

# Multiple levels of regulation specify the polarity of an asymmetric cell division in *C. elegans*

Jennifer Whangbo\*, Jeanne Harris† and Cynthia Kenyon§

Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, CA 94143-0448, USA

\*Present address: School of Medicine, University of California, Los Angeles, Los Angeles, CA 90095, USA

†Present address: Department of Botany and Agricultural Biochemistry, University of Vermont, Burlington, VT 05405-0086, USA

§Author for correspondence (e-mail: ckenyon@biochem.ucsf.edu)

Accepted 21 August; published on WWW 9 October 2000

## SUMMARY

Wnt signaling systems play important roles in the generation of cell and tissue polarity during development. We describe a Wnt signaling system that acts in a new way to orient the polarity of an epidermal cell division in *C. elegans*. In this system, the EGL-20/Wnt signal acts in a permissive fashion to polarize the asymmetric division of a cell called V5. EGL-20 regulates this polarization by counteracting lateral signals from neighboring cells that would otherwise reverse the polarity of the V5 cell division. Our findings indicate that this lateral signaling pathway

also involves Wnt pathway components. Overexpression of EGL-20 disrupts both the asymmetry and polarity of lateral epidermal cell divisions all along the anteroposterior (A/P) body axis. Together our findings suggest that multiple, inter-related Wnt signaling systems may act together to polarize asymmetric cell divisions in this tissue.

Key words: *egl-20*, Wnt pathway, Asymmetric cell division, Cell polarity, *C. elegans*

## INTRODUCTION

Asymmetric cell divisions play a key role in generating cell and tissue complexity during development. The mechanisms by which a single cell can divide to yield two cells with different developmental potentials have been studied in a wide range of organisms (Horvitz and Herskowitz, 1992; Jan and Jan, 1998; Knoblich, 1997). An important feature of many asymmetric cell divisions is the spatial orientation of the daughter cells relative to each other and to the body axis. Studies in *Caenorhabditis elegans* and *Drosophila* have implicated the Wnt signaling pathway in regulating the polarity of certain asymmetric cell divisions (reviewed in Cadigan and Nusse, 1997; Hawkins and Garriga, 1998).

In the *C. elegans* four-cell embryo, the EMS blastomere receives a polarizing signal from its neighbor, the germline precursor P<sub>2</sub> (Goldstein, 1992). This signal, encoded by the *mom-2/Wnt* gene, acts in a position-dependent manner to induce and orient the asymmetric division of the EMS cell (Goldstein, 1993; Thorpe et al., 1997). As a consequence, the anterior daughter of EMS adopts a mesodermal fate and the posterior daughter adopts an endodermal fate. The MOM-2/Wnt signal polarizes the EMS cell division by regulating known Wnt pathway components including MOM-5 (Frizzled), APR-1 (adenomatous polyposis coli-related) and WRM-1 ( $\beta$ -catenin/Armadillo) (Rocheleau et al., 1997; Thorpe et al., 1997).

During postembryonic development, a Wnt signaling pathway defined by LIN-44/Wnt and its putative receptor LIN-

17/Frizzled (Fz) control asymmetric cell divisions in certain tail blast cells, including B, F, U and T (Chamberlin and Sternberg, 1994; Herman and Horvitz, 1994; Herman et al., 1995; Sawa et al., 1996). This signaling system functions in a manner that is different from the embryonic MOM-2/Wnt signaling pathway. In this case, loss-of-function mutations in the *lin-44/Wnt* gene do not abolish asymmetry, but instead can reverse the polarity of these cell divisions. Loss-of-function mutations in the *lin-17/fz* gene cause these asymmetric cell divisions to become symmetric (Herman et al., 1995; Sternberg and Horvitz, 1988). Thus, LIN-17 acts in a pathway that can generate asymmetry independently of LIN-44/Wnt, and LIN-44/Wnt acts to orient this asymmetric cell division correctly.

In this study, we investigate the role of a different Wnt protein, EGL-20, in the orientation of other asymmetric cell divisions during postembryonic development. Previously, this Wnt protein has been shown to regulate the migrations of the QL and QR neuroblasts along the anteroposterior (A/P) body axis (Harris et al., 1996; Maloof et al., 1999; Whangbo and Kenyon, 1999). QL and QR undergo identical patterns of division, yet their descendants exhibit opposite migratory behaviors: QL and its descendants migrate posteriorly, whereas QR and its descendants migrate anteriorly (Harris et al., 1996; Maloof et al., 1999; Sulston and Horvitz, 1977). EGL-20 acts in a dose-dependent manner to specify these opposite migratory behaviors. High levels of EGL-20 activate a canonical Wnt signal-transduction pathway that leads to expression of the Hox gene *mab-5*, which in turn promotes posterior cell migration. In contrast, low levels of EGL-20

promote anterior migration by activating a separate, undefined pathway. *egl-20* is expressed by a small group of cells in the posterior body region. However, despite this localized expression in the animal, EGL-20 does not act as a position-dependent morphogen to specify these distinct migratory fates. Instead, QL and QR exhibit different responses because they have different response thresholds to EGL-20 signaling (Whangbo and Kenyon, 1999).

Here, we show that, in addition to regulating cell migrations, EGL-20 also influences the polarity of certain asymmetric cell divisions in the lateral epidermis of the animal. The six epidermal V cells (V1-V6), also known as seam cells, divide in a polarized pattern along the A/P axis during the postembryonic larval stages and give rise to cuticular and sensory structures. In wild-type animals, the first division of each V cell is an oriented asymmetric division: the posterior daughters (Vn.p cells) adopt the seam cell fate and continue to divide, whereas the anterior daughters (Vn.a cells) adopt the syncytial fate and fuse with the epidermal syncytium called *hyp7* (Sulston and Horvitz, 1977). We find that loss-of-function mutations in *egl-20* cause reversals in the polarity of the V5 daughter cell fates. In *egl-20* mutants, V5.a often becomes a seam cell while V5.p fuses with *hyp7*. Thus, *egl-20* mutants continue to display asymmetry in the V5 division, but are defective in the relative orientation of these two fates along the A/P axis.

We find that the EGL-20/Wnt polarization system behaves in a novel and unexpected manner. First, the location of the EGL-20/Wnt signal in the animal does not determine the polarity of the V5 cell division. Therefore, a different source of positional information must orient the V5 cell division, and EGL-20 is required in order for this system to function correctly. Moreover, we demonstrate that signals from neighboring cells are responsible for reversing the polarity of the V5 cell division in *egl-20* mutants. In the absence of both EGL-20 and the lateral signals, wild-type polarity is restored to the V5 cell division. This suggests the presence of an underlying system that is capable of polarizing the V5 cell division correctly. We propose that EGL-20 functions to counteract the effect of signals from neighboring cells that would otherwise mis-polarize the V5 cell division. Curiously, components of the Wnt signaling pathway that act with EGL-20 to activate Hox gene expression in the Q neuroblasts also participate in this polarizing system, but their relationship to EGL-20 is very different.

Finally, we find that very high levels of EGL-20 protein have a widespread effect on V cell polarity, interfering with both the orientation of asymmetric cell divisions, as well as the generation of asymmetry itself. This suggests the model that the underlying polarity system that orients V cell divisions may be a Wnt-regulated system that uses a receptor with low affinity for EGL-20. We propose that this underlying system may be a global regulator of asymmetric events along the A/P body axis.

## MATERIALS AND METHODS

### General procedures, nomenclature and strains

Methods for routine culturing and genetic analysis are described by Brenner (1974) and Sulston and Hodgkin (1988). All experiments were performed at 25°C, unless otherwise stated. The wild-type strain N2 is the parent of all strains used with the exception of *egl-*

*20(n1437)*, which was isolated in an MT2878 background. The following strains were used: N2 wild-type var. Bristol, MT1215: *egl-20(n585)IV*, MT3973: *egl-20(n1437)IV*, CF142: *egl-20(mu25)IV*, CF263: *egl-20(mu39)IV*, CF367: *mig-14(mu71)II*, NF19: *mig-14(k124)II*, CB3303: *mig-1(e1787)I*, NT3362: *mig-1(n687)I*, MT1306: *lin-17(n671)I*, MT1463: *lin-17(n677)I*, CF491: *pry-1(mu38)I*; *him-5(e1490)V*, CF731: *bar-1(ga80)X*, MT4051: *lin-44(n1792)I*; *him-5(e1490)V*, CB620: *lin-18(e620)X*, CF1135: *egl-20(n585)IV*; *muEx68* [*myo-2-egl-20::gfp+pPD10.46(unc-22 antisense)*], CF1048: *mulS53* [*hs-egl-20+pPD10.46*]; *egl-20(n585)IV*, CF1059: *mulS53*, CF192: *mig-1(e1787)I*; *egl-20(n585)IV*, CF546: *mig-1(n687)I*; *egl-20(n585)IV*, CF587: *mig-1(mu72) JeIn1 I*; *egl-20(n585)IV*, CF298: *lin-17(n671)I*; *egl-20(n585)IV*, CF540: *lin-17(n677)I*; *egl-20(n585)IV*, CF680: *mig-14(mu71)II*; *egl-20(n585)IV*, CF520: *mig-14(k124)II*; *egl-20(n585)IV*, CF853: *pry-1(mu38)I*; *egl-20(n585)IV*, CF1226: *egl-20(n585)IV*; *bar-1(ga80)X*, CF517: *lin-44(n1792)I*; *egl-20(n585)IV*, CF524: *egl-20(n585)IV*; *lin-18(e620)X*, CF588: *egl-20(n585)IV*; *lin-18(n1051)X*, CF1075: *lin-17(n677)I*; *mulS53*, CF1076: *mulS53*; *bar-1(ga80)X*, *mig-1(mu72) JeIn1* [*pRF4(rol-6)+mec7::lacZ*]*I*, *lin-18(n1051)X*, *mig-14(k124)II*; *mulS53*.

### Determining the fates of V5.a and V5.p

The V5 daughter cell fates were identified directly by Nomarski differential interference contrast (DIC) microscopy. In wild-type animals, V5.a and V5.p adopt syncytial and seam cell fates, respectively. Along with differences in developmental potential, these two cell types differ in the morphology and placement of their nuclei (Sulston and Horvitz, 1977). The nucleus of V5.a is placed slightly ventral to the seam cell nucleus and has a granular nucleoplasm. In addition, V5.a fuses with the *hyp7* syncytium and loses its cellular outline. The nucleus of the seam cell daughter (V5.p) is located laterally, in the focal plane directly below the alae (cuticular ridges). V5.p has a distinctive eye-shaped outline and a smooth nucleoplasm. Occasionally, in animals in which the fates of the V5 daughters were reversed, the seam cell nucleus was located ventrally rather than laterally.

### Cell ablations

Larvae were staged by collecting newly hatched animals at 30-minute intervals. The newly hatched animals were transferred to a fresh plate and allowed to feed for 30 minutes before mounting for ablation. Thus, the staged animals were 0.5 to 1 hour old at the start of ablations. The animals were ablated over the course of the next 30 minutes, so that all ablations were complete by 1.5 hours after hatching.

Individual cells were killed using a laser microbeam as described in Waring et al. (1992). The animals were immobilized for ablation on agarose pads containing 2-3 mM sodium azide. Operated animals were removed from the pad as soon as possible to minimize effects of the azide on development and viability, and then allowed to develop for 7-12 hours on a plate seeded with bacteria. Subsequently, the animals were remounted to confirm the absence of ablated cells and to determine the pattern of V5 daughter nuclei. The unablated sides of the experimental animals served as internal controls. In general, the ablation procedure produced only small developmental delays. Operated animals whose development appeared significantly retarded were discarded.

For following cell lineages after ablation, operated animals were allowed to recover for 3 hours on a seeded plate. The animals were then remounted and observed continuously until the V5 cell had divided and the fates of the daughters could be determined unambiguously based on nuclear morphology and position.

### Reporter genes and transgenic arrays

The extrachromosomal array *muEx68* [*myo-2-egl-20::gfp*] and integrated array *mulS53* [*hs-egl-20*] were described previously (Whangbo and Kenyon, 1999).

### Heat shock conditions

Staged populations of larvae grown at 20°C were collected at 0 to 30 minutes after hatching and were subjected to heat shock for various lengths of time on an aluminium block that was maintained at 33°C. Immediately after heat shock, the animals were placed at 20°C and allowed to grow until the V cell divisions were complete (end of L1 larval stage).

## RESULTS

### Mutations in *egl-20* reverse the polarity of the asymmetric V5 cell division

In wild-type animals, the first division of the epidermal V cells generates a polarized pattern of seam cells and syncytial nuclei along the A/P axis of the worm. These two cell types can be visualized using Nomarski optics and distinguished by differences in the morphologies of their nuclei and by the distinctive eye-shaped outline of the seam cells (Fig. 1A,B). The seam and syncytial fates also can be distinguished by using the monoclonal antibody MH27, which stains the apical junctions between epithelial cells (Francis and Waterston 1991). Immediately after the V cells divide, MH27 staining reveals the outlines of both daughter cells. However, the outlines of the syncytial daughter cells disappear as they fuse with *hyp7* and lose their cell-cell junctions (Fig. 1D,F).

In approximately 50% of *egl-20(n585)* animals, we observed that this alternating pattern of seam and syncytial fates was often disrupted by reversals in the relative positions of the V5 daughter cells. In these animals, the V5.a cell exhibited the morphology of a seam cell and the V5.p cell exhibited the morphology of a syncytial nucleus (Fig. 1B). Consistent with this observation, the V5.p cell fused with *hyp7* in 27/63 *egl-20(n585)* animals stained with the MH27 antibody (Fig. 1F). The *n585* mutation behaves as a strong reduction-of-function mutation in genetic tests (Harris et al., 1996). Thus, we infer that wild-type *egl-20* activity is required to establish the proper A/P polarity of the asymmetric V5 daughter fates.

The reversals in the relative positions of V5.a and V5.p could arise either because of a polarity reversal within the V5 cell division, or because of migration of the daughters after their birth. To distinguish these, we observed the V5 cell division in 14 *egl-20(n585)* animals until the daughter cell fates could be determined unambiguously. In six of these animals, the fates of V5.a and V5.p were reversed, and in eight animals the fates were in the wild-type orientation. The V5 daughters remained stationary in all cases. This finding indicates that the mispositioning of the V5 daughters in *egl-20* mutants arises from reversals in the polarity of the V5 cell division.

The V cells have a polarized asymmetry that can be observed by MH27 staining long before the cells divide (Austin and Kenyon, 1994). In wild-type animals, the V(2-6) cells each have a ventral projection that extends from the anterior half of the cell to the ventral midline (Fig. 1C). Only the anterior daughters inherit this projection following V cell division. To determine whether mutations in *egl-20* reversed the asymmetry of the V5 cell itself, we examined *egl-20(n585)* animals stained with MH27. We found that all V cell morphologies were indistinguishable from wild type, indicating that the initial asymmetry of V5 itself was normal in *egl-20* mutants (Fig. 1E). Thus mutations in *egl-20* appear to disrupt the polarity of the

**Table 1. The polarity of the V5 cell division is reversed in *egl-20* mutants**

Genotype	% Reversed		
	25°C	20°C	15°C
Wild type	0 (100)	0 (50)	0 (50)
<i>egl-20(n585)*</i>	50 (100)	39 (88)	6 (49)
<i>egl-20(n1437)</i>	49 (101)	4 (51)	10 (51)
<i>egl-20(mu25)</i>	36 (100)	12 (52)	8 (50)
<i>egl-20(mu39)</i>	0 (76)	0 (100)	0 (75)

The number of animals (*n*) examined at each temperature is shown in parentheses.

\*We also examined later divisions in the V5 lineage at 25°C. The seam cell daughter of V5 divided with reversed polarity in 42% of the animals scored (*n*=98). The seam cell daughter in the third division of the V5 lineage divided with reversed polarity in 30% of the animals scored (*n*=104). In addition, we observed polarity reversals in the division of the V6.pa cell (39%) and occasional reversals in the divisions of V3.pa, V4.pa and V4.pp (≤5%) (*n*=104).

V5 cell division rather than that of V5 itself. For simplicity, in this study, we refer to this phenotype as the 'V5 polarity' phenotype.

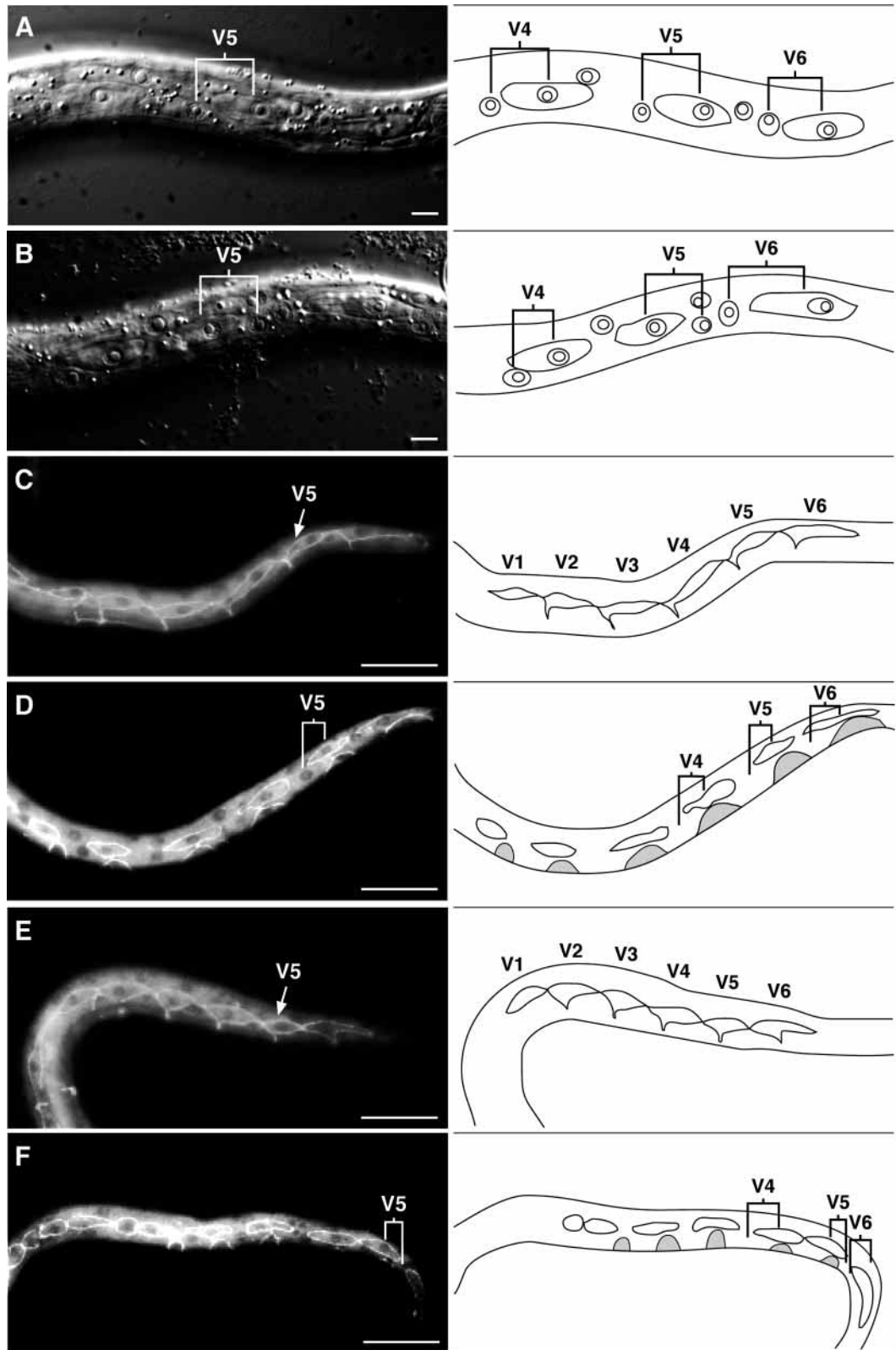
We examined the V5 polarity phenotype in three other *egl-20* mutants. The *egl-20(n1437)* and *egl-20(mu25)* alleles also caused cell fate reversals of the V5 daughters, whereas the weak loss-of-function allele *egl-20(mu39)* allele did not (Table 1). None of these mutations affected the divisions of V1-V4, V6 or T, a seam cell located in the tail. The establishment of V5 polarity in *egl-20* mutants appeared to be a temperature-sensitive process, since all of the *egl-20* alleles that exhibited V5 polarity reversals were temperature sensitive for the phenotype (Table 1). In contrast, these alleles are not temperature sensitive for the cell migration phenotype of *egl-20* mutants (J. Harris, PhD Thesis, University of California, 1995). Therefore it seems likely that an *egl-20*-independent system can polarize the V5 cell division correctly at low temperatures, but not at high temperatures.

*egl-20* activity is also required to specify the correct polarity of later asymmetric divisions in the V5 lineage. We examined the positions and morphologies of the V5 descendants in *egl-20(n585)* animals at the end of the L2 larval stage and observed additional polarity reversals in the second and third divisions of the V5 lineage. In addition, occasional polarity reversals occurred in the third division of the V3, V4 and V6 cell lineages (the second division of these V cells is symmetric and cannot be examined for polarity reversals). Since the frequency of polarity reversals in later V cell divisions is relatively low (see Table 1 footnotes), we have focused our studies on the V5 cell division in *egl-20(n585)* mutants.

### Genes that act with *egl-20* to activate Hox gene expression do not influence the polarity of the V5 cell division in the wild type

EGL-20/Wnt is required to activate expression of the Hox gene *mab-5* in the migratory neuroblast QL and its descendants (Harris et al., 1996; Maloof et al., 1999). EGL-20 appears to act in a canonical Wnt signaling pathway to activate Hox gene expression. Mutations in *lin-17/frizzled* (Sawa et al., 1996), *bar-1/β-catenin/armadillo* (Eisenmann et al., 1998), as well as several genes whose identity is not known, including *mig-1* and *mig-14*, also disrupt *mab-5* expression in QL and its





**Fig. 1.** *egl-20* activity is required to orient the V5 cell division. (A,B) Nomarski photomicrographs of L1 larvae on agarose pads containing 100 mM azide, which enables visualization of the seam cell outlines. (C-F) Fluorescence micrographs of L1 larvae stained with monoclonal antibody MH27 to visualize outlines of the V cells. Immunostaining of MH27 was performed as previously described (Austin and Kenyon, 1994). Tracings of the photographs are shown on the right. The epidermal P cells are shaded in gray. Animals are oriented with anterior to the left. (A) Wild type. The posterior V5 daughter (V5.p) has adopted the seam cell fate and has a visible cell outline. The anterior V5 daughter (V5.a) has adopted the syncytial fate. Its nucleus is slightly ventral to the V5.p nucleus, and its nucleoplasm has a grainy texture. (B) *egl-20(n585)*. The polarity of the V5 cell division is reversed: V5.a has the morphology and position of a seam cell and V5.p has the morphology and position of a syncytial nucleus. (C) Wild-type animal, 2-4 hours after hatching. The V(2-6) cells each have a ventral projection that extends from the anterior half of the cell to the ventral midline of the worm ( $n=20$ ). (D) Wild-type animal, 6-8 hours after hatching. The anterior daughters of the V cells have fused with the hyp7 syncytium and have lost their outlines ( $n=26$ ). (E) *egl-20(n585)* animal, 2-4 hours after hatching. V cell morphologies are normal ( $n=20$ ). (F) *egl-20(n585)* animal, 6-8 hours after hatching. A polarity reversal in the V5 cell division resulted in the fusion of V5.p with the hyp7 syncytium (47% of the animals examined showed a reversed fusion pattern in the V5 cell division,  $n=55$ ). Scale bars: 10  $\mu$ m.

**Table 2. V5 polarity phenotype of single and double mutants**

V5 polarity in single mutants		V5 polarity in double mutants		
Genotype	% Reversed	Genotype	% Reversed	P-value
Wild type	0 (100)			
<i>egl-20(n585)</i>	50 (100)			
<i>mig-14(mu71)</i>	0 (79)	<i>mig-14(mu71); egl-20(n585)</i>	68 (102)	0.02
<i>mig-14(k124)</i>	7 (81)	<i>mig-14(k124); egl-20(n585)</i>	64 (90)	0.08
<i>lin-17(n671)</i>	0 (100)	<i>lin-17(n671); egl-20(n585)</i>	2 (100)	<0.0001
<i>lin-17(n677)</i>	0 (79)	<i>lin-17(n677); egl-20(n585)</i>	0 (90)	<0.0001
<i>mig-1(e1787)</i>	0 (100)	<i>mig-1(e1787); egl-20(n585)‡</i>	72 (100)	0.003
<i>mig-1(n687)</i>	0 (75)	<i>mig-1(n687); egl-20(n585)</i>	51 (152)	>0.9999
<i>mig-1(mu72)</i>	0 (61)	<i>mig-1(mu72); egl-20(n585)</i>	33 (147)	0.008
<i>pry-1(mu38)*</i>	0 (50)	<i>pry-1(mu38); egl-20(n585)§</i>	80 (100)	<0.0001
<i>bar-1(ga80)</i>	0 (100)	<i>egl-20(n585); bar-1(ga80)¶</i>	36 (112)	0.09
<i>lin-44(n1792)*</i>	0 (100)	<i>lin-44(n1792); egl-20(n585)**</i>	40 (177)	0.01
<i>lin-18(e620)</i>	0 (80)	<i>egl-20(n585); lin-18(e620)</i>	30 (99)	0.0008
<i>lin-18(n1051)</i>	0 (68)	<i>egl-20(n585); lin-18(n1051)</i>	30 (108)	<0.0001

All animals were examined at 25°C. The number of animals (*n*) examined for each strain is shown in parentheses. The *P*-values were derived using the Fisher exact test. These values represent the probability that the frequency of V5 polarity reversals observed in the double mutant strains is a random sample of the frequency observed in *egl-20* mutants alone.

\*These strains also contain the *him-5(e1490)* mutation, which causes a high incidence of males.

‡We are unable to infer the role of *mig-1* in V5 polarity since mutations in *mig-1* did not show a consistent effect.

§The V6 cell divided symmetrically and yielded two syncytial daughters in two animals.

¶We observed one animal in which the V5 cell divided symmetrically and gave rise to two syncytial daughters.

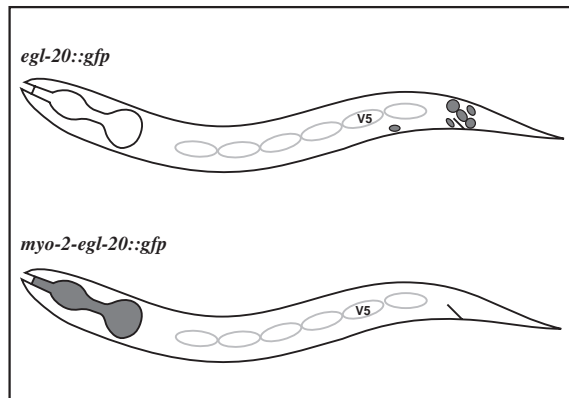
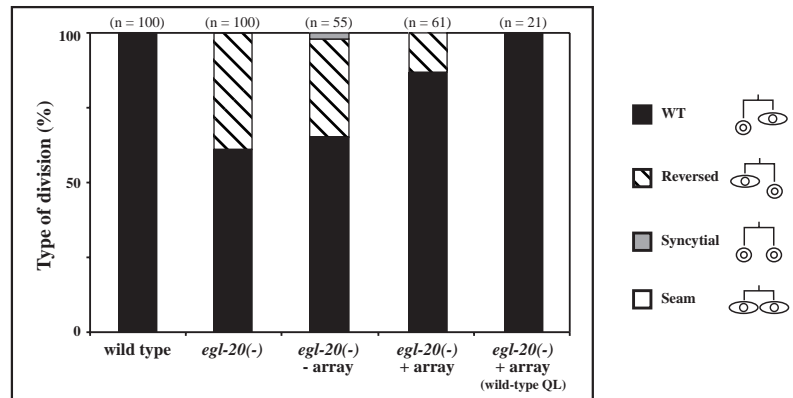
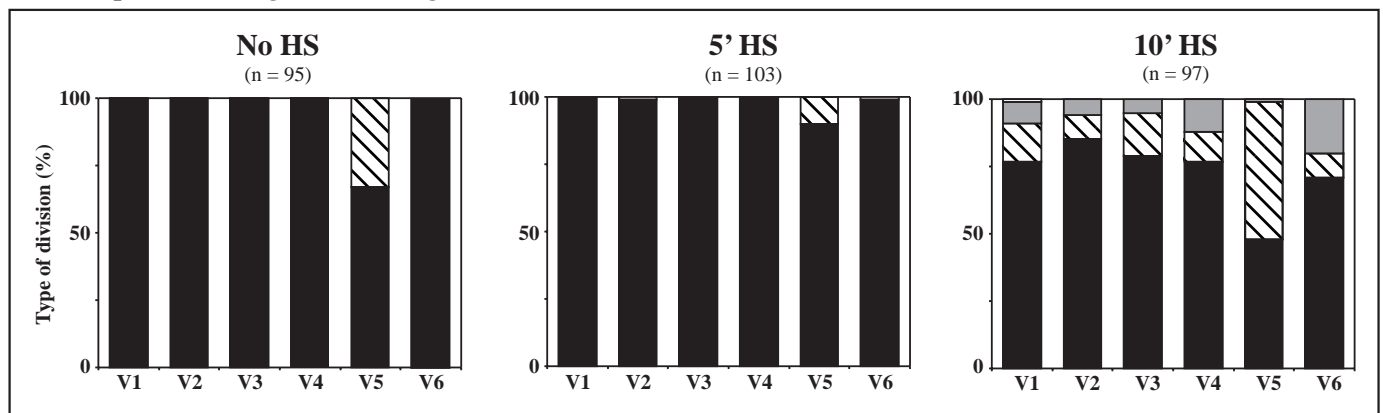
\*\*Occasional polarity reversals (3% of the animals examined) and symmetric divisions (4%) occurred in the V6 cell division.

descendants (Harris et al., 1996; Maloof et al., 1999). We examined mutations in all of these genes for their effects on V5 polarity and found that, with one exception, V5 developed normally. Only *mig-14(k124)* animals exhibited V5 polarity reversals at a low frequency (Table 2). Another gene, *pry-1*, acts as a negative regulator of this Wnt signaling pathway and appears to function downstream of EGL-20/Wnt and upstream of BAR-1/Arm. Reduction-of-function mutations in *pry-1* activate *mab-5* expression in both QL and QR in an *egl-20*-independent but *bar-1*-dependent fashion (Maloof et al., 1999). We found that mutations in *pry-1* also did not exhibit polarity defects in the V5 cell division. If EGL-20 polarized the V5 cell division by activating this Wnt pathway, then mutations in *lin-17*, *bar-1*, *mig-1* and *mig-14* also should have caused polarity reversals. Conversely, if EGL-20 polarized the V5 cell division by repressing this same pathway, then *pry-1* mutations should have caused V5 polarity reversals. Since none of these mutations (with the exception of *mig-14*) affected V5 polarity, we infer that EGL-20 exerts its effect on V5 polarity through a different, undefined pathway. This pathway could also involve known Wnt pathway components since there are other *frizzled*, *dishevelled* and *armadillo*-related genes in *C. elegans* (Ruvkun and Hobert, 1998).

We also examined mutations in two genes that affect the polarity of other asymmetric cell fate decisions in *C. elegans*: *lin-44*, a Wnt homolog required for normal polarity of the T seam cell lineage in the tail of the animal (Herman and Horvitz, 1994; Herman et al., 1995) and *lin-18*, which regulates the polarity of two vulval precursor cell lineages (Ferguson et al., 1987). These mutants were not defective in the polarity of the V5 or other V cell divisions (Table 2).

### Polarity of the V5 cell division is not dependent on the spatial localization of *egl-20* expression in the animal

How does *egl-20* activity orient the V5 cell division? Previously, we showed that a rescuing *egl-20::gfp* transgene is expressed exclusively within a group of epidermal and muscle cells located in the tail region posterior to the V5 cell (Whangbo and Kenyon, 1999). This suggested a model in which the asymmetric expression of EGL-20 along the A/P axis could lead to asymmetric levels of receptor activity in the V5 cell, and thus provide the positional information required to orient the V5 cell division. To test this model, we moved the source of EGL-20 from the posterior to the anterior, and examined the polarity of the V5 cell division (Fig. 2A). We did this by placing the *egl-20::gfp* fusion gene under the control of the *myo-2* promoter, which drives expression only in the anteriorly-located pharynx (Whangbo and Kenyon, 1999). We have shown that this fusion is able to rescue the Q cell migration defect of *egl-20* mutants. The descendants of QL never migrate correctly in *egl-20(-)* animals. In contrast, approximately 40% of the *egl-20(-)* animals carrying the *myo-2-egl-20::gfp* fusion exhibit normal cell migrations. In all of these animals (in which EGL-20 was clearly functional), V5 divided with the correct polarity. When all of the animals carrying the array were considered, we still observed significant rescue of V5 cell polarity (Fig. 2B). Therefore the polarity of the V5 division does not depend upon the posterior localization of EGL-20. This indicates that EGL-20 is not an instructive signal for V5 polarity determination.

**A EGL-20 localization****B V5 polarity in *egl-20(-)* + *myo-2-egl-20::gfp*****C V cell polarities in *egl-20(-)* + *hs-egl-20***

**Fig. 2.** EGL-20/Wnt is not an instructive signal for V5 polarity determination. All animals were examined at 20°C. *n* is the number of animals, *P* is the Fisher's exact *P*-value. (A) Schematic diagram of the *egl-20* expression pattern in *egl-20(-)* L1 larvae carrying the following transgenes: *egl-20::gfp* (top) and *myo-2-egl-20::gfp* (bottom). Cells expressing the *egl-20* fusion genes are filled in gray. The six V cells are represented by ovals. (B) Frequency of V5 reversals in CF1135 animals [*egl-20(n585)IV*; *muEx68 (myo-2-egl-20::gfp)*] that have retained (+) or lost (-) the *muEx68* extrachromosomal array. Wild-type and *egl-20(n585)* data are shown for reference. Not all *egl-20(-)* animals carrying the *muEx68* array were rescued for the QL migration phenotype. We scored V5 polarity in all animals carrying the *muEx68* array and in the subset of the animals carrying the *muEx68* array in which the QL migration defect was rescued (since we knew that EGL-20 was functional in these animals). In both cases, *egl-20(n585)* animals carrying the *muEx68* array are rescued for V5 polarity compared to animals that have lost the array ( $P=0.0082$  for all animals carrying *muEx68*;  $P=0.0009$  for animals with wild-type QL migrations). Black shading represents wild-type polarity. Hatched represents reversed polarity. Dark gray represents symmetric divisions in which both daughter cells adopt the syncytial fate. (C) Frequency of V cell polarity defects in *egl-20(n585)* animals following different doses of *hs-egl-20*. The frequency of V5 reversals in animals following a 5-minute heat shock is significantly suppressed compared with animals without heat shock ( $P=0.0001$ ). Surprisingly, a 10-minute heat pulse disrupts the polarities of all the V cell divisions. White shading represents symmetric divisions in which both daughter cells adopt the seam fate.

**Overexpression of EGL-20 causes polarity reversals in all V cells**

To ask whether EGL-20 might act in a dose-dependent manner to determine V5 polarity, we provided different levels of hs-EGL-20 by varying the duration of heat shock in *egl-20(-)* animals carrying a *hs-egl-20* fusion gene (Whangbo and Kenyon, 1999). In the absence of heat shock, V5 divided with reversed polarity at the frequency expected for *egl-20(n585)* animals at 20°C (Fig. 2C). We observed that a 5-minute pulse of *hs-egl-20* significantly suppressed the frequency of polarity reversals (Fig. 2C). This result is consistent with the interpretation that EGL-20 plays a permissive role in establishing the polarity of the V5 cell division. Surprisingly, we found that higher levels of hs-EGL-20 (10-minute pulse) could disrupt the polarities of all the V cell divisions (Fig. 2C).

Thus, although these other V cells normally do not require *egl-20* activity to establish their polarities, the system that establishes their polarities can be disrupted by high levels of EGL-20. Expression of hs-EGL-20 (30-minute pulse) also disrupts the polarities of the V5 and all other V cell divisions in a wild-type background, demonstrating that the effect of hs-EGL-20 in *egl-20* mutants is not due to a failure to rescue (Table 3). In addition to polarity reversals, high levels of EGL-20 led to symmetric divisions in which both V cell daughters adopted the syncytial fate. Thus, in addition to disrupting the orientation of an asymmetric cell division, high levels of EGL-20 can disrupt the generation of asymmetry itself.

What genes are required for high levels of hs-EGL-20 to cause these ectopic V cell polarity reversals? We provided hs-EGL-20 in various strains carrying mutations in known

**Table 3. Effect of Wnt pathway mutations on V cell polarities following *hs-egl-20***

Genotype	Type of division (%)	V1	V2	V3	V4	V5	V6
Wild type ( <i>n</i> =51)	Reversed	0	0	0	0	0	0
	Symmetric	0	0	0	0	0	0
<i>hs-egl-20</i> ( <i>n</i> =84)	Reversed	20	8	30	33	37	5
	Symmetric	11	8	19	15	6	8
<i>lin-17(n677); hs-egl-20</i> ( <i>n</i> =74)	Reversed	27	8	7	14	23	23
	Symmetric	22	7	18	9	20	38
<i>bar-1(ga80); hs-egl-20</i> ( <i>n</i> =79)	Reversed	19	20	38	35	27	13
	Symmetric	9	3	16	22	3	13
<i>mig-14(k124); hs-egl-20</i> ( <i>n</i> =75)	Reversed	0	0	0	0	0	0
	Symmetric	0	0	0	0	0	0

Newly hatched animals were heat shocked for 30 minutes at 33°C and allowed to recover at 20°C. Polarity of the V cell divisions was determined after the V cell daughters had differentiated (approx. 8 hours following heat shock).

The number of animals (*n*) examined for each strain is shown in parentheses.

Animals carrying the *hs-egl-20* array, *muls53*, without heat shock did not exhibit any V cell polarity defects (*n*>70 for each strain).

Symmetric divisions almost always gave rise to two syncytial daughters.

components of the EGL-20 signal transduction pathway for *mab-5* activation. We observed that a mutation in the *mig-14* gene was able to suppress the ectopic polarity reversals, whereas mutations in *lin-17* and *bar-1* did not alter the response of the V cells to hs-EGL-20 (Table 3). In other experiments, we also observed a slight enhancement of the V5 polarity defect in *mig-14(-)*; *egl-20* double mutants (Table 2). Together these results suggest that *mig-14* acts with *egl-20* to regulate cell polarization.

### Establishment of V5 polarity in the wild type does not appear to require lateral signaling between cells in the V lineages

To learn more about how the polarity of the V5 cell division is established, we tested whether intercellular signals between cells in the V lineages were involved. One possibility was that the V5 daughter cells are equivalent at birth and differentiate later as a result of lateral signaling between one another. We tested whether this was the case in either wild-type or *egl-20(-)* animals. Using a laser microbeam, we ablated one of the V5 daughters shortly after the V5 cell division and then examined the fate adopted by the remaining daughter. Following V5.a ablation in wild-type animals, V5.p always adopted the expected seam fate (*n*=5). Conversely when V5.p was ablated, V5.a always adopted the syncytial fate (*n*=8). In *egl-20(n585)* animals, each V5 daughter was able to adopt either the seam or syncytial fate in the absence of the other daughter. Following V5.a ablation, V5.p adopted the seam fate in 5/7 animals and adopted the syncytial fate in 2/7 animals. Similarly when V5.p was ablated, V5.a adopted the seam fate in 5/8 animals and adopted the syncytial fate in 3/8 animals. Together these results suggested that the fate of each daughter is established independently of its sister cell, although it remains possible that signaling between the daughter cells occurs as soon as they are born, prior to the time of these ablations.

We also addressed the possibility that the V5 cell division

was polarized by lateral signals from the neighbors of V5. This model was appealing for two reasons. First, all of the V cells exhibit a striking morphological polarity and as a result, the posterior and anterior environments of V5 (and its daughters) are different from one another (see Fig. 1). Second, the fates of certain cells that arise later within the V5 lineage are known to be regulated by lateral signals from cells in neighboring V cell lineages (Austin and Kenyon, 1994; Sulston and White, 1980; Waring et al., 1992). To test whether signals from neighboring V cells might influence the polarity of the V5 cell division, we ablated the seam cells adjacent to V5 in wild-type animals and subsequently examined the polarity of the V5 cell division (Table 4). We found that V5 divided with wild-type polarity after ablation of either its anterior (V1-V4) or posterior (V6 and T) neighbors. V5 also divided correctly after ablation of all the seam cell neighbors (V1-V4, V6 and T). Thus, we were unable to find any evidence that lateral signaling between V cells influences the polarity of the V5 cell division in wild-type animals.

### Lateral signals from posterior neighbors cause the V5 polarity reversals observed in *egl-20* mutants

To determine whether signals from neighboring V cells might influence the polarity of the V5 cell division in *egl-20* mutants, we ablated neighboring V cells in these animals. Surprisingly, when we ablated V6 and T at hatching, the descendants of V5 always divided with normal polarity (Table 4). To confirm that the V5 daughter cells remained stationary under these conditions, we observed the V5 division in six *egl-20(n585)* animals in which V6 and T had been ablated until the fates of the daughter cells could be distinguished clearly. In all six animals, the V5 daughter cells remained stationary and adopted the wild-type fates. Thus, we conclude that removal of posterior seam cell neighbors in *egl-20* mutants can restore wild-type polarity to the V5 division. These results implied that the polarity of the V5 cell division was reversed in *egl-20* mutants because V5 received 'polarity-reversing' signals from the posterior seam cells V6 and T.











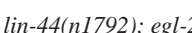



Next, we tested whether ablation of both V6 and T was necessary for rescue of the V5 polarity reversals in *egl-20* mutants. We found that ablation of V6 alone was not sufficient for complete rescue of the V5 polarity reversals in *egl-20* mutants. This finding is consistent with observations by Austin and Kenyon (1994) that non-adjacent seam cells are capable of forming contacts when their intervening neighbors have been removed by cell ablation. In addition, we found that ablation of T alone had no rescuing activity (Table 4). Together these results suggested that the signaling between V5 and its posterior neighbors occurs through direct cell-cell contact or short-range signals.

Are other V cells sensitive to the polarity-reversing signals? To ask whether signals from V6 and T could cause polarity reversals in V4, we allowed V4 to move closer to V6 by ablating the two intervening cells, Q and V5, at hatching. In all six ablated animals, V4 always divided with normal polarity. Thus, the V5 cell may be unique in its ability to respond to the polarity-reversing signals in *egl-20(n585)* animals.

Since signals from posterior seam cells were able to influence the polarity of the V5 cell division in *egl-20* mutants, we asked if anterior cells were also capable of signaling.



**Table 4. Polarity of the V5 cell division following ablations of neighboring seam cells**

Genotype/treatment	% Reversed	<i>P</i> -value*
<b>Wild type</b>		
 Unablated sides	0 (45)	
 V6, T ablated	0 (11)	
 V6 ablated	0 (15)	
 V2-V4 ablated	0 (10)	
 V1-V4, V6, T ablated	0 (11)	
<b><i>egl-20(n585)</i>‡</b>		
 Unablated sides	47 (81)	
 V6, T ablated	0 (32)	<0.0001
 V6 ablated	10 (10)	0.04
 T ablated	50 (10)	>0.9999
 V1-V4 ablated	56 (27)	0.51
 V1-V4, V6, T ablated	0 (12)	0.0012
<b><i>lin-44(n1792); egl-20(n585)</i>§¶</b>		
 Unoperated animals	40 (177)	
 V6, T ablated/ablated sides	13 (39)	0.0014
 V6, T ablated/unablated sides	13 (38)	0.0013
	V5 polarity	

The number of animals (*n*) examined for each treatment is shown in parentheses.

The intact sides of the operated animals were used as controls.

\*The *P*-values were derived using the Fisher exact test. For ablations in *egl-20(n585)*, these values represent the probability that the frequency of V5 polarity reversals observed in the ablated sides is a random sample of the frequency observed in the unablated control sides. For ablations in *lin-44(n1792); egl-20(n585)*, both the ablated and unablated control sides were compared with animals that did not undergo operation. Thus, the *P*-values represent the probability that the frequency of V5 polarity reversals observed in the operated animals is a random sample of the frequency observed in control animals that were not operated.

‡We also performed the posterior cell ablations (V6 and T) in another *egl-20* mutant, *egl-20(mu25)*. Of the ablated sides, V5 divided with reversed polarity in 1/14 animals. Of the unablated sides, V5 divided with reversed polarity in 7/16 animals. The Fisher exact *P*-value for this experiment was 0.02. Thus, posterior cell ablations had similar effects in this mutant and in *egl-20(n585)*.

§The posterior cell ablations on one side significantly affected the V5 polarity of the unablated side for ablations performed on either the left or right sides (*P*=0.019 and *P*=0.010, respectively).

¶To eliminate the possibility that these observations were an artifact of laser ablation, we ablated the V1 cell on one side and subsequently examined on both ablated and unablated sides. Of the ablated sides, V5 divided with reversed polarity in 4/13 animals. Of the unablated sides, V5 divided with reversed polarity in 5/12 animals. These frequencies of polarity reversals were not significantly different from the frequency observed in unoperated animals (*P*>0.9999).

Ablation of the anterior seam cells (V2-V4) did not significantly alter the frequency of V5 polarity reversals in *egl-20* mutants (Table 4). Ablation of both anterior and posterior neighbors completely rescued the V5 polarity reversals in *egl-20* mutants, as observed for posterior ablations alone (Table 4). Thus, the anterior seam cell neighbors did not appear to influence the polarity of the V5 division in *egl-20* mutants.

### LIN-44/Wnt spatially restricts polarity-determining signals

What is the pathway responsible for generating polarity reversals in *egl-20* mutants? Perhaps in the absence of EGL-20, another Wnt signal is able to provide polarity information to the V5 cell. One candidate is the LIN-44/Wnt signal, which is produced in the tail and orients the asymmetric divisions of certain tail epidermal cells (Herman and Horvitz, 1994; Herman et al., 1995). If *lin-44* encoded the polarity-reversing signal, then removing *lin-44* activity should suppress the

polarity reversals seen in *egl-20(-)* animals. However, *lin-44(n1792); egl-20(n585)* double mutants still exhibit V5 polarity reversals, indicating that LIN-44 is not the polarity-reversing signal (Table 2).

An alternate hypothesis was that *lin-44* encodes the 'polarity-rescuing' signal for the V5 cell following posterior cell ablations in *egl-20* mutants. Since *lin-44* is expressed posterior to the V6 and T seam cells, it was possible that receptors on these seam cells normally restrict the movement of the LIN-44/Wnt signal. Ablation of V6 and T in *egl-20* mutants could allow LIN-44 to diffuse further anteriorly and rescue the V5 polarity defect. We found that ablation of V6 and T could rescue the V5 polarity defect in *egl-20(n585)* animals that also carried the *lin-44(n1792)* mutation (Table 4). Thus, *lin-44* does not encode the polarity-rescuing signal.

Ablation of V6 and T in *egl-20* single mutants rescues the polarity of the V5 cell division only on the side of the animal on which the ablation was performed. The unablated sides



continue to exhibit V5 polarity reversals at the frequency expected for *egl-20* mutants. Thus, the polarity-determining signals appear to be spatially restricted to one side of the animal. Surprisingly, we found that ablating V6 and T in *lin-44(n1792); egl-20(n585)* double mutants could also rescue the V5 polarity defect of the unablated sides (Table 4). Therefore, *lin-44* activity may be required to prevent polarity signals from one side of the animal from affecting the other side of the animal.

### Mutations in *lin-17/frizzled* suppress the V5 polarity reversals in *egl-20/Wnt* mutants

To determine whether any of the genes that act with *egl-20* to activate Hox gene expression might act within the signaling system that generates polarity reversals in *egl-20* mutants, we constructed double mutants carrying mutations in these genes as well as *egl-20(n585)*. Most of the mutations we examined did not have a strong or consistent effect on the *egl-20(n585)* V5 polarity phenotype (Table 2). However, mutations in the putative Wnt receptor *lin-17/fz* completely suppressed the V5 polarity reversals observed in *egl-20(n585)* mutants. In *lin-17(-); egl-20(n585)* double mutants containing either the *lin-17(n671)* or the *lin-17(n677)* allele, the V5 daughter cells almost always divided normally. Mutations in *lin-17* produced the same effect as ablation of V6 and T in *egl-20* mutants: both suppressed the V5 polarity reversals. This suggests a role for LIN-17 in the lateral signaling system between V cells.

In addition, mutations in both alleles of *lin-18* consistently were able to suppress the V5 polarity reversals in *egl-20(-)* mutants from 50% to 30% (Table 2). Like *lin-17*, *lin-18* is also required to orient asymmetric cell divisions in the vulval precursor lineages (Ferguson et al., 1987; Sternberg and Horvitz, 1988). Therefore, it is possible that these two genes may be acting together to generate the V5 polarity reversals in *egl-20* mutants.

### Mutations in *pry-1* enhance the frequency of V5 polarity reversals in *egl-20* mutants

As described above, the *pry-1* gene functions as a negative regulator of the Hox gene activation pathway (Maloof et al., 1999). We examined the polarity of the V5 cell division in *pry-1; egl-20* double mutants and found that V5 divided with reversed polarity in a large majority of animals (80%) (Table 2). Thus *pry-1*, like *lin-17*, appears to function in the pathway that reverses the polarity of the V5 cell division in *egl-20* mutants. We attempted to order these two genes in a pathway, however the *lin-17 pry-1; egl-20* triple mutant animals were too unhealthy to analyze.

## DISCUSSION

In this study, we have examined the effects of Wnt pathway components and cell-cell interactions on the polarity of the asymmetric V5 cell division in *C. elegans*. This cell polarity system appears to involve three inter-related levels of regulation. First, a pathway involving EGL-20 acts to polarize the V5 cell division correctly in wild-type animals. Second, a polarity-reversing pathway incorrectly polarizes the V5 cell division in the absence of EGL-20. Third, an underlying polarity-determining system operates independently of both

EGL-20 and of signals from neighboring cells to polarize the V5 cell division correctly. We now discuss each of these three pathways in turn.

### The role of EGL-20 in the polarization of the V5 cell division

We were interested in learning whether EGL-20 functions as a permissive or instructive signal in determining the polarity of V5. In *Drosophila*, ectopic *wingless* (*wg*) expression has been shown to reorganize dorsoventral pattern in the leg imaginal disc, indicating an instructive role for the Wg signal (Struhl and Basler, 1993). Likewise, further analyses of *wg* function in imaginal discs have demonstrated that Wnt signals can function as concentration-dependent morphogens, which generate pattern directly by specifying distinct spatial domains of gene expression at different threshold activity levels (Lecuit and Cohen, 1997; Neumann and Cohen, 1997; Zecca et al., 1996). In the early *C. elegans* embryo, the MOM-2/Wnt acts instructively, since the position of the Wnt signal determines the polarity of the asymmetric EMS cell division (Goldstein, 1993; Thorpe et al., 1997). However, Wnt signals have also been shown to act in a permissive fashion. Expression of *wg* under the control of a heat shock promoter can restore segmentation in *wg-* embryos, suggesting a permissive role for this Wnt protein (Sampedro et al., 1993). In addition, the EGL-20/Wnt protein acts in a permissive, position-independent fashion to regulate the direction and extent of neuronal Q cell migration in *C. elegans* (Whangbo and Kenyon, 1999).

In this study, we have found that EGL-20 functions permissively to orient the V5 cell division. Our findings indicate that although EGL-20 is synthesized only on the posterior side of the V5 cell, EGL-20 itself does not serve as the polarity cue. If the source of EGL-20 is moved to the anterior side of the V5 cell, it can still orient the polarity of the division properly. This indicates that the spatial information required to polarize the V5 cell division must be provided by another source, and the function of EGL-20 is to ensure that V5 responds correctly to this information.

Because EGL-20 regulates a canonical Wnt pathway to activate Hox gene expression in migrating neuroblasts, we asked whether EGL-20 might activate this same pathway to regulate the V5 cell division. One of the genes that acts in the Hox gene activation pathway, *mig-14*, also acts in the EGL-20 pathway for polarity determination. However, mutations in other genes in the Hox gene activation pathway do not generate V5 polarity reversals, indicating that they do not act with EGL-20 in the V5 polarity pathway. EGL-20 also functions independently of genes in the Hox gene activation pathway for its function in promoting anterior migration of the QR descendants. Whether these two pathways share any common components is not clear.

Since EGL-20 is not the polarity cue for the V5 cell, what is the source of polarity information? Our experiments reveal the existence of an underlying polarity system that is able to orient the V5 cell division correctly in the absence of both EGL-20 and lateral, polarity-reversing signals from posterior neighbors. Perhaps the information that orients the V5 cell division correctly resides within this system.

What is the role of EGL-20 in the wild type? Since ablation of neighboring cells relieves the requirement for EGL-20, the role of EGL-20 may be to prevent the polarity-reversing signals

from influencing the intrinsic response that V5 has to the underlying information. In principle, EGL-20 could act in several ways to block these lateral signals. One possibility is that it could act directly on the lateral signaling system to repress it. Alternatively, EGL-20 activity could override the effect of the lateral signaling system by amplifying the strength of the underlying polarity-determining system, or by ensuring that cells respond correctly to this underlying positional information. It is also conceivable that EGL-20 is part of yet another polarity system that provides positional information independently of the underlying polarization system; however, this model seems less economical to us.

### How does EGL-20 activity counteract the lateral signaling pathway?

In the absence of *egl-20* activity, the V5 cell can respond to polarity-reversing signals from neighboring posterior seam cells. Our findings suggest that the lateral signaling pathway involves the LIN-17 receptor, since mutations in *lin-17* have the same effect as ablation of the posterior neighbors of V5: both suppress the polarity reversals caused by *egl-20* mutations. In this model, LIN-17 activity could be required within the lateral signaling system for the production of or response to the polarity-reversing signals.

In *lin-17; egl-20* double mutants, normal polarity is restored to the V5 cell division. A simple interpretation of this genetic interaction might be that in wild-type animals, EGL-20/Wnt binds to the LIN-17/Fz receptor and turns its activity OFF. This would prevent LIN-17 from transmitting polarity-reversing signals. However, we cannot reconcile this model with the involvement of PRY-1 in V5 polarity determination. If LIN-17 is a negative regulator of PRY-1 in this pathway, as seems to be the case in the Hox gene activation pathway, then mutations in *pry-1* should cause the polarity-reversing pathway to be constitutively active in an EGL-20-independent manner. Thus *pry-1* mutations should reverse the polarity of the V5 cell in otherwise wild-type animals. However, we did not observe any V5 polarity defects in *pry-1* mutants. For this reason, we do not favor the model that EGL-20 silences the lateral signaling pathway by binding to the LIN-17 receptor and turning it off. Instead, we propose that EGL-20 inhibits the lateral signaling pathway in a different, possibly indirect manner. For example, EGL-20 could amplify the strength of the underlying polarization pathway so that it overrides the lateral signaling pathway. Alternatively, EGL-20 could activate a separate pathway that shields V5 from the effects of lateral signals.

### How does the lateral signaling pathway influence the polarity of V5?

Based on our ablation studies, the lateral signaling system between V5 and the posterior seam cells appears to involve direct cell-cell contact or short-range signals. In addition, our genetic analyses suggest that the LIN-17/Fz and PRY-1 proteins play a role in this signaling pathway. An obvious candidate for the Wnt protein that activates LIN-17 in this pathway would be LIN-44, which is produced in the tail of the animal. However, mutations in *lin-44* do not efficiently suppress the polarity reversals that occur in *egl-20* mutants, indicating that LIN-44 cannot be the polarity-reversing signal. Possibly one of the three remaining *Wnt* genes in the *C. elegans* genome (Ruvkun and Hobert, 1998) is involved. Surprisingly,

we found that LIN-44/Wnt has a role in preventing polarity-determining signals on one side of the animal from affecting the polarity of the V5 cell on the other side of the animal. Thus, LIN-44 allows the polarities of the V5 cells on each side of the animal to be determined by independent mechanisms.

In *lin-17; egl-20* double mutants, V5 divides with normal polarity. However, in *pry-1; egl-20* double mutants, V5 divides with reversed polarity at a very high frequency (80%). Thus a simple model is that, in the absence of EGL-20, PRY-1 activity promotes the correct V5 polarization, and LIN-17 activity reverses V5 polarity by downregulating PRY-1 activity. It is possible that both of these gene products act together in the signaling cells to regulate the synthesis of the polarity-reversing signal. Another possibility is that these genes might act within V5, where they respond to lateral signals and, in turn, change the way V5 interprets the underlying polarity information.

### Wnt signals may act locally to orient specific lateral epidermal cell divisions

Our findings indicate that EGL-20 acts in a permissive way to polarize the V5 cell division, and also to regulate the migrations of the Q cells, which are adjacent to the V5 cell at hatching. Interestingly, an analogous situation may exist for the activities of the *lin-44/Wnt* gene. *lin-44* is expressed in a group of epidermal cells located in the tail, whereas *egl-20* is expressed in a group of cells located anteriorly to the site of *lin-44* expression. Like EGL-20, LIN-44 influences cells located just anterior to its site of expression. In principle, the asymmetric localization of LIN-44 in the animal could provide these cells with the positional information that orients their divisions. However, previous studies have shown that expression of LIN-44 from a heat-shock promoter that is expected to act globally can rescue the *lin-44* mutant phenotype. It is formally possible that this heat-shock promoter produced a non-uniform distribution of LIN-44 protein along the body axis (Herman et al., 1995). However, together with our findings, the data support the idea that LIN-44, like EGL-20, acts in a permissive rather than instructive fashion to orient cell divisions.

The analogy of the LIN-44 and EGL-20 polarity systems raises the question of whether other Wnt systems act locally on other seam cells in the animal. This may be the case, since the CAM-1 protein, a Ror tyrosine kinase receptor, has been shown to regulate the polarity of the V1 cell division (Forrester et al., 1999). The extracellular domain of Ror tyrosine kinases exhibit homology to the Frizzled cysteine-rich domain (Xu and Nusse, 1998), which has been shown to be necessary and sufficient for binding to Wnt ligands in vitro (Bhanot et al., 1996).

### Does a global regulatory system orient the V cell divisions?

If not EGL-20 or LIN-44, then what positional information allows the V cells to distinguish anterior from posterior? As described above, an attractive possibility is that this information is part of the underlying system that orients the V5 cell division in the absence of both EGL-20 and posterior neighbors. It is even possible that this underlying polarizing information acts in a global fashion to orient the LIN-44-dependent cell divisions, and potentially other A/P cell divisions as well. There is

evidence for a global system of positional information that generates cell asymmetry along the A/P body axis. With few exceptions, the TCF homolog POP-1 appears to be present at higher levels in the anterior daughters than the posterior daughters of each cell that divides along the A/P axis. This asymmetry in POP-1 localization appears to be required for many sister cells to adopt different fates (Lin et al., 1995, 1998). In the early embryo, the A/P localization of POP-1 is regulated by the MOM-2/Wnt protein, which orients and polarizes the EMS cell division (Rocheleau et al., 1997; Thorpe et al., 1997). Thus, in this case, a Wnt family member is capable of providing the spatial information that allows a cell to distinguish anterior from posterior. One model is that the underlying system that orients the V5 cell division in the absence of EGL-20 and lateral signals is the same system that polarizes the asymmetric POP-1 segregation. If a Wnt protein acts in this global system to provide the V cells with spatial information, it may have escaped detection if it also has an essential function in patterning the A/P body axis during development.

In this study, we found that high levels of EGL-20 disrupted both the generation as well as the orientation of asymmetric cell divisions all along the A/P body axis. This finding is consistent with the model that high levels of EGL-20 interfere with the ability of this underlying system to provide cells with positional information. This could occur if the underlying positional system uses a Wnt receptor that has only a low affinity for EGL-20.

Another biological process that requires a positioning system to orient cells along the A/P body axis is neuronal cell migration. In separate studies, we have analyzed the role of Wnt signals and Hox gene activities in the anteroposterior migrations of the Q neuroblasts. Although many proteins have been shown to regulate these migrations, including Wnt signals, Hox proteins and other extracellular factors, none of these proteins functions to allow cells to distinguish anterior from posterior (see Sym et al., 1999; Whangbo and Kenyon, 1999). Interestingly, high levels of EGL-20 protein can randomize the migrations of the Q neuroblasts along the A/P axis (Whangbo and Kenyon, 1999). These features are reminiscent of the situation that we observe for the regulation of V cell polarization. Perhaps a single, global system of positional information involving some form of Wnt signaling orients both asymmetric cell divisions and cell migration along the A/P body axis.

We thank Lisa Williams for construction of the *pry-1(mu38); egl-20(n585)* double mutant. We also thank Javier Apfeld, Joy Alcedo, Scott Alper, Queelim Ch'ng, Craig Hunter, Lucie Yang and other members of the Kenyon laboratory for extensive discussions and for critical comments on the manuscript. J. W. was supported by a NSF Predoctoral Fellowship. C. K. is the Herbert Boyer Professor of Biochemistry and Biophysics. This work was supported by NIH grant GM37053 to C. K.

## REFERENCES

- Austin, J. and Kenyon, C. (1994). Cell contact regulates neuroblast formation in the *Caenorhabditis elegans* lateral epidermis. *Development* **120**, 313-323.
- Bhanot, P., Brink, M., Samos, C. H., Hsieh, J., Wang, Y., Macke, J. P., Andrew, D., Nathans, J. and Nusse, R. (1996). A new member of the frizzled family from *Drosophila* functions as a Wingless receptor. *Nature* **382**, 225-230.
- Brenner, S. (1974). The genetics of *C. elegans*. *Genetics* **77**, 71-94.
- Cadigan, K. M. and Nusse, R. (1997). Wnt signaling: a common theme in animal development. *Genes and Development* **11**, 3286-3305.
- Chamberlin, H. M. and Sternberg, P. W. (1995). Mutations in the *Caenorhabditis elegans* gene *vab-3* reveal distinct roles in fate specification and unequal cytokinesis in an asymmetric cell division. *Dev. Biol.* **170**, 679-689.
- Eisenmann, D. M., Maloof, J. N., Simske, J. S., Kenyon, C. and Kim, S. K. (1998). The beta-catenin homolog BAR-1 and LET-60 Ras coordinately regulate the Hox gene *lin-39* during *Caenorhabditis elegans* vulval development. *Development* **125**, 3667-3680.
- Ferguson, E. L., Sternberg, P. W. and Horvitz, H. R. (1987). A genetic pathway for the specification of the vulval cell lineages of *Caenorhabditis elegans* [published erratum appears in *Nature* **327**, 82]. *Nature* **326**, 259-267.
- Forrester, W. C., Dell, M., Perens, E. and Garriga, G. (1999). A *C. elegans* Ror receptor tyrosine kinase regulates cell motility and asymmetric cell division. *Nature* **400**, 881-885.
- Francis, R. and Waterston, R. H. (1991). Muscle cell attachment in *Caenorhabditis elegans*. *J. Cell Biol.* **114**, 465-479.
- Goldstein, B. (1992). Induction of gut in *Caenorhabditis elegans* embryos. *Nature* **357**, 255-257.
- Goldstein, B. (1993). Establishment of gut fate in the E lineage of *C. elegans*: the roles of lineage-dependent mechanisms and cell interactions. *Development* **118**, 1267-1277.
- Harris, J., Honigberg, L., Robinson, N. and Kenyon, C. (1996). Neuronal cell migration in *C. elegans*: regulation of Hox gene expression and cell position. *Development* **122**, 3117-3131.
- Hawkins, N. and Garriga, G. (1998). Asymmetric cell division: from A to Z. *Genes Dev.* **12**, 3625-3638.
- Herman, M. A. and Horvitz, H. R. (1994). The *Caenorhabditis elegans* gene *lin-44* controls the polarity of asymmetric cell divisions. *Development* **120**, 1035-1047.
- 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.
- Horvitz, H. R. and Herskowitz, I. (1992). Mechanisms of asymmetric cell division: two Bs or not two Bs, that is the question. *Cell* **68**, 237-255.
- Jan, Y. N. and Jan, L. Y. (1998). Asymmetric cell division. *Nature* **392**, 775-778.
- Knoblich, J. A. (1997). Mechanisms of asymmetric cell division during animal development. *Curr. Opin. Cell Biol.* **9**, 833-841.
- Lecuit, T. and Cohen, S. M. (1997). Proximal-distal axis formation in the *Drosophila* leg. *Nature* **388**, 139-145.
- Lin, R., Hill, R. J. and Priess, J. R. (1998). POP-1 and anterior-posterior fate decisions in *C. elegans* embryos. *Cell* **92**, 229-239.
- Lin, R., Thompson, S. and Priess, J. R. (1995). *pop-1* encodes an HMG box protein required for the specification of a mesoderm precursor in early *C. elegans* embryos. *Cell* **83**, 599-609.
- Maloof, J. N., Whangbo, J., Harris, J. M., Jongeward, G. D. and Kenyon, C. (1999). A Wnt signaling pathway controls hox gene expression and neuroblast migration in *C. elegans*. *Development* **126**, 37-49.
- Neumann, C. J. and Cohen, S. M. (1997). Long-range action of Wingless organizes the dorsal-ventral axis of the *Drosophila* wing. *Development* **124**, 871-880.
- Rocheleau, C. E., Downs, W. D., Lin, R., Wittmann, C., Bei, Y., Cha, Y. H., Ali, M., Priess, J. R. and Mello, C. C. (1997). Wnt signaling and an APC-related gene specify endoderm in early *C. elegans* embryos. *Cell* **90**, 707-716.
- Ruvkun, G. and Hobert, O. (1998). The taxonomy of developmental control in *Caenorhabditis elegans*. *Science* **282**, 2033-2041.
- Sampedro, J., Johnston, P. and Lawrence, P. A. (1993). A role for wingless in the segmental gradient of *Drosophila*? *Development* **117**, 677-687.
- Sawa, H., Lobel, L. and Horvitz, H. R. (1996). The *Caenorhabditis elegans* gene *lin-17*, which is required for certain asymmetric cell divisions, encodes a putative seven-transmembrane protein similar to the *Drosophila* frizzled protein. *Genes Dev.* **10**, 2189-2197.
- Sternberg, P. W. and Horvitz, H. R. (1988). *lin-17* mutations of *Caenorhabditis elegans* disrupt certain asymmetric cell divisions. *Dev. Biol.* **130**, 67-73.
- Struhl, G. and Basler, K. (1993). Organizing activity of wingless protein in *Drosophila*. *Cell* **72**, 527-540.
- Sulston, J. and Hodgkin, J. (1988). Methods. In *The Nematode*

- Caenorhabditis elegans (ed. W. B. Wood), pp. 587-605. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.
- Sulston, J. and Horvitz, H.** (1977). Post-embryonic cell lineages of the nematode, *C. elegans*. *Dev. Biol.* **56**, 110-156.
- Sulston, J. E. and White, J. G.** (1980). Regulation and cell autonomy during postembryonic development of *Caenorhabditis elegans*. *Dev. Biol.* **78**, 577-597.
- Sym, M., Robinson, N. and Kenyon, C.** (1999). MIG-13 positions migrating cells along the anteroposterior body axis of *C. elegans*. *Cell* **98**, 25-36.
- Thorpe, C. J., Schlesinger, A., Carter, J. C. and Bowerman, B.** (1997). Wnt signaling polarizes an early *C. elegans* blastomere to distinguish endoderm from mesoderm. *Cell* **90**, 695-705.
- Waring, D. A., Wrischnik, L. and Kenyon, C.** (1992). Cell signals allow the expression of a pre-existent neural pattern in *C. elegans*. *Development* **116**, 457-466.
- Whangbo, J. and Kenyon, C.** (1999). A Wnt signaling system that specifies two patterns of cell migration in *C. elegans*. *Mol. Cell* **4**, 851-858.
- Xu, Y. K. and Nusse, R.** (1998). The Frizzled CRD domain is conserved in diverse proteins including several receptor tyrosine kinases. *Curr. Biol.* **8**, R405-R406.
- Zecca, M., Basler, K. and Struhl, G.** (1996). Direct and long-range action of a wingless morphogen gradient. *Cell* **87**, 833-844.