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# *mig-5/Dsh* controls cell fate determination and cell migration in *C. elegans*

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**Abstract**

Cell fate determination and cell migration are two essential events in the development of an organism. We identify *mig-5*, a Dishevelled family member, as a gene that regulates several cell fate decisions and cell migrations that are important during *C. elegans* embryonic and larval development. In offspring from *mig-5* mutants, cell migrations are defective during hypodermal morphogenesis, QL neuroblast migration, and the gonad arm migration led by the distal tip cells (DTCs). In addition to abnormal migration, DTC fate is affected, resulting in either an absent or an extra DTC. The cell fates of the anchor cell in hermaphrodites and the linker cells in the male gonad are also defective, often resulting in the cells adopting the fates of their sister lineage. Moreover, 2° vulval precursor cells occasionally adopt the 3° vulval cell fate, resulting in a deformed vulva, and the P12 hypodermal precursor often differentiates into a second P11 cell. These defects demonstrate that MIG-5 is essential in determining proper cell fate and cell migration throughout *C. elegans* development.


*Keywords:* Wnt signaling; Dishevelleds; *C. elegans*; Distal tip cells; Vulval precursor cells; Hypodermal morphogenesis; P11/P12; Q neuroblast; *mig-5*; Migration

## Introduction

A fundamental question in developmental biology is how a single-celled zygote develops into a multicellular organism composed of a variety of cell types arrayed into complex patterns. Two mechanisms that are employed to accomplish this feat are the determination of specific cell fates and the migration of cells to their final destination within the organism. One way to diversify cell types during development is through asymmetric cell divisions, in which sister cells adopt different fates (reviewed in Betschinger and Knoblich, 2004). This asymmetry can be accomplished intrinsically within the dividing cell through molecular segregation of localized determinants, through asymmetric placement of the mitotic spindle to create daughters of different sizes, or extrinsically via cell–cell signaling. When cell signaling is involved, identical sister cells containing equivalent developmental potentials become differentiated as the consequence of intercellular communication between one or both of the sister cells and their neighboring cells. Feedback mechanisms within each cell quickly reinforce the differences between the two cells, resulting in separate cell lineages. After cell fate determination, many cells migrate to distant sites to eventually interact with clonally distinct cells in order to form functional tissues within the developing embryo. Such migrations are also often regulated by cell signaling.

One signaling pathway known to regulate both cell fate decisions and cell migration is Wnt signaling. Multiple Wnt signaling pathways have been shown to direct both cell fate decisions and cell migration in many multicellular organisms. Wnt signaling functions through a Wnt ligand that interacts with a Frizzled (Fz) transmembrane receptor. Fzs transduce the signal to the intracellular protein Dishevelled (Dsh), which can activate at least two Wnt signaling pathways. Wnt/β-catenin signaling regulates the fate of cells, e.g., during the determination of body

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axes (reviewed in Schier and Talbot, 2005; Weaver and Kimelman, 2004; Yamaguchi, 2001) and regulation of myogenesis in flies and frogs (Kazanskaya et al., 2004; Kozopas and Nusse, 2002; Shi et al., 2002). The Wnt/planar cell polarity (PCP) pathway is a regulator of convergent extension cell movements during gastrulation in frogs and fish (reviewed in Keller, 2002), germband extension in flies (Irvine and Wieschaus, 1994), formation of the Organ of Corti in the mammalian ear (Wang et al., 2005) and neural tube closure (reviewed in Copp et al., 2003).

Studies in *C. elegans* have shown that regulation of cell fate decisions and cell migrations by Wnt signaling is pervasive throughout development, including gut induction, blastomere orientation, neuronal migration, and organogenesis (reviewed in Eisenmann, 2005; Herman and Wu, 2004; Korswagen, 2002). The *C. elegans* genome contains three Dsh family members, *dsh-1*, *dsh-2*, and *mig-5*. The Dshs have been shown to act redundantly in the regulation of cell fate and spindle orientation in the early embryo (Walston et al., 2004), and *mig-5(RNAi)* has demonstrated that MIG-5 acts in the Wnt/β-catenin pathway to control the migration of the QL neuroblast during larval development (Korswagen et al., 2002). MIG-5 has also recently been shown to regulate the polarity of the B cell through a PCP-like pathway (Wu and Herman, 2006).

We have conducted a genetic screen for mutations that result in defects in cell migrations during larval development and have identified two alleles of the gene *mig-5*. In this study, we characterize two mutant alleles of *mig-5*. We find that maternal loss of *mig-5* results in cell migration defects in the dorsal and ventral hypodermal cells, Q neuroblasts, and the distal tip cells of the gonad, and also causes cell fate defects in multiple cells, including the distal tip cells, the anchor cell, the linker cell, vulva precursor cells and the P11/P12 hypodermal cells. These studies indicate that MIG-5 functions throughout embryonic and larval development to direct both cell fate and cell migration.

## Materials and methods

### *Strains and alleles*

The Bristol strain N2 was used as wild type. Strains were maintained and cultured as described (Brenner, 1974). Other strains used included NJ301 (*dpy-10(e128), mig-5*(*rh94*), *unc-4(e120))*, NJ532 (*dpy-10(e128), mig-5*(*rh147*), *unc-4(e120))* and KS432 (*him-5(e1490); mnEx11(MIG-5*::*GFP*, *STR-1*::*GFP)*). The deficiency strains *mnDf84*, *mnDf91*, *mnDf92*, *mnDf93*, *mnDf94*, *mnDf97*, *mnDf98*, *mnDf99*, *mnDf101*, *mnDf103*, *mnDf104*, *mnDf106*, *mnDf108*, and *mnDf109* were obtained from the *Caenorhabditis* Genetics Ceneter. Nomarski imaging was used to examine the phenotypes of *mig-5* mutant and wild-type worms. In some cases, 0.5–1% 1-phenoxy-2-propanol was added to the buffer on the pad as an anesthetic to slow movement.

### *Deficiency testing*

In deficiency complementation mapping, *mnDf* strains, containing a deficiency chromosome marked with *unc-4(e120)*, balanced with the inversion *mnC1*, and marked with *dpy-10(e128)* and *unc-52(e444)*, were crossed with strains containing a *mig-5* allele (either *rh94* or *rh147*) flanked by *dpy-10(e128)* and *unc-4(e120)*. For non-complementation, homozygous *unc-4*, heterozygous *dpy-10* F1s were collected and the progeny were examined for abnormal distal tip cells (DTCs).

### *Cosmid and phage DNA*

Cosmids clones were obtained from the Laboratory of Molecular Biology, Medical Research Council (MRC), England. Cosmids were grown in 2X YT buffer and purified by CsCl ultracentrifugation or using an ion exchange column (Qiagen, Valencia, CA). Phage DNA was prepared using Magic Lambda Preps DNA Purification System (Promega, Madison, WI).

### *Southern blot analysis*

Genomic DNA was obtained from N2, 13 deficiency strains, and *mig-5* mutants (*rh94* and *rh147*). It was digested overnight with *Bam*HI and *Hin*dIII and separated by electrophoresis on a 0.7% agarose gel. The DNA was transferred to a positively-charged nylon membrane and UV-cross-linked. Probes for cosmid or phage DNA were labeled with $^{32}$P or digoxigenin (DIG). Probe labeling and hybridization were performed as previously described (Sambrook et al., 1992 and Genius System User's Guide for Filter Hybridization, Boehringer Mannheim Corp., Indianapolis, IN). Membranes were stripped and reused several times.

### *Cloning and subcloning*

The cosmid T05C12 was digested with the restriction enzyme *Age*I. Fragments were subcloned into the plasmid vector Bluescript II SK or the cosmid vector Lorist 2. The Bluescript vector was digested with *Xma*I to produce an *Age*I compatible overhang and was dephosphorylated using calf intestinal alkaline phosphatase (CIP). Subcloned fragments contained in the Lorist 2 vector were obtained by recircularizing the vector following *Age*I digestion.

### *Transgenic strains*

Cosmid DNA (100 μg/ml) was injected with the dominant marker *rol-6 (su1006)* (200 μg/ml) into the gonads of hermaphrodites to create transgenic strains. F1 progeny displaying the rolling phenotype of mutant *rol-6* were isolated and F2 progeny were examined for the rolling phenotype to demonstrate the presence of a transgenic line. The migration of the descendants of the QL neuroblast was examined to identify rescue of the *mig-5* phenotype.

## Results

### *Mapping and cloning of the mig-5 gene*

In a screen for cell migration defects, two alleles of *mig-5*, *rh94* and *rh147,* were isolated and are allelic, based on complementation testing. Two-factor and three-factor crosses localized the *mig-5* alleles to chromosome II between *dpy-10* and *unc-4*. Deficiency complementation testing demonstrated that *mig-5* is bounded by *fer-15* and *emb-27* on the left and *zyg-9* and *let-252* on the right (Fig. 1A). Southern blot analysis, using cosmids as probes, identified cosmid F14F5 as containing at least part of the gene encoded by *mig-5*. Rescue of the migration defects by a pool of nearby cosmids and F14F5 alone demonstrated that F14F5 contains the entire *mig-5* gene. Thus, the location of *mig-5* within F14F5 is shown by physical mapping and by functional rescue of the *mig-5* mutant phenotype.

Further cosmid rescue experiments demonstrate that *mig-5* lies between F10B5 and T07C3, and is completely contained within T05C12 (Fig. 1B). In order to further narrow the location of *mig-5*, T05C12 was digested with *Age*I to create two fragments (12.2 kb and 17.2 kb) and the remaining

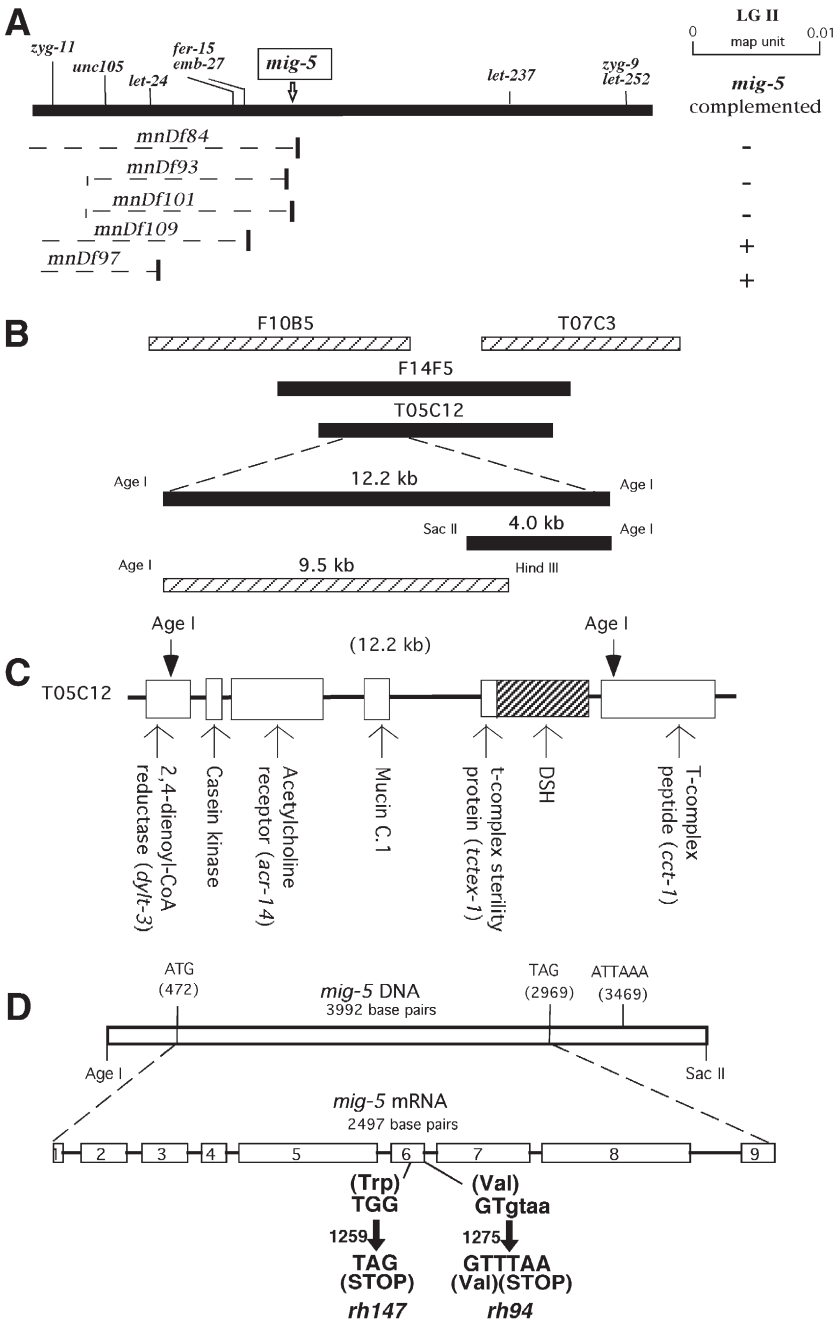


Fig. 1. Identification and cloning of *mig-5* alleles. (A) In chromosomal deficiency tests, *mig-5* failed to complement *mnDf84, mnDf93,* and *mnDf101*. The top thick line represents the genetic map of LG II and the location of *mig-5* based on deficiency mapping. The dashed lines mark the region of LG II missing in each chromosomal deficiency. (B) Rescue with cosmids and cosmid subclones indicates that *mig-5* is completely contained within a 4.0 kb region of the cosmid T05C12. The solid bars indicate rescue of the QL migration defect and the patterned bars indicate a failure to rescue the QL migration defects. T05C12 was digested with *Age*I to create a 12.2 kb rescuing fragment that was further digested with *Sac*II to create a 4.0 kb rescuing fragment. A *Hin*dIII digestion of the 12.2 kb *Age*I fragment failed to rescue the QL migration defects. (C) A gene map of the 12.2 kb *Age*I fragment of T05C12 shows that a Dsh homolog is wholly contained within the 4.0 kb *Hin*dIII–*Age*I rescuing fragment. (D) The lesions in *mig-5(rh147)* and *mig-5(rh94)* were identified by sequencing. *mig-5(rh147)* is the product of a G to A point mutation at base 1259, resulting in a Trp codon becoming a stop codon. *mig-5(rh94)* is the product of a G to a T point mutation at base 1275 that abrogates a splice site, resulting in read-through into intron 6 where the first codon is a stop codon.

religated cosmid, which was 14.8 kb. The 12.2 kb fragment and the religated 14.8 kb cosmid were injected into *mig-5* mutant worms and scored for rescue. Only the 12.2 kb fragment rescued. Therefore, *mig-5* is within the 12.2 kb *Age*I fragment, which contains only five genes, a Dsh protein, *tctex-1* (a t-complex sterility protein), a mucin C.1 homolog, *acr-14* (an acetylcholine receptor), and a casein kinase homolog (Fig. 1C). In order to determine which gene is *mig*-5, the 12.2 kb *Age*I-digested fragment was further digested with *Sac*II to create a 4.0 kb clone that contained all of the Dsh gene. This smaller 4.0 kb fragment rescued the migration defects. Deletion of 2.7 kb of the fragment

abrogates rescue, suggesting that *mig-5* is T05C12.6, a Dishevelled homolog.

Sequencing of T05C12.6 showed that the *mig-5(rh147)* allele is the product of a nonsense mutation, changing a G to an A at base 1259 of the gene (Fig. 1D). The *mig-5(rh94)* allele contains a point mutation, changing a G to a T at base 1275, resulting in a loss of the 5′ splice site, i.e., the splice donor site, of intron 6. The net effect is a premature stop at the following codon from read-through into the intron. Therefore, the mutant lesions of both alleles are located within T05C12.6/*mig-5*.

### *MIG-5 is a C. elegans dishevelled family member*

MIG-5 is predicted to be a protein of 666 amino acids. It contains homology to the Dsh family of proteins. Besides MIG-5, *C. elegans* has two other Dsh family members, DSH-1 and DSH-2. All three *C. elegans* Dshs contain the three major domains found in all Dshs, DIX, PDZ, and DEP. Alignment of the overall protein sequences shows that MIG-5 is the most divergent of the three *C. elegans* Dshs and that DSH-1 and DSH-2 are more similar to each other than to MIG-5 (Fig. 2A). The similarity of the *C. elegans* Dshs to human and fly Dshs is generally much higher within the three canonical domains. DSH-1 and DSH-2 show a high degree of identity with one another within the DIX domain; however, all three *C. elegans* Dshs show comparable identity with Dshs from other species (Fig. 2A). Interestingly, MIG-5 shows low levels of identity within the PDZ domain compared to the other Dshs, including DSH-1, which is highly similar to the fly and human Dshs (Fig. 2B). However, DSH-1 shows less conservation in the C-terminal DEP domain, while DSH-2 and MIG-5 display as much identity to the human Dshs as the fly Dsh does (Fig. 2B). Although DSH-1 is more divergent within the DEP domain when compared to the human Dshs, all three *C. elegans* Dshs are equally similar with each other within the DEP. In summary, the presence of the three main conserved domains found in Dsh family members suggests that MIG-5 is also a Dsh.

**A**

Amino acid identity among Dishevelleds - Full length and DIX domain

| | *D.m.* DSH | Human DVL1 | Human DVL2 | Human DVL3 | *C.e.* DSH-1 | *C.e.* DSH-2 | *C.e.* MIG-5 |
|---|---|---|---|---|---|---|---|
| *Drosophila* DSH | | 67.1 | 69.4 | 67.1 | 43.5 | 41.2 | 40.0 |
| Human DVL1 | 44.3 | | 74.4 | 81.4 | 38.4 | 38.4 | 31.4 |
| Human DVL2 | 44.9 | 63.1 | | 77.4 | 42.9 | 42.9 | 38.1 |
| Human DVL3 | 46.4 | 67.3 | 69.6 | | 37.3 | 41.0 | 32.5 |
| *C. elegans* DSH-1 | 28.3 | 26.6 | 24.6 | 24.9 | | 71.8 | 40.0 |
| *C. elegans* DSH-2 | 28.3 | 26.7 | 26.6 | 27.4 | 47.8 | | 35.3 |
| *C. elegans* MIG-5 | 24.1 | 22.7 | 22.1 | 22.9 | 23.9 | 20.4 | |

Percent identity among DIX domains

Percent identity of full length proteins

**B**

Amino acid identity among Dishevelleds - PDZ domains and DEP domains

| | *D.m.* DSH | Human DVL1 | Human DVL2 | Human DVL3 | *C.e.* DSH-1 | *C.e.* DSH-2 | *C.e.* MIG-5 |
|---|---|---|---|---|---|---|---|
| *Drosophila* DSH | | 52.1 | 52.1 | 56.2 | 28.1 | 34.4 | 37.5 |
| Human DVL1 | 61.1 | | 75.8 | 75.8 | 37.4 | 50.5 | 53.8 |
| Human DVL2 | 64.1 | 73.5 | | 85.7 | 37.4 | 50.5 | 53.8 |
| Human DVL3 | 64.1 | 78.0 | 85.4 | | 34.1 | 44.0 | 51.6 |
| *C. elegans* DSH-1 | 52.0 | 51.0 | 47.2 | 50.0 | | 53.2 | 53.2 |
| *C. elegans* DSH-2 | 36.9 | 38.5 | 38.2 | 43.0 | 43.7 | | 51.6 |
| *C. elegans* MIG-5 | 25.8 | 25.5 | 24.1 | 23.5 | 27.9 | 22.2 | |

Percent identity among DEP domains

Percent identity among PDZ domains

Fig. 2. MIG-5 is a Dishevelled family member. Matrices comparing the identity between MIG-5 and other known Dsh family members, including *Drosophila* DSH, the three human Dvls and the other two *C. elegans* Dshs. Alignment percentages were obtained using ClustalW alignment of the relevant regions of the proteins. (A) Bottom left, identity between the full length proteins; top right, identity between the proteins in the DIX domain. (B) Bottom left, identity between the proteins in the PDZ domain; top right, identity between the proteins in the DEP domain.

*mig-5 regulates spindle orientation in the early embryo*

It has been previously shown that a β-catenin-independent Wnt signaling pathway orients the mitotic spindle of two blastomeres in the early embryo, EMS and ABar (Rocheleau et al., 1997; Schlesinger et al., 1999; Thorpe et al., 1997; Walston et al., 2004). In the EMS blastomere of wild-type 4-cell embryos, the centrosomes initially set up in a dorsal-ventral orientation, but then the spindle quickly rotates to an anterior–posterior orientation (Fig. 3A, Hyman and White, 1987). In Wnt signaling mutants, the spindle of EMS fails to rotate into the proper anterior–posterior orientation until anaphase (Fig. 3B). In 8-cell embryos, three of the four granddaughters of AB divide in parallel with each other, but the fourth, ABar, divides perpendicular to its sister cells (Fig. 3C). In Wnt signaling mutants that result in ABar spindle misalignment, the ABar blastomere divides parallel to the other three AB descendants (Fig. 3D). This aberrant spindle alignment results in the posterior daughter of ABar ultimately adopting a position in the embryo more anterior than its normally anterior sister.

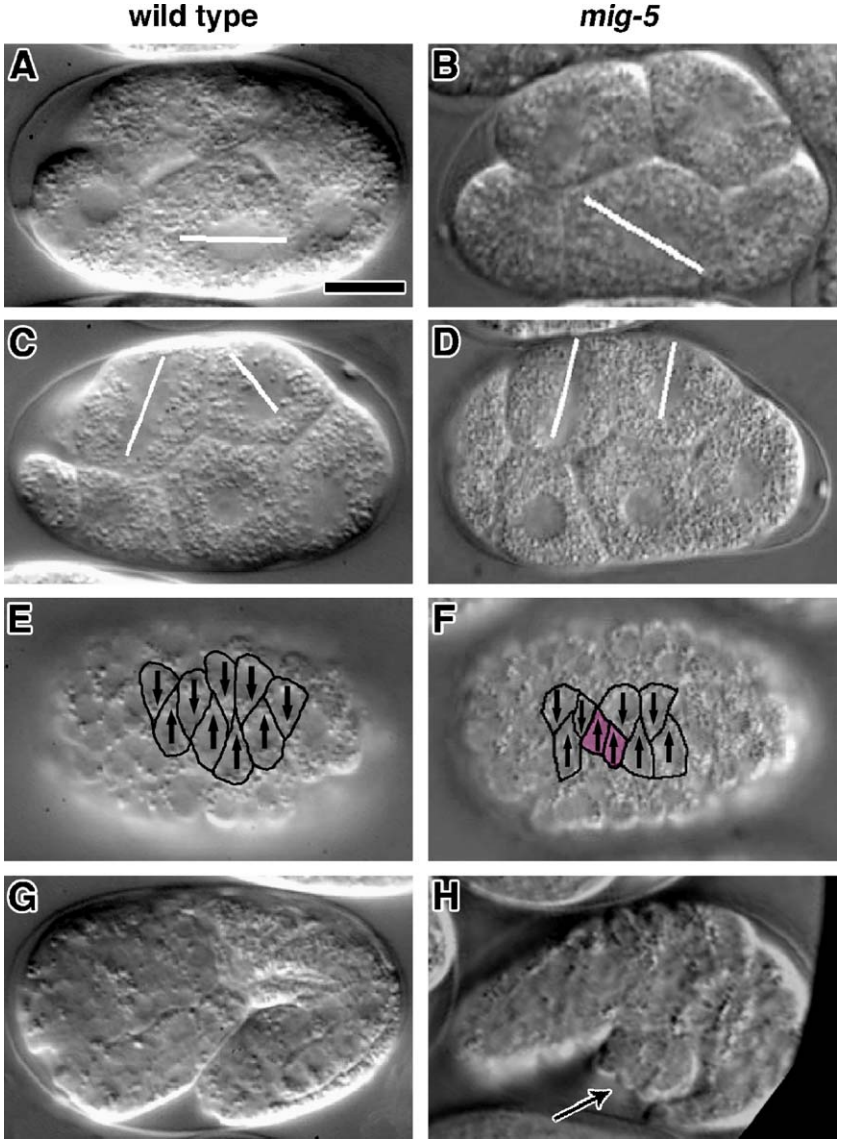


Fig. 3. Embryonic defects resulting from *mig-5* alleles. Wild-type embryos are on the left, *mig-5* mutants are on the right. (A, B) EMS spindle defects. (A) Although it initially sets up in a dorsal–ventral orientation, the mitotic spindle of EMS quickly rotates to an anterior–posterior orientation prior to division of EMS in wild-type embryos. (B) In an embryo from a *mig-5(rh94)* mutant mother, the EMS spindle is skewed from an anterior–posterior orientation during early mitosis, but rotates to the proper orientation prior to the completion of anaphase. (C, D) ABar spindle defects. (C) In wild-type embryos, the mitotic spindle of ABar is oriented perpendicular and transverse to the other AB grand-daughters. (D) In an embryo from a *mig-5(rh147)* mutant mother, the ABar spindle aligns parallel with the mitotic spindles of the other AB granddaughters. (E, F) Dorsal intercalation defects. (E) During dorsal intercalation, two rows of dorsal hypodermal cells alternately intercalate between one other to form a single row of dorsal cells. (F) In an embryo from a *mig-5(rh147)* mutant, dorsal cells are seen migrating as groups in the same direction rather than intercalating between their contralateral partners (pink cells). (G, H) Ventral enclosure defects. (G) Ventral enclosure is driven by the migration of hypodermal cells from the dorsal to the ventral side of the embryo. When the cells meet at the ventral midline, they form junctions or fuse, successfully enclosing the embryo in an epithelial sheet. (H) In an embryo from a *mig-5(rh94)* mutant, the cells at the ventral midline fail to meet, resulting in rupture of internal contents and leaking of cells (arrow) from the embryo. Scale bar = 10 μm.

In both EMS and ABar, we demonstrated that MIG-5 acts redundantly with the other two Dshs, *dsh-1* and *dsh-2,* based on *mig-5(RNAi)* loss of function experiments (Walston et al., 2004). Embryos from *mig-5(rh147)* mutant hermaphrodites show a stronger loss of function regarding spindle orientation of both cells, resulting in 14.3% ($n=7$) spindle misalignment in EMS and 81.8% ($n=11$) misalignment in ABar (Figs. 3B, D, Table 1). Embryos from hermaphrodites homozygous for the weaker *mig-5(rh94)* allele display 23.1% ($n=16$) spindle misalignment in EMS and 56.3% ($n=13$) spindle misalignment in ABar. The mutant alleles of *mig-5* confirm the RNAi loss of function data by demonstrating that Wnt signaling, acting partially through MIG-5, is responsible for orienting the mitotic spindles of some blastomeres in the early embryo.

### *mig-5 controls cell migration during hypodermal morphogenesis*

Morphogenesis of the hypodermis in wild-type *C. elegans* embryos involves two major cell migration events, dorsal intercalation and ventral enclosure. During dorsal intercalation, two rows of ten cells each intercalate between one another to form a single row of twenty cells that contacts the lateral seam cells on both sides (Fig. 3E, Williams-Masson et al., 1998). In embryos from *mig-5(rh147)* and *mig-5(rh94)* mothers, dorsal cells intercalate in a disorganized manner (65.4%, $n=26$ and 55.8%, $n=36$, respectively) (Fig. 3F, Table 1). Failure of dorsal intercalation has previously been shown to result in defects in elongation later in morphogenesis (Heid et al., 2001). Many of the *mig-5* mutant embryos displaying dorsal intercalation defects eventually arrest at the 2-fold stage due to a failure of elongation. During ventral enclosure, cells on the outermost edges of the hypodermal sheet migrate ventrally across underlying neuroblasts towards the ventral midline (Williams-Masson et al., 1997). The cells from each side meet at the ventral midline, resulting in complete enclosure of the embryo in an epithelial monolayer (Fig. 3G). In some embryos from *rh147* and *rh94* mutant worms, ventral enclosure fails, resulting in a rupture from the ventral surface (4.5%, $n=116$ and 6.0%, $n=116$, respectively) (Fig. 3H). The defects in hypodermal morphogenesis (ventral enclosure and elongation failures) in embryos from *mig-5* mutant hermaphrodites result in 9.5% ($n=116$) embryonic lethality in *mig-5(rh147)* and 8.0% ($n=116$) embryonic lethality in *mig-5(rh94)*. Thus, *mig-5* is involved in cell migrations of both the dorsal and ventral hypodermal cells during morphogenesis of *C. elegans* embryos.

Table 1
Frequency of various defects in offspring of *mig-5* mutant hermaphrodites (*n*)

| Phenotype | *mig-5(rh94)* | *mig-5(rh147)* |
|---|---|---|
| *Spindle orientation* | | |
| EMS | 23.1 (16) | 14.3 (7) |
| ABar | 56.3 (13) | 81.8 (11) |
| *Cell migration* | | |
| Dorsal intercalation | 55.8 (36) | 65.4 (26) |
| Ventral enclosure | 6.0 (116) | 4.5 (116) |
| QL neuroblast | 100.0 (50) | 100.0 (50) |
| DTC[a] | 15.0 (119) | 5.0 (250) |
| *Cell fates* | | |
| DTC (hermaphrodites) | | |
| Loss of 1 | 17.6 (119) | 36.6 (101) |
| Loss of both | 0.8 (119) | 5.9 (101) |
| DTC (males)[1] | 87.0 (100) | 63.0 (100) |
| LC/VD (males) | 46.0 (100) | 38.0 (100) |
| 2° VPC (loss of 1) | 6.0 (100) | 39.0 (100) |
| 2° VPC (loss of 2) | 0.0 (100) | 12.0 (100) |
| P11/P12 | 56.0 (100) | 90.0 (100) |

[a] Scored for Glp *(abnormal GermLine Proliferation)* phenotype, denoting loss of both DTCs.

### *mig-5 polarizes the migration of the QL neuroblast*

In wild-type *C. elegans* larvae, the neuroblasts QL and QR are left–right homologs, which undergo identical cell divisions during the first larval stage (Sulston and Horvitz, 1977). Each of the neuroblasts generates three differentiated neurons (AQR/PQR, AVM/PVM and SDQR/SDQL) and two cells that undergo apoptosis. Prior to division, the neuroblasts undergo long-range migrations, with QL migrating to the posterior and QR to the anterior (Fig. 4A). Wnt signaling regulates the migration of QL by activating the expression of the Hox gene *mab-5* within QL (Maloof et al., 1999; Salser and Kenyon, 1992). Loss of function of *mig-5* via *mig-5(RNAi)* has been shown to result in QL migrating anteriorly (Gleason et al., 2002). Likewise, we find that 100% ($n=50$) of QL descendants migrate towards the anterior in larvae from hermaphrodites homozygous for either *mig-5* mutant allele (Fig. 4B, Table 1). The QL migration phenotype can be completely rescued with one copy of either maternal or zygotic wild-type *mig-5* gene product (Figs. 4C, D). This demonstrates that only low levels of *mig-5* expression are required to maintain proper QL polarity and migration.

### *mig-5 controls the fate of the Z1 and Z4 somatic gonad lineages*

In wild-type hermaphrodites, the somatic gonad precursors, Z1 and Z4, have similar early lineages that initially generate 12 cells (Kimble and Hirsh, 1979). An asymmetric division of Z1 and Z4 results in the more distal daughters ultimately forming the distal tip cells (DTCs), while the lineage of one of the more proximal daughters ultimately forms the anchor cell. The DTCs are responsible for leading the elongation and reflection of the anterior and posterior gonad arms and for regulating germ cell proliferation by exerting a mitogenic influence on the germ cells in their immediate proximity.

In some *mig-5* hermaphrodites, one or both DTCs are missing (18.5%, *rh94*, $n=119$; 42.6%, *rh147*, $n=101$) resulting in the loss of the corresponding gonad arm(s) (Figs. 6A, B, Table 1). The loss of anterior or posterior DTCs occurs at similar frequencies, suggesting that MIG-5 is required for formation of both DTCs. A single copy of wild-type *mig-5* can almost completely rescue DTC loss in *mig-5(rh147)* larvae

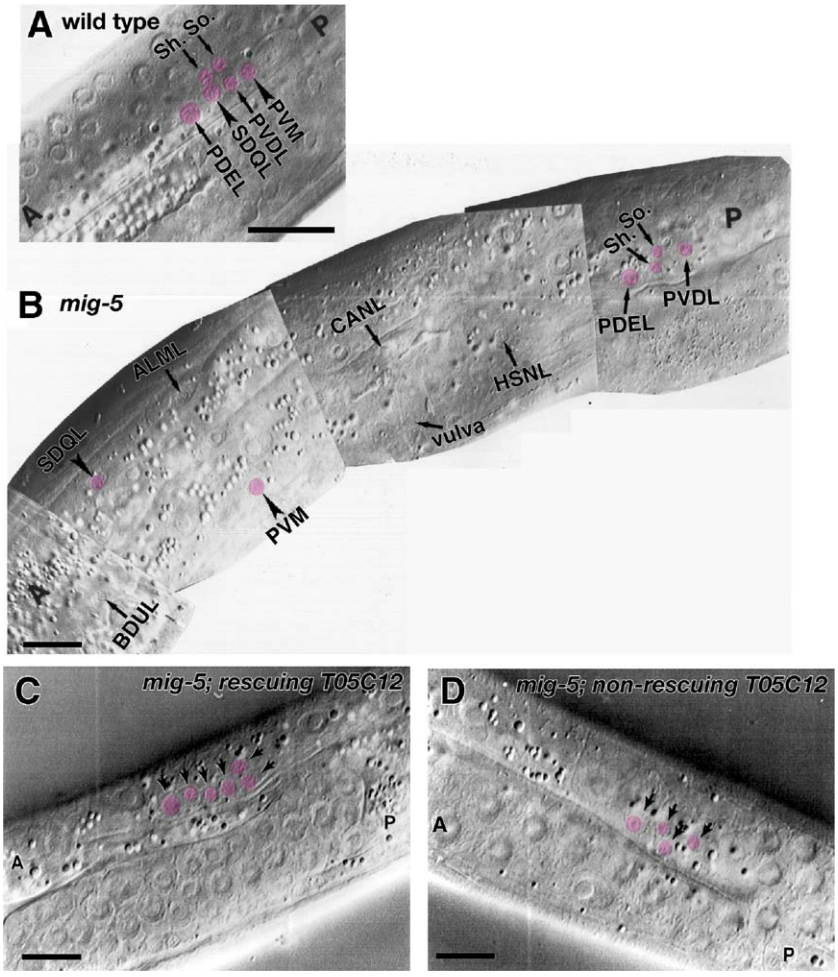


Fig. 4. *mig-5* results in neuroblast migration defects in larvae. (A) On the left side of a wild-type young adult hermaphrodite, SDQL and PVM (arrowheads) are located in the posterior, adjacent to PDEL, PVDL, the sheath (Sh.) and socket (So.) cells. (B) In an offspring from a *mig-5* mutant hermaphrodite, SDQL and PVM are absent from the posterior on the left side, and instead are located at the anterior near BDUL and ALML. (C) The QL migration defect is rescued in the progeny of a *mig-5* mutant that is also expressing a 7.0 kb region of T05C12. On the left side (bottom), SDQL and PVM are located adjacent to PDEL and PVDL and the sheath and socket cells. (D) A 12.0 kb subclone of T05C12, that does not include *mig-5*, does not rescue the QL migration defects in progeny from *mig-5* mutants. On the left side of the worm (bottom), only the PDEL and PVDL neurons are present with the sheath and socket cells. Scale bars = 10 μm.

when contributed either maternally (2.0%, $n=100$) or zygotically (0%, $n=100$). Rarely, three DTCs are found in the gonadal arms in offspring from *mig-5* mutants (1.7%, rh94, $n=119$; 0%, rh147, $n=101$). In these few cases, two DTCs were observed to be associated with the posterior arm of the gonad. A loss of asymmetry in the Z1 and Z4 daughters has been shown to occur in other Wnt signaling mutants, including *lin-17/Wnt, sys-1/β-catenin, wrm-1/β-catenin, lit-1/NLK* and *pop-1/Tcf* (Miskowski et al., 2001; Siegfried and Kimble, 2002; Siegfried et al., 2004; Sternberg and Horvitz, 1988). This suggests that Wnt/β-catenin signaling, possibly acting partially through MIG-5, controls the asymmetric cell divisions that generate the somatic gonad.

Because neither allele showed a fully penetrant loss of DTCs, the alleles were tested against a chromosomal deletion, *mnDf99,* that covers *mig-5*. In homozygous *mig-5* mutant offspring from mothers who are heterozygous for the mutant allele and the deficiency, *mig-5(rh94)* larvae have an increase in loss of DTCs (17.0%, $n=100$), while DTC loss is not increased in *mig-5(rh147)* larvae (23.5%, $n=220$). When both the mothers and the offspring are heterozygous for a mutant *mig-5* allele and the deficiency, loss of DTCs is similar for both *mig-5* alleles (24.0%, *rh94*, $n=100$; 22.1%, *rh147*, $n=275$). These results demonstrate a dose-sensitive response for *mig-5(rh94)*, but not for *mig-5(rh147)*. Therefore, *mig-5(rh94)* and *mig-5 (rh147)* are both recessive loss of function mutations, but *mig-5 (rh147)* is a stronger allele and behaves as a null allele for the DTC loss phenotype when the larva comes from a *mig-5 (rh147)/mnDf99* mother.

In wild-type males, Z1 and Z4 undergo equivalent early divisions to generate a total of 10 cells (Kimble and Hirsh, 1979). Two of these cells, Z1.a and Z1.p, become DTCs. In male offspring from *mig-5* mutant hermaphrodites, germ line proliferation defects due to loss of DTCs are seen for both alleles (87%, *rh94*, $n=100$; 63%, *rh147*, $n=100$), suggesting that DTC fates are affected in males more prominently than hermaphrodites (data not shown, Table 1). The more proximal sisters from the Z1 and Z4 lineages, Z1.paa and Z4.aaa, are

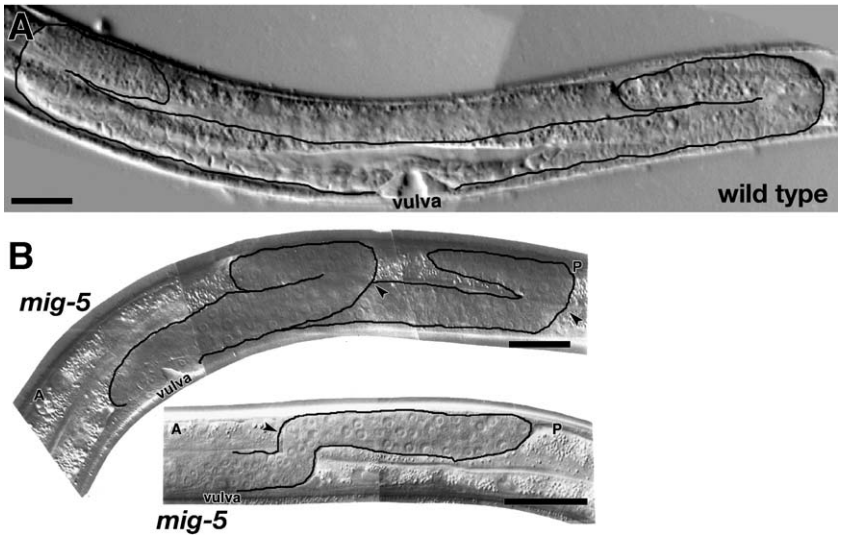


Fig. 5. Distal tip cell migrations are aberrant in *mig-5* mutants. (A–C) Distal tip cell (DTC) migration defects. (A) In a wild-type gonad, the DTCs initially migrate away from each other along the ventral surface of the worm. They then turn dorsally and subsequently reflex back on themselves to migrate towards each other again. Scale bar = 20 μm. (B) In a young adult offspring from a *mig-5* mutant worm, the anterior DTC has led the anterior gonad arm posteriorly, resulting in two gonad arms in the posterior and none in the anterior. (C) In a L3 larva from a *mig-5* mutant worm, the posterior DTC makes a precocious dorsalward turn (arrowhead) at early L2 prior to posterior migration. Scale bars = 20 μm.

equivalent and can adopt the 1° fate of the linker cell (LC), a terminally differentiated cell responsible for leading gonad elongation, or the 2° fate of becoming a vas deferens precursor cell (VD) (Kimble and Hirsh, 1979). Similar to the hermaphrodite AC/VU decision, cell–cell signaling randomly determines the fate of these cells such that only one cell becomes the LC, while the other becomes a VD. In male offspring from *mig-5* mutants, 46% ($n$ = 100) of *mig-5(rh94)* males and 38% ($n$ = 100) of *mig-5(rh147)* males have two LCs leading two gonadal arms, instead of one LC and one gonadal arm as in wild-type larvae (data not shown, Table 1). Therefore, mutations in *mig-5* result in cell fate defects in the lineage of several cells in the developing somatic gonad of *C. elegans*. These fate transformations from 2° to 1° fates could be due to an interruption of the signaling occurring through cell–cell contacts or through intrinsic cell lineage transformations. Additional experiments would be required to distinguish these possibilities.

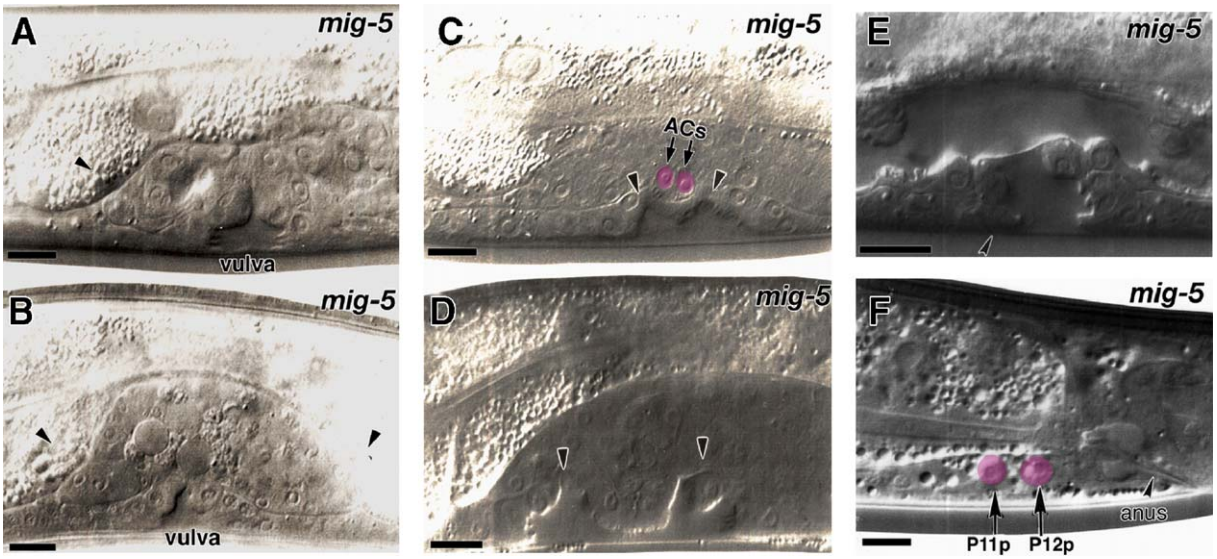


Fig. 6. *mig-5* regulates cell fate during larval development. (A, B) Loss of DTC fates in the offspring from a *mig-5(rh94)* mutant results in the loss of gonad arms. (A) Loss of the anterior DTC and anterior gonad arm. (B) Loss of both DTCs and gonad arms. (C, D) Duplication of the anchor cell in offspring from a *mig-5(rh147)* mutant results in vulval defects from faulty cell signaling. (C) Formation of two anchor cells, resulting in two fused vulvae. (D) Formation of two vulvae due to multiple anchor cells. (E) 2° VPC fate defects. Loss of the anterior 2° VPC results in an asymmetric vulva in a larva from a *mig-5(rh147)* mutant. It is missing the symmetrical anterior structures seen in the wild-type vulva in (A). (F) P11/P12 fate defects. In offspring from *mig-5* mutants, when P12 adopts the fate of the P11 cell, P12p migrates anterior and positions itself next to P11p and then divides with cell fates similar to P11p daughters. Scale bars = 10 μm.

*DTC migration is regulated by mig-5*

In wild-type hermaphrodites, the migrations of the DTCs follow precise pathways with specific timing (Fig. 5A, Kimble and White, 1981). During the second larval stage, the DTCs lead the gonad arms in opposite directions towards the anterior or posterior of the worm. In the third larval stage, the DTCs turn and migrate from their ventral position towards the dorsal body wall. In the fourth larval stage, the DTCs turn and migrate towards one another again. In offspring from *mig-5* mutant hermaphrodites, one of the DTCs sometimes migrates abnormally (15% *rh94*, $n=119$; 5% *rh147*, $n=250$) (Fig. 5B, Table 1). Often in these cases, a defective DTC prematurely migrates dorsally during the second larval stage or both DTCs are observed migrating in the same direction, either anteriorly or posteriorly. Similar frequencies of migrations defects were seen between the anterior and posterior arms of the gonad. Therefore, *mig-5* regulates both the cell fate and migration decisions of the DTCs during gonad development.

*mig-5 affects 2° Vulval Precursor Cell (VPC) fate*

In wild-type hermaphrodites, all six vulval precursor cells (VPCs) have the potential to generate vulval tissue and can adopt 1°, 2°, or 3° VPC fates (Sternberg and Horvitz, 1986). The AC activates Ras signaling in P6.p, such that it adopts the 1° fate (Hill and Sternberg, 1992). Notch signaling from P6.p subsequently induces the 2° fate in neighboring P5.p and P7.p (Chen and Greenwald, 2004). The remaining VPCs, P3.p, P4.p and P8.p, adopt the 3° fate. The progeny of the 1° and 2° cells generate a total of 22 cells that collectively form the vulva, while the 3° cells or their daughters fuse with the hypodermis. Wnt signaling has been shown to be a permissive requirement for cells to adopt the 1° and 2° fates. Mutations in *bar-1/β-catenin, apr-1/APC*, and *pop-1/Tcf* result in all of the VPCs fusing with the hypodermis (i.e., they adopt 3° fates), while a mutation in *pry-1/Axin* results in additional VPCs adopting 1° and 2° fates (Eisenmann et al., 1998; Gleason et al., 2002; Hoier et al., 2000). Larvae from *mig-5* hermaphrodites display loss of one (6%, *rh94*, $n=100$; 39%, *rh147*, $n=100$) or both 2° VPCs (0%, *rh94*, $n=100$; 12%, *rh147*, $n=100$) resulting in an asymmetric vulva or an abnormal mini-vulva (Fig. 6E, Table 1). Interestingly, with the exception of one case, when only one 2° VPC was lost, it is always P5.p that transforms its lineage. (Additionally, among larval offspring of homozygous *mig-5 (rh147)* mutants, hermaphrodites rarely display two ACs, resulting in the induction of two vulvas (3% ($n=100$); Figs. 6C, D)). Therefore, *mig-5* probably regulates a Wnt/β-catenin pathway to allow specification of the fate of the VPCs.

*mig-5 establishes the fate of P12*

In wild-type hermaphrodites, a bilaterally symmetric pair of hypodermal cells, P11/12L and P11/12R, migrate to the ventral midline (Sulston and White, 1980). Typically, P11/12L adopts a more anterior position and becomes P11, while P11/12R moves more posteriorly and becomes P12. The resulting lineages of P11 and P12 differ such that the posterior daughter of P11 (P11.p) becomes a hypodermal cell that fuses with the hyp7 syncytium, whereas the posterior daughter of P12 (P12.p)

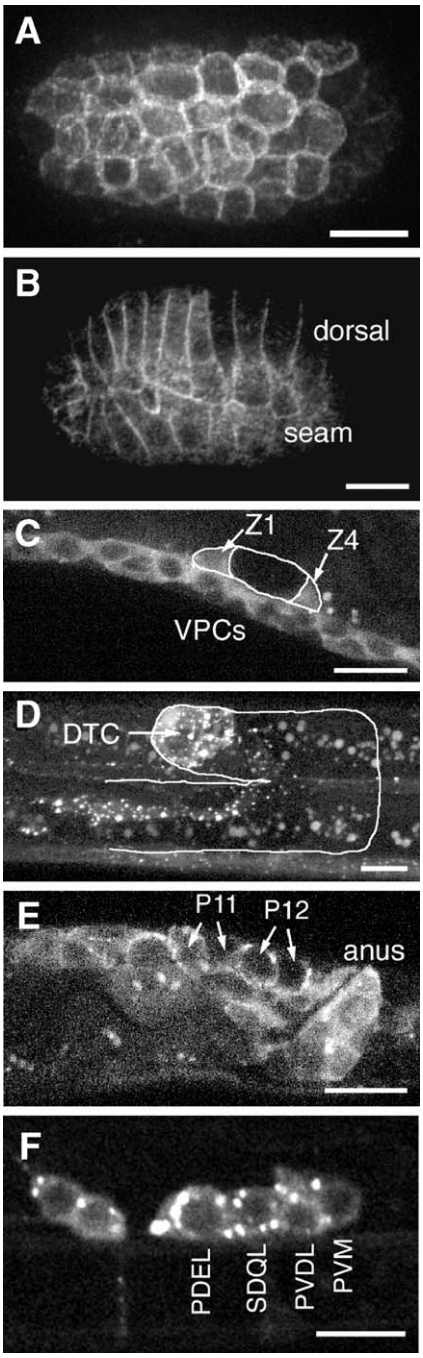


Fig. 7. MIG-5::GFP is expressed throughout development. (A, B) Anti-GFP immunostaining for MIG-5::GFP in embryos. (A) MIG-5::GFP is seen in almost all cells in an approximately 100-cell embryo, and localizes cortically in most cells. (B) During hypodermal morphogenesis, MIG-5::GFP localizes cortically in all hypodermal cells. (C–E) MIG-5::GFP expression in larvae. (C) In an L1 larva, MIG-5::GFP is expressed in Z1 and Z4 of the four-cell gonad. It is also found in the underlying vulval precursor cells (VPCs). (D) In an early L4 larva, MIG-5 is present in the distal tip cell of a migrating gonad. (E) MIG-5 is localized cortically in the daughters of the P11 and P12 cells. (F) In a late L4 larva, MIG-5::GFP is expressed in several neurons, including the QL progeny, SDQL and PVM. Scale bars = 10 μm.

goes through another division. That division results in a hypodermal cell (P12.pa) named hypP12, which is located just anterior to the anus, and a cell that undergoes apoptosis (P12.pp). In larvae from *mig-5* mutants, P12 adopts the P11 fate (56% *rh94*, $n=100$; 90% *rh147*, $n=100$) resulting in two P11.p cells and the loss of P12.pa from its preanal location (Fig. 6F, Table 1). Thus, cell signaling is defective for multiple processes in offspring from *mig-5* mutant hermaphrodites.

### *MIG-5::GFP is expressed throughout embryonic and larval development*

MIG-5::GFP, driven by the endogenous *mig-5* promoter (Wu and Herman, 2006), is expressed throughout embryonic development. It is found in all cells of the early embryo (Fig. 7A). In most cells, the fusion protein is localized to the cell cortex, with only low levels remaining in the cytoplasm. This localization is consistent with *mig-5* mutant defects, which suggest that MIG-5 is acting in many cells throughout early embryogenesis.

During hypodermal morphogenesis, MIG-5::GFP expression is seen in most cells within the embryo. Notably, it is cortically localized in all hypodermal cells (dorsal and lateral seam, Fig. 7B; ventral, data not shown). Interestingly, shortly after dorsal intercalation is completed, MIG-5 leaves the cortex and becomes more cytoplasmic in dorsal cells. This change in subcellular localization suggests that MIG-5 either stops acting or changes its activity, and possibly its function, in the dorsal hypodermis following intercalation.

In L1 larvae, the four-cell gonadal primordium is being established and polarized. MIG-5::GFP is expressed in both Z1 and Z4, which will eventually form the distal tip cells (Fig. 7C). During gonad migration, the DTCs display high levels of MIG-5::GFP (Fig. 7D). MIG-5::GFP is also present in the cells underlying the gonadal primordium, which become the VPCs (Fig. 7C) and later it is present in the fully developed vulva (data not shown). This expression pattern supports roles for *mig-5* in fate decisions in the gonad and vulva, and in migration of the gonad.

MIG-5::GFP is also present and cortically localized in both P11 and P12 and their progeny, P11.a, P11.p, P12.a and P12.p (Fig. 7E), and in SDQL and PVM, two of the neurons derived from the QL neuroblast (Fig. 7F). Again, MIG-5 is expressed in cells that display phenotypic defects when the function of *mig-5* is removed.

## Discussion

Wnt signaling is used at many stages of development to control both cell fates and cell migrations. We demonstrate here that two mutant alleles of *mig-5*, one of three Dishevelleds (Dshs) in *C. elegans,* cause multiple cell fate and cell migration defects during embryonic and larval development. Although Wnt signaling has been shown to regulate neuroblast migration in *C. elegans,* Wnt-dependent regulation of the migration of epithelial cells during hypodermal morphogenesis and the migration of the distal tip cells (DTCs) during gonad development have not been previously described in *C. elegans*.

### *Redundancy among Dshs or other pathways may result in less penetrant defects*

Although we report multiple mutant defects, none of them are fully penetrant. There are several possibilities for why the observed penetrance of the defects was not 100%, even when the putative null allele *mig-5(rh147)* is placed over a chromosomal deficiency that deletes *mig-5*. First, there may be redundancy of the molecules controlling these events. *mig-5* is one of three Dshs in *C. elegans*, and redundancy between the three Dshs may result in incomplete penetrance of mutant defects. We have shown this to be the case for Dsh-dependent regulation of spindle alignment in the early embryo, where both MIG-5 and DSH-2 contribute to controlling the orientation of the spindle in the blastomere ABar (Walston et al., 2004). Indeed, removal of the function of both *mig-5* and *dsh-2* results in embryonic lethality prior to the developmental stages that defects in *mig-5(rh94)* and *mig-5(rh147)* mutants are observed (T.W. and J.H., unpublished data). This early lethality precludes a study of the effects of redundancy between the Dshs during later cell fate and cell migration decisions at the present time. However, removal of the function of all three Dshs is incompletely penetrant for B cell polarity defects (M.W. and M.H., unpublished data), suggesting that other mechanisms, in addition to Wnt signaling, may be involved in polarizing some cells in *C. elegans*.

A separate signaling pathway may be acting in parallel with Wnt signaling to polarize certain cells, determine cell fates, and direct cell migrations. Bei et al. demonstrated that a Src tyrosine kinase pathway acts redundantly with Wnt signaling in the early embryo to specify endoderm and to control spindle alignment of the EMS blastomere (Bei et al., 2002). Src signaling or another functionally redundant signaling pathways could also be cooperating with Wnt signaling throughout later development.

### *Dsh as a regulator of cell migration*

Recent discoveries point to several potential mechanisms by which Wnt signaling may direct cell migrations. In mammals, Wnts and Fzs can act as neuronal guidance cues that direct anterior–posterior migration of growth cones (Liu et al., 2005; Lyuksyutova et al., 2003). In *Drosophila,* commissural axon guidance is regulated, in part, by Derailed/Ryk, an atypical receptor tyrosine kinase, which can act as a receptor for a Wnt5 (Yoshikawa et al., 2003). Additionally, *C. elegans* Wnts, Fzs and LIN-18/Ryk have recently been shown to regulate both neuronal polarity and anterior–posterior growth cone guidance, with EGL-20/Wnt acting specifically as a repulsive guidance cue (Hilliard and Bargmann, 2006; Pan et al., 2006). Mammalian Ryk has the ability to bind to Dsh (Lu et al., 2004), which suggests that MIG-5 could potentially transduce guidance cues downstream of both Fzs and LIN-18/Ryk.

MIG-5 may also be regulating cell migration using more traditional guidance cues. In *Xenopus* embryos, Dsh can

mediate cell repulsion by binding to both ephrins and Eph receptors and activating a planar cell polarity (PCP) pathway acting through *Daam1* (Lee et al., 2006; Tanaka et al., 2003). Migrating cells in *mig-5* mutants may similarly be unable to transduce the guidance cues from Wnts and other guidance molecules, resulting in improper polarization of migrating cells.

### *Dsh signals to multiple pathways during development*

Once migrating cells receive MIG-5-dependent guidance cues, an unanswered question is how MIG-5 transduces such signals. Dsh proteins act at the crossroads of at least two downstream pathways, the Wnt/β-catenin pathway and the PCP pathway. Several defects in *mig-5* mutant animals are similar to mutant phenotypes observed when other genes of the Wnt/β-catenin pathway are removed. For proper migration of the QL neuroblast, Wnt/β-catenin signaling regulates expression of *mab-5/Hox*. Loss of function of *bar-1/β-catenin* or *pop-1/Tcf* results in defective migration of QL and loss of *mab-5* expression (Herman, 2001; Maloof et al., 1999). Presumably upstream of MIG-5, loss of *egl-20/Wnt, lin-17/Fz,* or *mig-1/Fz* results in migratory defects of the QL neuroblast (Harris et al., 1996; Maloof et al., 1999). Additionally, loss of *lin-17/Fz* results in QR descendants migrating too far to the anterior (Whangbo and Kenyon, 1999). However, loss of a different Fz, *cfz-2*, results in wild-type QL migrations and the anterior migration of the QR neuroblast ceases prematurely (Zinovyeva and Forrester, 2005). This suggests that migration of neuroblasts in *C. elegans* is a highly coordinated event involving Wnt/β-catenin signaling acting through multiple Fzs, MIG-5/Dsh, and other downstream components to regulate transcription within the migrating cells.

The proper establishment of the vulva involves parallel signaling from a Wnt/β-catenin pathway and a LET-60/Ras pathway to increase expression of *lin-39/Hox* (Eisenmann et al., 1998). Downstream of MIG-5, components of a classical Wnt/β-catenin destruction complex, PRY-1/Axin and APR-1/APC, have been shown to control the fate of the VPCs (Gleason et al., 2002; Hoier et al., 2000). Additionally, BAR-1/β-catenin and POP-1/Tcf also control VPC fate by activating expression of *lin-39/Hox* (Eisenmann and Kim, 2000; Eisenmann et al., 1998; Gleason et al., 2002). The defects seen in *mig-5* mutants suggest that it is acting within this Wnt/β-catenin pathway to direct the cell fates of the VPCs to form the adult vulva.

Likewise, the fates of Z1 and Z4 are also regulated by components of Wnt/β-catenin signaling. Mutants of *sys-1/β-catenin* and *pop-1/Tcf* display a loss of gonad arms and DTCs, resulting in extra anchor cells (ACs) (Siegfried et al., 2004; Siegfried and Kimble, 2002). Additionally, alleles for one of the other *C. elegans* Dshs, *dsh-2*, display a loss of DTCs and one or both gonad arms and the production of extra ACs in hermaphrodites and extra linker cells (LCs) in males (Chang et al., 2005) The localization of DSH-2 was also observed in the same places that we report for MIG-5 expression, i.e. Z1, Z4, and DTCs during gonad migration. The similarity in phenotypes between the *mig-5* alleles and *dsh-2/Dsh*, *sys-1/β-catenin*, and *pop-1/Tcf* mutants suggests that the cell signaling events that direct the fate of the somatic gonad include a Wnt/β-catenin pathway that includes at least two of the three Dshs in *C. elegans*. However, DTC migration defects have not been reported for *dsh-2* alleles or for downstream Wnt/β-catenin components. This may demonstrate a non-redundant, gene-specific division of labor between the Dsh family members, with MIG-5 potentially regulating a Wnt/β-catenin-independent signaling pathway, such as a PCP-like pathway.

Mammalian tissue culture studies have demonstrated that Dsh regulates neurite outgrowth during the early stages of neuronal differentiation using β-catenin-independent mechanisms. However, multiple potential pathways have been suggested, including the Wnt/$Ca^{2+}$ pathway acting through the DIX domain of Dvl (Fan et al., 2004) and PCP signaling acting either through the PDZ domain to regulate Rac and JNK (Rosso et al., 2005) or through a region between the PDZ and DEP domains to activate Rho (Kishida et al., 2004). The cell migration defects that we describe in the *mig-5* alleles could be the result of removing the function of a PCP or other similar pathway to direct migration and polarity of the cells that contain MIG-5.

Indeed, in *C. elegans*, the lack of reported migration phenotypes in loss-of-function backgrounds for downstream Wnt/β-catenin components suggests that MIG-5 may be directing cell migration through a Wnt/β-catenin-independent pathway. Wu and Herman demonstrated that the PCP components, RHO-1/RhoA and LET-502/ROCK, act through MIG-5 to coordinate spindle orientation and polarity of the B cell and its daughters in *C. elegans* males (Wu and Herman, 2006). Other PCP homologs, including *cdh-3* and *cdh-4* (Ds and Fat), *cdh-6* (Fmi) and *tag-15* (Pk) were also shown to be active in the B cell, but perhaps of less significance. These results suggest that MIG-5 may direct a PCP-like signaling pathway to specify both lineage and polarity.

Another possibility is that MIG-5 may regulate migration by directly binding to actin. A region of the DIX domain in the mammalian Dsh, Dvl2, contains an actin-binding domain (Capelluto et al., 2002). When Dsh is bound by actin, it functions in Wnt/β-catenin- and JNK-independent manners, and instead acts through casein kinase Iε (Capelluto et al., 2002; Torres and Nelson, 2000). When actin-associated Dvl2 function is removed, the actin becomes disorganized and motility of cells decreases (Wechezak and Coan, 2005). This pathway may be similar to the Wnt/spindle orientation pathway in blastomeres of early *C. elegans* embryos. Regulation of migration of the DTCs and neuroblasts by MIG-5 may likewise be acting directly on the cytoskeleton rather than through more elaborate signal transduction pathways.

In summary, we have shown that MIG-5 directs cell polarity, cell fate and cell migration during embryonic and larval development in *C. elegans*. In the future, we anticipate using structure-function analysis of individual Dsh domains to more closely study the role of the Dshs, including *mig-5*, and their potential downstream effectors in these processes.

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