals were identified as segregants from strain MH809 *[ncl-1 unc-36; let-60(sy101 sy127); him-5; kuEx72(ncl-1$^+$ unc-36$^+$ let-60$^+$)]*. *kuEx72* is composed of cosmid C33C3 (*ncl-1*$^+$) and plasmids R1p16 (*unc-36*$^+$) (Herman et al., 1995) and pMH90 (*let-60*$^+$) (see below). Further details pertaining to the construction of MH809 will be published elsewhere (J. Y., M. S. and M. H, unpublished data). Mid-L3 stage hermaphrodites in which spontaneous somatic loss of the *kuEx72* extrachromosomal array had occurred were recognized by examining the following cells for the cell autonomous Ncl-1 (enlarged nucleolus) phenotype (Hedgecock and Herman, 1995): ASKL, ADLL (from AB.alp); ILsoDL, ASIL, P(3/4,5/6,7/8).p (from AB.pla); excretory cell, DVB, hyp8/9, F (from AB.plp); ASKR, ADLR, ILsoDR, ASIR, P(3/4,5/6,7/8).P (from AB.pra); hyp8/9, B, DVA (from AB.prp); posterior distal tip cell, AC (from MS.ap); anterior distal tip cell, AC (from MS.pp); and body muscles (from AB.prp, MS, C and D). Additional cells scored to determine more precisely the point of *kuEx72* loss in mosaic animals included: ALA, RID (from AB.ala); m3L, m3VL (from AB.alp); mc2DL, mc2DR, I5, mc2V (from AB.ara); TL.pa(a/p) (from AB.pla); TR.pa(a/p) (from AB.pra); m3DL (from MS.aa); and coelomocytes (from MS). Because the Ncl phenotype cannot be scored reliably in the SMs, we assessed SM genotypes indirectly by scoring the Ncl phenotype of related M-derived body muscles and coelomocytes. Approximately 2500 animals were screened in order to identify the mosaic animals described here. Only mosaic animals for which SM positions were recorded are shown.

Construction of *let-60 ras* transgenic lines

Germ-line injections were performed as described (Mello et al., 1991). The host strain for all microinjections was of genotype *dpy-20; him-5*. pMH90, a *let-60(+)*-bearing plasmid, is derived from pMH82 (Han and Sternberg, 1990), with the addition of a 5 kb *Bam*HI fragment bearing additional 5′ flanking sequences. pMH132, a *let-60(G13E)*-bearing plasmid, is derived from pMH106 (Han and Sternberg, 1991), with codon 13 changed from GGA (Gly) to GAA (Glu). Either pMH90 (10 μg/ml) or pMH132 (1 μg/ml) was coinjected with a plasmid containing *dpy-20(+)* [pMH86, 10 μg/ml; Han and Sternberg, 1991] and bluescript SK(+) (100 μg/ml) to obtain stably transmitting non-Dpy Multivulva lines. Trangenic animals from such lines were gamma-irradiated at a dose of 3600 rads, and their F_1 non-Dpy progeny were picked singly to plates to identify those in which the transgene had integrated into the genome (giving 75% transmittance to the F_2). Lines bearing integrated transgenes were obtained at a frequency of approximately 1 in 200 F_1s picked. A total of 5 *let-60(+)*-bearing lines and 4 *let-60(G13E)*-bearing lines were initially analyzed; SM positioning was normal in all lines. Two transgenic lines of each type were chosen for further analysis: *kuIs11* and *kuIs12 [let-60(+)*] and *kuIs13* and *kuIs14 [let-60(G13E)]*. Each line was backcrossed one time to the parent *dpy-20; him-5* strain before analysis. *kuIs17* is a control transgenic array containing pMH86, pSK(+) and a non-*let-60*-bearing plasmid.

RESULTS

Mutations that reduce Ras pathway activity cause a broadening of final SM positions similar to that seen after gonad ablation in wild type

To quantify the extent of SM migration in various mutants, we have defined compartments along the anterior-posterior axis of the animal based on the positions of the VPCs during the mid-L3 larval stage (Materials and Methods). In wild-type animals, 100% of the SMs terminate migration in the compartment nearest P6.p (Table 1A, Fig. 2A,B). In gonad-ablated animals, the SMs adopt a broader range of final positions, with some stopping in the P7.p compartment while others continue on to the P5.p compartment (Thomas et al., 1990; Table 1A). However, a majority of the SMs still are found in the proper P6.p compartment even after gonad ablation.

We examined a number of mutants previously characterized as having reduced levels of Ras pathway activity and found that many have a broadened range of final SM positions (Table 1, Fig. 2C,D). The anteroposterior distribution of SMs in these mutants resembles that of gonad-ablated wild-type animals and

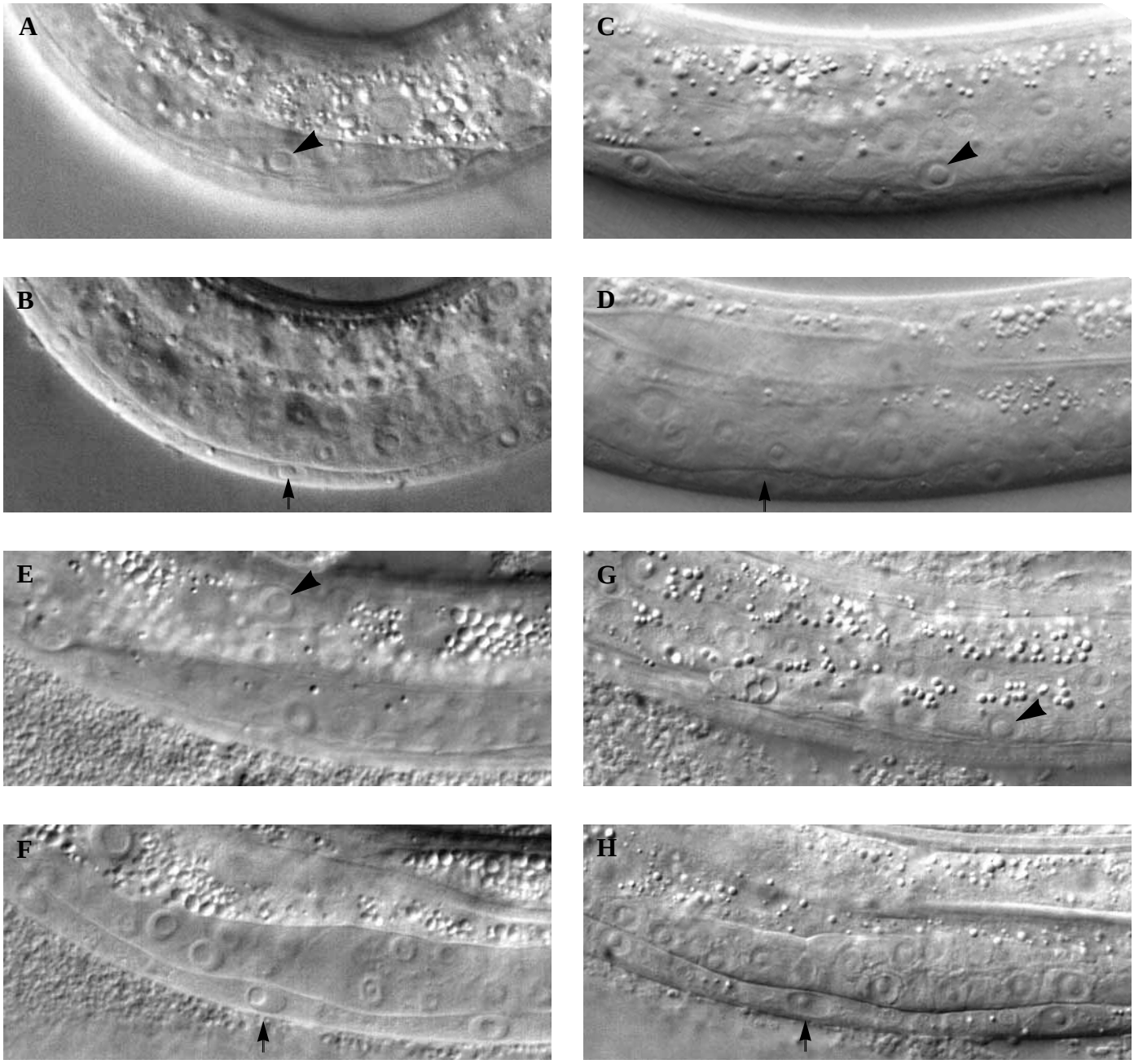


Fig. 2. Photomicrographs of representative animals having mispositioned SMs. Lateral views of the mid-body region of mid-L3 stage hermaphrodites. For each animal, two focal planes are shown. (A,C,E,G) Left or right focal plane showing one SM (indicated by arrowhead). (B,D,F,H) Central focal plane showing VPCs, with arrow indicating P6.p. (A,B) N2 (Wild type), SM positioned near P6.p. (C,D) *ksr-1(ku68),* SM posterorly displaced. (E,F) *ksr-1(ku68),* SM dorsally displaced. (G,H) *let-60(sy101 sy127)* mosaic animal lacking *kuEx72* in the M lineage, SM posteriorly displaced.

differs significantly from that of *egl-15* or *egl-17* mutants. Furthermore, in some mutants the SMs occasionally adopted abnormal dorsal or lateral positions (Fig. 2E,F and Table 1 legend).

We examined the effects of the strong *let-60 ras* loss-of-function mutations *let-60(s1124)* and *let-60(s1155)* (Clark et al., 1988; Beitel et al., 1990) by looking at rare escaper animals that survive past the time that SM migration occurs. The SMs adopt a broadened range of final positions and sometimes adopt dorsal positions in such animals; however, most of these animals are quite sick and have other defects which could be affecting SM migration secondarily. We also examined the effects of mutations that only partially reduce Ras pathway activity. Surviving *let-60(n2035)* and *lin-45(sy96)* homozygotes are fairly vigorous but partially Vulvaless (Beitel et al., 1990; Han et al., 1993), whereas most surviving *ksr-1(ku68), ksr-1(ku83), lin-45(ku112), mek-2(ku159), mek-2(ku114)* and *sur-1/mpk-1(ku1)* homozygotes are healthy and have wild-type vulval and gonadal morphology (Wu and Han, 1994; Sundaram and Han, 1995; Wu et al., 1995; D. Sieburth and M.

Table 1. Effects of Ras pathway mutations on SM positioning

	Genotype[1]	gonad	vulva	AC / P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	
A	+/+	+	WT				100%			many[2]
	+/+	–	Vul			5%	57%	35%	3%	n = 60
B	*let-60(s1124)*	+	Vul			6%	47%	47%		n = 17
	let-60(s1155)	+	Vul			7%	50%	32%	11%	n = 28
	let-60(n2035)	+	Vul				85%	15%		n = 33
	let-60(n1046gf)	+	Muv				92%	6%	2%	n = 133
C	*ksr-1(ku68)*	+	WT			8%	82%	10%		n = 109
	ksr-1(ku68)	–	Vul			2%	36%	44%	9%	n = 66
	ksr-1(ku83)	+	WT			7%	71%	22%		n = 59
D	*lin-45(sy96)*	+	Vul/WT			3%	76%	21%		n = 38
	lin-45(ku112)	+	WT			7%	87%	5%		n = 40
E	*mek-2(ku114)*	+	WT			2%	77%	20%		n = 84
	mek-2(ku159)	+	WT			6%	74%	20%		n = 34
F	*sur-1(ku1)*[3]	+	WT/Vul			9%	88%	3%		n = 75

Final SM positions in (A) wild type, (B) *let-60 ras*, (C) *ksr-1*, (D) *lin-45 raf*, (E) *let-537/mek-2* and (F) *sur-1/mpk-1* mutants. In all figures, anterior is to the left and ventral is down. The positions of the VPCs (P3.p-P8.p) at the mid-L3 stage are indicated. The gonadal anchor cell (AC) was located above P6.p as shown for all animals scored. Vertical lines represent compartment boundaries that we arbitrarily defined relative to the VPCs (see Materials and Methods). The percentage of SMs that adopted final positions in a given compartment is indicated within that compartment. Percentages are rounded to the nearest whole number and therefore do not always add up to 100. +, gonad intact. –, gonad precursors ablated (Materials and Methods). Muv, Multivulva. Vul, Vulvaless. WT, wild-type vulva. n, number of SMs scored. In the following mutants, a small percentage of SMs were observed in abnormal dorsal or lateral positions: 2/17 in *let-60(s1124)*, 1/28 in *let-60(s1155)*, 1/133 in *let-60(n1046gf)*, 3/109 in *ksr-1(ku68)*, 1/66 in gonad-ablated *ksr-1(ku68)*, 1/59 in *ksr-1(ku83)*, 5/84 in *mek-2(ku114)*.

[1]Animals are unmarked and were obtained from homozygous mutant mothers unless otherwise indicated below.
let-60(s1124): let-60(s1124) unc-22 unc-31, from *let-60(s1124) unc-22 unc-31/nT1* mothers.
let-60(s1155): let-60(s1155) unc-22 unc-31, from let-60(s1155) unc-22 unc-31/nT1 mothers.
let-60(n2035): let-60(n2035), from *let-60(n2035)/DnT1* mothers.
lin-45(ku112): lin-45(ku112) dpy-20.
lin-45(sy96): lin-45(sy96); him-5.

[2]As controls for markers used in this paper, we have shown that the *dpy-20, him-5, lon-2* and *unc-22* mutations do not perturb sex myoblast positioning (n>30 for each).

[3]15°C. As a control, we have shown that wild-type hermaphrodites grown at 15°C have completely normal SM positioning (n=54).

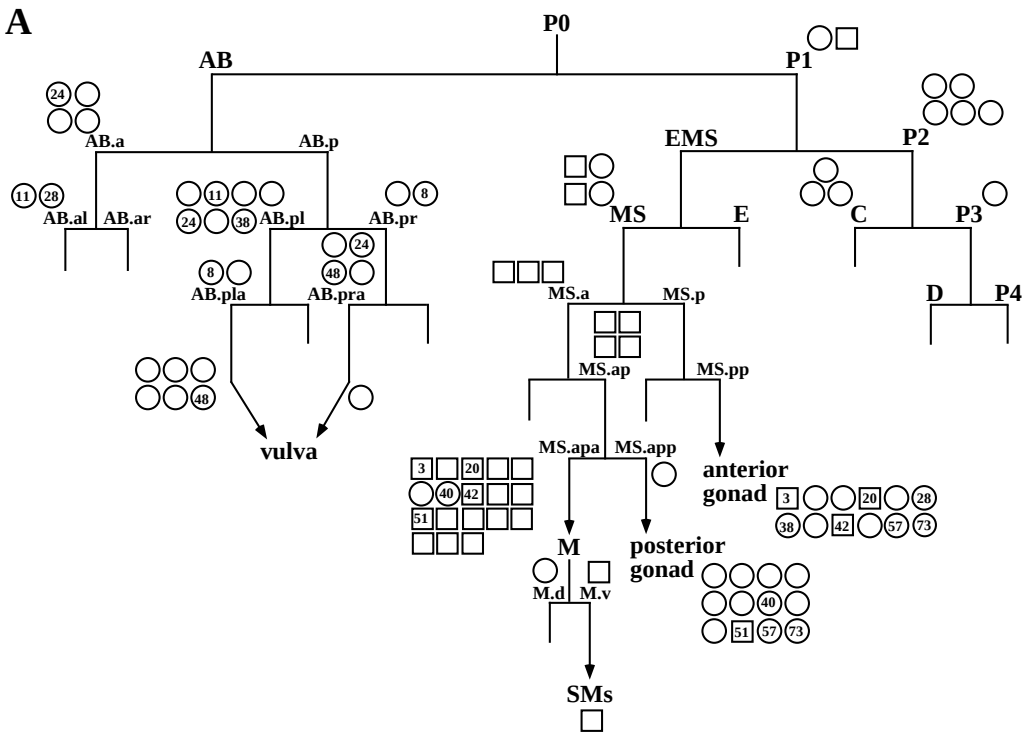
H., unpublished data). All of these mutants have defects in SM positioning, suggesting that the effects of Ras pathway mutations on SM positioning are likely to be direct.

We did not observe significant SM positioning defects in *let-537/mek-2(h294)* animals (n=19) or *sur-1/mpk-1(oz140)* animals (n=37) segregating from heterozygous mothers, a fact that might be attributable to maternal rescue, which is commonly associated with other Ras pathway mutant phenotypes (i.e., Beitel et al., 1990; Han et al., 1993; Kornfeld et al., 1995a). Since homozygotes for either allele are sterile, the phenotypes of mutants segregating from mutant mothers could not be assessed. Neither *let-537/mek-2(h294)* nor *sur-1/mpk-1(oz140)* is thought to eliminate gene activity (Church et al., 1995).

lin-3 and *let-23* encode the putative ligand and receptor controlling vulval induction (Aroian et al., 1990; Hill and Sternberg, 1992), while *lin-1* and *lin-31* encode putative transcription factors that function downstream of the Ras pathway during vulval induction (Miller et al., 1993; Beitel et al., 1995) (see Fig. 1C). We did not find any SM positioning defects in *lin-3(n1058)* animals segregating from heterozygous mothers (n=28), or in *let-23(sy1), let-23(sy97), lin-1(e1275), lin-1(ar147)* or *lin-31(n301)* mutant animals segregating from homozygous mutant mothers (n>30 for each). Additionally, neither *lin-1(e1275)* nor *lin-1(ar147)* suppressed the SM positioning defects of *ksr-1* mutants (data not shown). These data suggest that the Ras pathway may use different upstream regulators and downstream targets for vulval induction versus SM migration (Fig. 1D) and further indicate that neither loss of vulval cell fates nor the presence of ectopic vulval cell fates perturbs SM positioning. Thomas et al. (1990) have shown previously that ablation of the vulval precursor cells does not significantly perturb SM positioning.

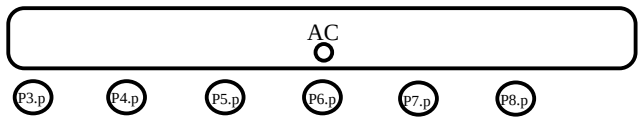
ksr-1 mutations affect gonad-dependent attraction

The similar SM migration defects seen in Ras pathway mutants and gonad-ablated wild-type animals suggest that the Ras pathway may mediate gonad-SM interactions. To test this hypothesis further, we constructed double mutants between *ksr-1* and *dig-1*. In approximately 10% of *dig-1* hermaphrodites, the gonad is located dorsally rather than ventrally; in such animals, the SMs first migrate anteriorly as usual, but then



B

let-60 ras Genotype of



SMs	gonad	P5.p	P6.p	P7.p	P8.p	
+	+		100%			n = 326[1,2]
+	+/–		100%			n = 34[3]
–	+		15%	77%	8%	n = 26[1]
–	+/–		25%	71%	4%	n = 24[1]
–	–	30%	50%	20%		n = 10[1]
All SMs lacking *kuEx72*		5%	25%	65%	5%	n = 60[1]

Fig. 3. Mosaic analysis of *let-60 ras*. Mosaic animals were identified by screening segregants from strain MH809 *[ncl-1 unc-36; let-60(sy101 sy127); him-5; kuEx72(ncl-1$^+$ unc-36$^+$ let-60$^+$)]* for cells with a Ncl-1 phenotype (see Materials and Methods). We also examined segregants from strain MH888 (*ncl-1 unc-36; him-5; kuEx72*) as controls. Unc segregants from MH888 had wild-type SM positioning (*n*=44), as did three mosaic animals lacking *kuEx72* in the SMs and one mosaic animal lacking *kuEx72* in the SMs and the posterior half of the gonad. (A) Summary of mosaic classes obtained. Circles, mosaic animals with normal SM positioning; squares, mosaic animals in which one or both SMs were abnormally positioned. Symbols are located near the cell in which the *kuEx72* array was initially lost (for early losses), or below the cell type that lacked *kuEx72* (for late losses). Because *kuEx72* is an unstable array, many mosaic animals had experienced two or more independent losses of *kuEx72*; such multiple losses are typical of mosaic analyses using extrachromosomal arrays (i.e., Herman et al., 1995). Secondary losses that occurred in diagrammed parts of the lineage are indicated by the identifying number of the affected individual. Some mosaic animals, including approximately half of those lacking *kuEx72* in the SMs, had additional secondary losses (not shown) in lineages that we have demonstrated are not involved in *let-60 ras* control of SM migration. Most anterior or posterior gonad mosaics lacked *kuEx72* in the relevant distal tip cell as well as the anchor cell; exceptions are mosaic animals #3, 20, 38, 40 and 51, in which the anchor cell was genotypically wild-type. (B) Summary of mosaic data, expressed as in Table 1. +, *kuEx72* present. –, *kuEx72* absent. +/–, *kuEx72* missing from some somatic gonad cells.

[1]In several animals not included in this figure, the AC was mispositioned over P5.p or P7.p and the SMs were mispositioned correspondingly. The AC and SMs were positioned close to P7.p in two non-mosaic animals, one mosaic animal lacking *kuEx72* in all the VPCs (#24) and two mosaic animals lacking *kuEx72* in half of the VPCs. The AC and SMs were positioned close to P5.p in two mosaic animals lacking *kuEx72* in half of the VPCs. In one P1 mosaic animal, not all of the VPCs could be identified but both SMs were positioned very close to the AC. In one M mosaic animal, the AC was positioned near P7.p; one SM was positioned near P7.p and the other SM was positioned near P8.p. In an M.vr mosaic animal, the AC was positioned between P6.p and P7.p; the genotypically wild-type SM was positioned near P7.p while the genotypically mutant SM was positioned near P8.p.

[2]This category includes 200 SMs from non-mosaic animals and 126 SMs from mosaic animals lacking *kuEx72* in tissues other than the SMs and gonad.

[3]This category includes 4 SMs from mosaic animals (#57, #73) lacking *kuEx72* in both distal tip cells and the AC and thus potentially lacking *kuEx72* in the entire somatic gonad.

often migrate dorsally to flank the center of the displaced gonad (Thomas et al., 1990). If the dorsal gonad is ablated prior to the time of SM migration, this dorsal excursion does not occur (Thomas et al., 1990). We found that in *dig-1* animals having dorsal gonads, 40% of the SMs adopted dorsal positions, 25% adopted lateral positions, and 35% remained ventral (*n*=20). In contrast, in *dig-1; ksr-1* animals with dorsal gonads, only 6% of the SMs adopted dorsal postions and 4% adopted lateral positions, while 90% remained ventral (*n*=48). These results suggest that *ksr-1* mutations reduce a gonad-dependent attractive guidance mechanism.

To examine possible effects of the Ras pathway on gonad-dependent repulsion, we constructed double mutants between *ksr-1* and *egl-15* or *egl-17* mutations. In *egl-15* or *egl-17*

Table 2. Double mutant analyses

	Genotype[1]	gonad	AC P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	
A	*egl-15*	+			2%	4%	26%	68%	n = 50
	egl-15	–			4%	44%	48%	4%	n = 25
	ksr-1(ku68); egl-15	+					29%	70%	n = 73
	lin-45(sy96); egl-15	+				7%	30%	63%	n = 30
	let-60(n1046gf); egl-15	+				15%	35%	50%	n = 46
	kuIs12; egl-15	+				17%	57%	25%	n = 40
	kuIs13; egl-15	+			2%	57%	35%	6%	n = 51
	kuIs17; egl-15	+			2%	20%	79%		n = 61
B	*egl-17*	+				3%	32%	65%	n = 62
	egl-17	–				20%	46%	35%	n = 46
	egl-17 ksr-1(ku68)	+					34%	66%	n = 32
	lin-45(sy96); egl-17	+				6%	65%	30%	n = 54
	let-60(n1046gf); egl-17	+				53%	25%	22%	n = 40
	kuIs12; egl-17	+			3%	31%	50%	17%	n = 36
	kuIs13; egl-17	+				56%	32%	12%	n = 59
	kuIs17; egl-17	+				2%	42%	56%	n = 48
C	*sem-5*	+			3%	64%	27%	6%	n = 33
	let-60(n1046gf); sem-5	+				54%	28%	18%	n = 82
	kuIs12; sem-5	+				97%	3%		n = 36
	kuIs13; sem-5	+			3%	81%	14%	3%	n = 73
	kuIs17; sem-5	+				50%	34%	16%	n = 32

See explanation in Table 1 legend. (A) *egl-15* data are for the viable partial loss-of-function allele *egl-15(n484)* (Stern and Horvitz, 1991). *egl-15* null alleles are lethal (DeVore et al., 1995). (B) *egl-17* data are for the molecular null allele *egl-17(n1377)* (M. Stern, personal communication). (C) *sem-5* data are for the viable partial loss-of-function allele *sem-5(n1779)* (Clark et al., 1992a). In several mutants, a small percentage of SMs were observed in abnormal dorsal or lateral positions: 2/30 in *lin-45; egl-15*, 3/46 in *let-60(n1046gf); egl-15*, 2/40 in *kuIs12; egl-15*, 1/51 in *kuIs13; egl-15*, 8/48 in *let-60(n1046gf); egl-17*, 3/82 in *let-60(n1046gf); sem-5*.

[1]All *egl-15* and *sem-5* chromosomes are marked with *lon-2*. The *lin-45(sy96)* single mutant strain is *him-5*. All *kuIs* transgenic strains contain *dpy-20* and *him-5* mutations. The Dpy-20 phenotype is rescued by the *kuIs* array (Materials and Methods). *kuIs17* is a non-*let-60*-bearing control transgene (Materials and Methods).

mutants, the SMs adopt abnormal posterior positions and appear to be repulsed by the gonad; gonad ablation allows the SMs to migrate more anteriorly (Stern and Horvitz, 1991; Table 2A,B). A *ksr-1* mutation did not significantly affect SM positions in *egl-15* or *egl-17* mutant backgounds (Table 2A,B), indicating that it did not perturb the ability of SMs to respond to repulsive gonadal cues. *lin-45(sy96)* also had little effect on *egl-15*, although it did partially suppress *egl-17* (Table 2A,B).

In gonad-ablated *ksr-1* mutant animals, the SMs adopted a range of anteroposterior postions that resembled those in gonad-ablated wild-type animals (Table 1A,C), suggesting that *ksr-1* mutations do not significantly affect gonad-independent guidance mechanisms. However, whereas SMs were never observed to adopt dorsal positions in gonad-ablated wild-type animals, approximately 5% of the SMs do adopt dorsal positions in *ksr-1* mutants (Fig. 2E,F and Table 1 legend). The dorsal displacement phenotype seen in *ksr-1* and other Ras pathway mutants might reflect a defect in a gonad-independent dorsoventral positioning mechanism.

let-60 ras functions within the SMs to control their proper positioning

In order to determine which cells require *let-60 ras* activity for proper SM positioning, we performed a mosaic analysis using the strongest existing *let-60 ras* allele, *sy101 sy127* (Han et al., 1990, 1991). Homozygotes for this allele arrest as L1 larvae, prior to the time that SM migration occurs. Mosaic animals were identified as viable segregants from strain MH809 *[ncl-1 unc-36; let-60(sy101 sy127); him-5; kuEx72(ncl-1$^+$ unc-36$^+$ let-60$^+$)]* in which spontaneous somatic loss of the *kuEx72* extrachromosomal array had occurred (see Materials and Methods). Mosaic animals retaining *kuEx72* in the SMs but lacking *kuEx72* in the anterior or posterior halves of the somatic gonad, in the vulval precursor cells or in other parts of the lineage all had wild-type SM positioning (Fig. 3). In contrast, 27 out of 32 mosaic animals lacking *kuEx72* in both SMs had defects in SM positioning (Figs 2G,H, 3). An additional mosaic animal lacked *kuEx72* in only one of the two SMs; the genotypically mutant SM in this animal was displaced whereas the genotypically wild-type SM was positioned normally. We therefore conclude that *let-60 ras* is required cell autonomously within the SMs to control proper anteroposterior positioning.

The absolute range of final SM positions in *let-60(sy101 sy127)* mosaic animals is similar to that observed in viable *let-60 ras* mutants or gonad-ablated wild-type animals in that very few SMs are displaced by greater than one compartment distance from the normal position. However, the percentage of

Table 3. Constitutive *let-60 ras* activity renders SM positioning gonad-independent

	Genotype[1]	gonad	vulva	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	
				AC						
A	+	+	WT				100%			n = many
	kuIs11[let-60(+)]	+	Muv				100%			n = 29
	kuIs12[let-60(+)]	+	Muv				100%			n = 40
	kuIs13[let-60(G13E)]	+	Muv				100%			n = 46
	kuIs14[let-60(G13E)]	+	Muv				100%			n = 61
	kuIs17[control]	+	WT				100%			n = 34
	lin-15(n765)	+	Muv				88%	2%	10%	n = 51
	lin-1(ar147)	+	Muv				100%			n = 28
B	+	–	Vul			5%	57%	35%	3%	n = 60
	kuIs13[let-60(G13E)]	–	Muv			2%	90%	7%		n = 41
	kuIs14[let-60(G13E)]	–	Muv			1%	90%	9%		n = 79
	kuIs17[control]	–	Vul		2%	6%	59%	33%		n = 54
	lin-15(n765)	–	Muv		3%	10%	45%	31%	10%	n = 29
	lin-1(ar147)	–	Muv		6%	20%	33%	41%		n = 36

See explanation in Table 1 legend. (A) Unoperated animals; (B) animals in which the gonad precursors were ablated (Materials and Methods).
[1]The *kuIs14* gonad ablation experiment was performed in an unmarked strain. All other *kuIs* transgenic strains are *dpy-20; him-5; kuIs.* The Dpy-20 phenotype is rescued by the *kuIs* array (Materials and Methods). *kuIs17* is a non-*let-60*-bearing control transgene (Materials and Methods).

SMs that are posteriorly displaced is higher for the mosaic animals. Additionally, although as mentioned above dorsal diplacement of the SMs was sometimes seen in viable *let-60 ras* mutants and other Ras pathway mutants, we did not observe dorsally positioned SMs in any type of *let-60(sy101 sy127)* mosaic animal.

Our mosaic analysis does not exclude the possibility that *let-60 ras* also functions within the somatic gonad to control SM positioning, since the nature of the lineage precluded us from identifying mosaics that lacked *kuEx72* in the entire somatic gonad but retained *kuEx72* in the SMs. In this regard, it is interesting to note that mosaic animals lacking *kuEx72* in both the gonad and SMs appear to have a somewhat different SM distribution than mosaic animals lacking *kuEx72* in the SMs only (Fig. 3B).

The activated *let-60(n1046gf)* allele causes a weak SM migration defect, but constitutively active *let-60 ras* transgenes do not

The *let-60 ras* gain-of-function (gf) allele *n1046gf* results in a Glycine to Glutamic acid change at codon 13 of the LET-60 RAS protein (Beitel et al., 1990); this change is similar to that seen in some oncogenic forms of N-Ras in mammals (Bos et al., 1985). Previous genetic characterization of *let-60(n1046gf)* in the vulval induction pathway has shown that this allele is constitutively active (Beitel et al., 1990; Han et al., 1990). We and others have observed that SM migration is normal in the majority of *let-60(n1046gf)* animals (DeVore et al., 1995); however, approximately 10% of the SMs do terminate their migration prematurely in the P7.p or even occasionally in the P8.p compartment (Table 1B). *let-60(n1046gf)* also dramatically enhances the SM positioning defects caused by loss-of-function mutations in *ksr-1, lin-45 raf, let-537/mek-2* or *sur-1/mpk-1* (M. S. and M. H., unpublished data).

As an alternative approach to study the effects of elevating *let-60 ras* activity, we constructed transgenic lines bearing integrated arrays of either *let-60(+)* (*kuIs11* or *kuIs12*) or *let-60(G13E)* (*kuIs13* or *kuIs14*) DNA expressed under the control of the *let-60* promoter (Materials and Methods). A high percentage of animals from both types of transgenic lines are Multivulva (Sundaram and Han, 1995), showing that they have constitutive *let-60 ras* activity in the vulva induction pathway. In the case of *kuIs13* and *kuIs14* animals, we have shown that the Multivulva phenotype is more severe and more completely signal-independent than that seen in *let-60(n1046gf)* animals, suggesting that these animals have a higher level of constitutive *let-60 ras* activity (Sundaram and Han, 1995; M. S. and M. H., unpublished data). However, none of the transgenic animals displays any SM migration defect (Table 3A). Furthermore, unlike *let-60(n1046gf), kuIs13* does not enhance the SM positioning defects of *ksr-1* or *lin-45* mutants (data not shown). We therefore conclude that the endogenous *let-60(n1046gf)* allele behaves anomalously and that constitutive *let-60 ras* activity does not pertub SM migration or positioning.

Constitutive *let-60 ras* activity renders SM positioning gonad-independent

Although constitutive *let-60 ras* transgenes have no obvious effect on SM migration in a wild-type background, we found that such trangenes allow fairly precise SM positioning to occur in the absence of the gonad. Whereas gonad ablation in wild-type animals caused 39% of SMs to terminate their migrations outside of the P6.p compartment, the same ablation in *kuIs13* or *kuIs14* animals caused only 10% to do so (and those SMs were only slightly displaced) (Table 3B). To a large degree, therefore, constitutive *let-60 ras* activity is epistatic to gonad ablation; that is, it bypasses the need for the somatic gonad in specifying the endpoint of migration. This result suggests that a major role of gonadal signaling is to stimulate *let-60 ras* activity in the SMs (see Discussion).

To rule out the possibility that the attenuating effects of

kuIs13 or *kuIs14* were due to the ability of these transgenes to promote gonad-independent vulval fates, we showed that in two other Multivulva mutants, *lin-1(ar147)* and *lin-15(n765)*, gonad ablation caused defects in SM positioning that were at least as severe as those seen after gonad ablation in wild type (Table 3B).

Increasing *let-60 ras* activity partially suppresses SM migration defects in *egl-15*, *egl-17* and *sem-5* mutants

egl-15 encodes an FGFR-like protein (DeVore et al., 1995), and in other systems Ras is thought to act downstream of the FGFR (Whitman and Melton, 1992; Wood et al., 1992; Reichman-Fried et al., 1994). If *let-60 ras* acts downstream of the *egl-15* FGFR to regulate SM positioning, then constitutive *let-60 ras* activity might be predicted to suppress the *egl-15* SM migration defect. The endogenous *let-60(n1046gf)* allele has only a slight effect on the *egl-15* mutant phenotype; most SMs in the double mutants are still posteriorly displaced but some now adopt dorsal positions (DeVore et al., 1995; Table 2A and Table 2 legend). Since we had observed that the endogenous *let-60(n1046gf)* allele behaves anomalously with respect to SM migration, we tested the ability of our *let-60 ras* transgenes to suppress *egl-15*. We found that the *kuIs12* or *kuIs13* transgenes did partially suppress *egl-15* in that a greater percentage of SMs migrated to the proper P6.p compartment (Table 2A). A small percentage of SMs adopted dorsal positions (Table 2 legend). Notably, SM positions in *kuIs13; egl-15* double mutants are more posterior than in gonad-ablated *kuIs13* animals (compare Tables 2A and 3B), suggesting that the EGL-15 FGFR does not function solely to transduce gonadal signals to LET-60 RAS (see Discussion).

egl-17 mutations cause SM migration defects similar to those caused by *egl-15* mutations (Stern and Horvitz, 1991), but the molecular identity of *egl-17* has not been reported yet. We found that *let-60(n1046gf)*, *kuIs12* or *kuIs13* partially suppressed the *egl-17* migration defect (Table 2B). Again, some SMs adopted dorsal positions (Table 2 legend), and SM positions in *kuIs13; egl-17* double mutants are more posterior than in *kuIs13* gonad-ablated animals (Tables 2B, 3B).

sem-5 encodes a GRB-2-like adaptor protein that is important for both vulval induction and SM migration (Clark et al., 1992a). *let-60(n1046gf)* can suppress the Vulvaless phenotype of strong *sem-5* mutants, suggesting that *let-60 ras* acts downstream of *sem-5* in the vulval induction pathway (Clark et al., 1992b). However, *let-60(n1046gf)* does not suppress the SM migration defects of *sem-5* mutants (Stern and DeVore, 1995; Table 2C). In contrast, we found that the *kuIs12 let-60(+)*-transgene does suppress the SM migration defects caused by *sem-5(n1779);* interestingly, however, the *kuIs13 let-60(G13E)*-transgene is not able to suppress *sem-5(n1779)* as effectively (Table 2C).

DISCUSSION

Several recent studies have implicated the small GTPase Ras in the control of cell migration. For example, dominant negative Ras protein has been found to interfere with wounding responses in tissue culture cells (Sosnowski et al., 1993). In *Drosophila*, constitutively activated Ras transgenes can partially suppress the tracheal migration defects of *breathless* mutants (Reichman-Fried et al., 1994), and both constitutive and dominant-negative Ras transgenes perturb border cell migration (Lee et al., 1996). However, one weakness of such studies has been the exclusive reliance on Ras proteins with altered rather than reduced function, since such altered proteins could potentially affect processes in which Ras is not normally involved.

Here we have used endogenous loss-of-function mutations as well as constitutively activated Ras transgenes to analyze the role of *let-60 ras* and other Ras pathway genes in regulating SM migration in *C. elegans*. Previous experiments revealed two separable components of SM guidance: a gonad-independent mechanism sufficient for the initial anterior migration to the mid-body region, and a gonad-dependent mechanism required for precise final positioning near the site of the future vulva (Thomas et al., 1990). We find that mutations reducing Ras pathway activity result in errors in final SM positioning similar to those seen after gonad ablation in wild-type animals. Ras pathway activity does not appear to be required for the initial anterior migration of SMs to the mid-body region, but it is both necessary and sufficient for the gonad-dependent regulation of SM positioning. Our results argue that *let-60 ras* plays a permissive role in regulating SM positioning by making SMs competent to perceive or respond to cues that control the precise extent of migration.

Gonad ablation and mutations reducing Ras pathway activity cause similar SM positioning defects

We have shown that loss-of-function mutations in *let-60 ras, ksr-1, lin-45 raf, let-537/mek-2* or *sur-1/mpk-1* cause the SMs to adopt a broadened range of final positions. In all viable mutants we examined, the SMs began migrating normally but then often stopped prematurely or continued migrating past their normal stopping point. Occasionally the SMs adopted abnormal dorsal or lateral positions. A mosaic analysis using a severe loss-of-function allele demonstrated that *let-60 ras* is required cell autonomously within the SMs to control proper anteroposterior positioning, and confirmed that *let-60 ras* activity is not required for other aspects of the SM fate nor for the process of SM migration per se. Rather, *let-60 ras* activity is necessary for SMs to know exactly where to stop migrating.

Since the anteroposterior SM positioning defects associated with loss of Ras pathway activity resemble those caused by gonad-ablation in wild-type animals, the Ras pathway may mediate the gonad-dependent control of SM positioning. *let-60 ras* appears to function on the receiving end of gonad-SM interactions since most mosaic animals lacking *let-60 ras* activity in the SMs had positioning defects while all mosaic animals retaining *let-60 ras* activity in the SMs had wild-type positioning. However, in mosaic animals lacking *let-60 ras* activity in the SMs but retaining it in the gonad, the percentage of SMs that were posteriorly displaced was higher than we had observed for viable *let-60 ras* mutants or gonad-ablated wild-type animals, and no SMs were anteriorly displaced. In contrast, in mosaic animals lacking *let-60 ras* activity in both the gonad and SMs, many SMs adopted correct positions while others were displaced either anteriorly or posteriorly; this pattern more closely resembles what we observe in viable *let-60 ras* mutants or gonad-ablated wild-type animals. These data

suggest that *let-60 ras* could have a second function in the gonad that influences SM positioning. We are currently exploring this possibility.

The dorsoventral SM positioning defects associated with some Ras pathway mutants may indicate an additional role for the pathway in a gonad-independent guidance mechanism. However, our mosaic analysis of *let-60 ras* did not identify any mosaic animals in which the SMs adopted dorsal positions. Dorsal displacement may occur only when *let-60 ras* activity is missing from multiple tissues; alternatively, it is possible that dorsal displacement results only from incomplete loss of *let-60 ras* activity and is not a null phenotype.

let-60 ras plays a permissive role rather than an instructive role in controlling SM positioning

We utilized integrated arrays of *let-60(+)* or *let-60(G13E)* DNA to assay the effects of elevating *let-60 ras* activity. We found that although these transgenes caused vulval phenotypes associated with high levels of constitutive Ras activity, they did not perturb SM migration. This result is consistent with previous findings in *Drosophila* that constitutively active forms of the breathless FGFR or of Ras1 do not perturb tracheal cell migrations (Reichman-Fried et al., 1994) and argues against models in which localized Ras activation directly orients SM migration. Instead, Ras must play a permissive role in allowing SMs to properly perceive or respond to positional cues. For example, *let-60 ras* activation may stimulate the expression or activity of other genes that play more direct, instructive roles in SM positioning.

let-60 ras acts downstream of gonadal signaling to regulate SM positioning

In transgenic animals having constitutive *let-60 ras* activity, gonad ablation had little effect on SM positioning. We draw two conclusions from these ablation experiments. First, *let-60 ras* is likely to be activated in response to gonadal signaling. Second, in wild-type animals, the effects of gonadal ablation on SM positioning may be due primarily to a block in LET-60 RAS activation rather than to the loss of any unique guidance information. Although observations of *dig-1* mutants argue that the gonad does provide instructional guidance cues (Thomas et al., 1990), sufficient non-gonadal cues were present to guide most SMs to their correct final positions when LET-60 RAS was constitutively activated. Instructional cues may therefore be provided redundantly by both gonadal and non-gonadal cells.

Based on our genetic, transgenic and ablation data, we propose a model in which gonadal signals stimulate *let-60 ras* activity in the receiving SM cell, thereby making the SM competent to perceive or respond to certain instructive guidance cues in the mid-body environment. Our model suggests that SM fate determination is a multistep process. A *let-60 ras*-independent process initially specifies the SM fate with respect to cell lineage (i.e. Greenwald et al., 1983). *let-60 ras*-dependent cell signaling events then further refine this fate to allow proper migratory responses.

The EGL-15 FGFR and LET-60 RAS do not act together in a single linear pathway to control SM migration

An important question that we have tried to address is whether the Ras pathway acts downstream of the EGL-15 FGFR to control SM migration. Our genetic analysis suggests that *egl-15* and *let-60 ras* do not act together in a single linear pathway since the loss-of-function phenotypes of these two genes appear to be fundamentally different. In *egl-15* mutants, the SMs seem to be repulsed by the somatic gonad and terminate migration at strikingly abnormal posterior positions (Stern and Horvitz, 1991). Two explanations for this phenotype have been proposed (Stern and Horvitz, 1991; DeVore et al., 1995): *egl-15* mutations may eliminate an attractive guidance system, thereby revealing an underlying repulsive influence of the gonad, or *egl-15* mutations may cause an abnormal response to a normally attractive gonadal cue. In contrast, we have shown that in *let-60 ras* or other Ras pathway mutants, the SMs adopt a more subtly broadened anteroposterior range of final positions similar to what is seen after gonad ablation in wild type; this phenotype is most simply interpreted as a loss of gonad-dependent guidance mechanisms. Additionally, the SMs in some Ras pathway mutants were found in abnormal dorsal or lateral positions, suggeting a role for the Ras pathway in a gonad-independent guidance mechanism.

DeVore et al. (1995) have shown that the activated *let-60(n1046gf)* allele can suppress the larval lethality of strong *egl-15* loss-of-function mutants, suggesting that *let-60 ras* does act downstream of *egl-15* in a pathway required for viability. *let-60 ras* could also act downstream of *egl-15* in a pathway required for SM migration since we found that constitutively active *let-60 ras* transgenes partially suppress the *egl-15* mutant phenotype, allowing SMs to overcome the repulsive influence of the gonad and migrate further anteriorly. However, the fact that *egl-15* mutations have more severe effects than gonad ablation on SM migration in both wild-type and *let-60 ras* transgenic backgrounds argues that the EGL-15 FGFR does not function solely to transduce gonadal signals to LET-60 RAS. Thus, while *egl-15* and *let-60 ras* may act together in one pathway controlling SM migration, one or both probably has independent roles in SM migration as well.

We thank Darrin Green, Yuming Han, Mitsunobu Hara and Derek Sieburth for mutant alleles, David Waring for C33C3, Mike Herman and Bob Herman for R1p16, and the Horvitz, Stern and Wood labs for strains. We especially thank Michael Stern for sharing unpublished data and for critical reading of an earlier version of the manuscript and helpful discussions throughout the course of this work. Some strains were provided by the Caenorhabditis Genetics Center, which is funded by the NIH National Center for Research Resources. M. S. is a Boehringer Mannheim fellow of the Life Sciences Research Foundation. M. H. is a Lucille P. Markey Scholar and a Searle Scholar. This research has been supported by grants from the U. S. Public Health Service (R01 GM47869), the March of Dimes Foundation, the Searle Scholar program and the Lucille P. Markey Charitable Trust.

REFERENCES

Aroian, R. V., Koga, M., Mendel, J. E., Ohshima, Y., and Sternberg, P. W. (1990). The *let-23* gene necessary for *Caenorhabditis elegans* vulval induction encodes a tyrosine kinase of the EGF receptor subfamily. *Nature* **348**, 693-699.

Aroian, R. V. and Sternberg, P. W. (1991). Multiple functions of *let-23*, a *Caenorhabditis elegans* receptor tyrosine kinase gene required for vulval induction. *Genetics* **128**, 251-267.

Avery, L. and Horvitz, H. R. (1987). A cell that dies during wild-type *C.*

elegans development can function as a neuron in a *ced-3* mutant. *Cell* **51**, 1071-1078.

Beitel, G. J., Clark, S. G. and Horvitz, H. R. (1990). *Caenorhabditis elegans ras* gene *let-60* acts as a switch in the pathway of vulval induction. *Nature* **348**, 503-509.

Beitel, G. J., Tuck, S., Greenwald, I. and Horvitz, H. R. (1995). The *C. elegans* gene *lin-1* encodes an ETS-domain protein and defines a branch of the vulval induction pathway. *Genes Dev*. **9**, 3149-3162.

Bos, J. L., Toksoz, D., Marshall, C. J., Verlaan-de Vries, M., Veeneman, G. H., van der Eb, A. J., van Boom, J. H., Janssen, J. W. G. and Steenvoorden, A. C. M. (1985). Amino acid substitutions at codon 13 of the N-ras oncogene in human acute myeloid leukaemia. *Nature* **315**, 726-730.

Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics* **77**, 71-94.

Church, D. L., Guan, K. L. and Lambie, E. J. (1995*). mek-2, mpk-1/sur-1* and *let-60 ras* are required for meiotic cell cycle progression in *C. elegans*. *Development* **121**, 2525-2535.

Clark, D. V., Rogalski, T. M., Donati, L. M. and Baillie, D. L. (1988). The *unc-22(IV)* region of *Caenorhabditis elegans*: genetic analysis of lethal mutations. *Genetics* **119**, 345-353.

Clark, S. G., Lu, X. and Horvitz, H. R. (1994). The *Caenorhabditis elegans* locus *lin-15*, a negative regulator of a tyrosine kinase signaling pathway, encodes two different proteins. *Genetics* **137**, 987-997.

Clark, S. G., Stern, M. J. and Horvitz, H. R. (1992a). *C. elegans* cell-signaling gene *sem-5* encodes a protein with SH2 and SH3 domains. *Nature* **356**, 340-344.

Clark, S. G., Stern, M. J. and Horvitz, H. R. (1992b). Genes involved in two *Caenorhabditis elegans* cell-signaling pathways. *Cold Spring Harbor Symp. Quant. Biol*. **57**, 363-373.

DeVore, D. L., Horvitz, H. R. and Stern, M. J. (1995). An FGF Receptor signaling pathway is required for the normal migrations of the sex myoblasts in *C. elegans* hermaphrodites. *Cell* **83**, 611-620.

Ferguson, E. L. and Horvitz, H. R. (1985). Identification and characterization of 22 genes that affect the vulval cell lineages of the nematode *Caenorhabditis elegans*. *Genetics* **110**, 17-72.

Glazer, L. and Shilo, B. (1991). The *Drosophila* FGF-R homolog is expressed in the embryonic tracheal system and appears to be required for directed tracheal cell extension. *Genes Dev*. **5**, 697-705.

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

Han, M. and Sternberg, P. W. (1990). *let-60*, a gene that specifies cell fates during *C. elegans* vulval induction, encodes a Ras protein. *Cell* **63**, 921-931.

Han, M. and Sternberg, P. W. (1991). Analysis of dominant-negative mutations of the *Caenorhabditis elegans let-60 ras* gene. *Genes Dev.* **5**, 2188-2198.

Han, M., Aroian, R. V. and Sternberg, P. W. (1990). The *let-60* locus controls the switch between vulval and nonvulval cell fates in *Caenorhabditis elegans*. *Genetics* **126**, 899-913.

Han, M., Golden, A., Han, Y. and Sternberg, P. W. (1993). *C. elegans lin-45 raf* gene participates in *let-60 ras*-stimulated vulval differentiation. *Nature* **363**, 133-140.

Hedgecock, E. M. and Herman, R. K. (1995). The *ncl-1* gene and genetic mosaics of *Caenorhabditis elegans*. *Genetics* **141**, 989-1006.

Herman, M. A., Vassilieva, L. L., Horvitz, H. R., Shaw, J. E. and Herman, R. K. (1995). The *C. elegans* gene *lin-44*, which controls the polarity of certain asymmetric cell divisions, encodes a Wnt protein and acts cell nonautonomously. *Cell* **83**, 101-110.

Hill, R. J. and Sternberg, P. W. (1992). The gene *lin-3* encodes an inductive signal for vulval development in *C. elegans*. *Nature* **358**, 470-476.

Hodgkin, J., Horvitz, H. R. and Brenner, S. (1979). Nondisjunction mutants of the nematode *Caenorhabditis elegans*. *Genetics* **91**, 67-94.

Hosono, R., Hirahara, K., Kuno, S. and Kurihara, T. (1982). Mutants of *Caenorhabditis elegans* with dumpy and rounded head phenotype. *J. Exp. Zool*. 224, 135-144.

Huang, L., Tzou, P. and Sternberg, P. W. (1994). The *lin-15* locus encodes two negative regulators of *Caenorhabditis elegans* vulval development. *Mol. Biol. Cell* **5**, 395-411.

Kayne, P. S. and Sternberg, P. W. (1995). Ras pathways in *Caenorhabditis elegans*. *Curr. Opin. Gen. Dev*. **5**(1), 38-43.

Klambt, C., Glazer, L. and Shilo, B. (1992). breathless, a Drosophila FGF Receptor homolog, is essential for migration of tracheal and specific midline glial cells. *Genes Dev*. **6,** 1668-1678.

Kornfeld, K., Guan, K. L. and Horvitz, H. R. (1995a). The *Caenorhabditis elegans* gene *mek-2* is required for vulval induction and encodes a protein similar to the protein kinase MEK. *Genes Dev*. **9**, 756-768.

Kornfeld, K., Hom, D. B. and Horvitz, H. R. (1995b). The *ksr-1* gene encodes a novel protein kinase involved in Ras-mediated signaling in *C. elegans*. *Cell* **83**, 903-913.

Lackner, M., Kornfeld, K., Miller. L., Horvitz, H. R. and Kim, S. K. (1994). A MAP kinase homolog, *mpk-1*, is involved in ras-mediated induction of vulval cell fates in *Caenorhabditis elegans*. *Genes Dev*. **8**, 160-173.

Lee, T., Feig, L. and Montell, D. (1996). Two distinct roles for Ras in a developmentally regulated cell migration. *Development* **122**, 409-418.

MacNicol, A. M., Muslin, A. J. and Williams, L. T. (1993). Raf-1 kinase is essential for early *Xenopus* development and mediates the induction of mesoderm by FGF. *Cell* **73**, 571-583.

McKim, K. S. (1990). Analysis of chromosome rearrangements in *Caenorhabditis elegans*. Ph.D. thesis, The University of British Columbia, Vancouver.

Mello, C. C., Kramer, J. M., Stinchcomb, D. and Ambros, V. 1991. Efficient gene transfer in *C. elegans* after microinjection of DNA into germline cytoplasm: Extrachromosomal maintenance and integration of transforming sequences. *EMBO J*. **10**, 3959-3970.

Miller, L. M., Gallegos, M. E., Morisseau, B. A. and Kim, S. K. (1993). *lin-31*, a *Caenorhabditis elegans* HNF-3/forkhead transcription factor homolog, specifies three alternative cell fates in vulval development. *Genes Dev*. **7**, 933-947.

Moerman, D. and Baillie, D. (1979). Genetic organization in *Caenorhabditis elegans*: fine structure analysis of the *unc-22* gene. *Genetics* **91**, 95-103.

Reichman-Fried, M., Dickson, B. Hafen, E. and Shilo, B. (1994). Elucidation of the role of breathless, a *Drosophila* FGF receptor homolog, in tracheal cell migration. *Genes Dev*. **8**, 428-439.

Sosnowski, R. G., Feldman, S. and Feramisco, J. R. (1993). Interference with enodgenous Ras function inhibits cellular responses to wounding. *J. Cell Biol*. **121**, 113-119.

Stern, M. J. and DeVore, D. L. (1994). Extending and connecting signaling pathways in *C. elegans*. *Dev. Biol*. **166**, 443-459.

Stern, M. J. and Horvitz, H. R. (1991). A normally attractive cell interaction is repulsive in two *C. elegans* mesodermal cell migration mutants. *Development* **113**, 797-803.

Sternberg, P. W. (1993). Intercellular signaling and signal transduction in *C. elegans*. *Annu. Rev. Genet.* **27**, 497-521.

Sulston, J. and Horvitz, H. R. (1977). Postembryonic cell lineages of the nematode *Caenorhabditis elegans*. *Dev. Biol*. **56**, 110-156.

Sundaram, M. and Han, M. (1995). The *C. elegans ksr-1* gene encodes a novel Raf-related kinase involved in ras-mediated signal transduction. *Cell* **83**, 889-901.

Sundaram, M. and Han, M. (1996). Control and integration of cell signaling pathways during *C. elegans* vulval development. *BioEssays* **18**, 473-480.

Thomas, J. H., Stern, M. J. and Horvitz, H. R. (1990). Cell interactions coordinate the development of the *C. elegans* egg-laying system. *Cell* **62**, 1041-1052.

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

Tuck, S. and Greenwald, I. (1995). *lin-25*, a gene required for vulval induction in *Caenorhabditis elegans*. *Genes Dev.* **9**, 341-357.

Umbhauer, M., Marshall, C. J., Mason, C. S., Old, R. W. and Smith, J. C. (1995). Mesoderm induction in *Xenopus* caused by activation of MAP kinase. *Nature* **376**, 58-62.

Whitman, M. and Melton, D. (1992). Involvement of p21 ras in *Xenopus* mesoderm induction. *Nature* **357**, 252-255.

Wood, K. W., Sarnecki, C., Roberts, T. and Blenis, J. (1992). Ras mediates nerve growth factor receptor modulation of three signal transducing proteins: MAP kinase, Raf-1, and RSK. *Cell* **68**, 1041-1050.

Wu, Y. and Han, M. (1994). Suppression of activated Let-60 Ras protein defines a role of *Caenorhabditis elegans* Sur-1/mpk-1 MAP kinase in vulval differentiation. *Genes Dev*. **8**, 147-159.

Wu, Y., Han, M. and Guan, K. L. (1995). MEK-2, a *Caenorhabditis elegans* MAP kinase kinase, functions in Ras-mediated vulval induction and other developmental events. *Genes Dev*. **9**, 742-755.

(Accepted 24 June 1996)