

A Wnt signaling pathway controls Hox gene expression and neuroblast migration in *C. elegans*

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SUMMARY

The specification of body pattern along the anteroposterior (A/P) body axis is achieved largely by the actions of conserved clusters of Hox genes. Limiting expression of these genes to localized regional domains and controlling the precise patterns of expression within those domains is critically important for normal patterning. Here we report that *egl-20*, a *C. elegans* gene required to activate expression of the Hox gene *mab-5* in the migratory neuroblast QL, encodes a member of the Wnt family of secreted glycoproteins. We have found that a second Wnt pathway gene, *bar-1*, which encodes a β -catenin/Armadillo-like protein, is also required for activation of *mab-5* expression in QL. In addition, we describe the gene *pry-1*,

which is required to limit expression of the Hox genes *lin-39*, *mab-5* and *egl-5* to their correct local domains. We find that *egl-20*, *pry-1* and *bar-1* all function in a linear genetic pathway with conserved Wnt signaling components, suggesting that a conserved Wnt pathway activates expression of *mab-5* in the migratory neuroblast QL. Moreover, we find that members of this Wnt signaling system play a major role in both the general and fine-scale control of Hox gene expression in other cell types along the A/P axis.

Key words: Wnt signaling, Hox gene, Cell migration, *C. elegans*

INTRODUCTION

Conserved Hox genes are required to specify regional identity along the metazoan anterior/posterior (A/P) axis. Three of the four *C. elegans* Hox genes, like their *Drosophila* and vertebrate homologs, have been shown to be expressed in broad domains along the A/P axis, and to function where they are expressed to specify regional identity: *lin-39*, a *Sex combs reduced* homolog, patterns the mid-body; *mab-5*, an *Antennapedia* homolog, patterns the posterior body region; and *egl-5*, an *Abdominal-B* homolog, patterns the tail (Kenyon, 1986; Costa et al., 1988; Chisholm, 1991; Clark et al., 1993; Wang et al., 1993).

Limiting the expression of the Hox genes to their appropriate regions is essential for correct patterning; in many organisms, misexpression causes dramatic homeotic transformations of one body region into another. For example, in *C. elegans*, expression of *lin-39* in the mid-body of hermaphrodites directs one particular ectodermal cell type, the Pn.p cells, to make a vulva in response to inductive signals; if the posterior Hox gene *mab-5* is expressed in the mid-body in place of *lin-39*, then mid-body Pn.p cells adopt posterior-specific fates and make a mating structure normally produced by Pn.p cells in the posterior of males (Maloof and Kenyon, 1998). Restriction of *mab-5* to the posterior is also important in the V cells, lateral

ectodermal cells that extend in a row along each side of the worm. Normally *mab-5* is only expressed in the descendants of the posteriormost V cells. In males this expression specifies the production of sensory structures known as rays instead of cuticular structures known as alae, which are made by anterior cells. Ectopic expression of *mab-5* in the anterior causes the anterior V cells to adopt the posterior fate, making rays instead of alae (Salser and Kenyon, 1996).

In some cases Hox genes must be expressed in precise spatial and temporal patterns within their general domains of function to achieve correct development. For example, *Drosophila Ubx* is expressed in an intricate pattern in parasegment 5, and this expression is crucial for giving parasegment 5 its unique identity (Castelli-Gair and Akam, 1995). Hox expression patterns are very dynamic in vertebrates, and it is likely that in at least some cases this dynamic expression is required for proper development (e.g. Nelson et al., 1996). In *C. elegans*, spatio-temporal control of *mab-5* and *egl-5* expression is required for proper fate specification (Salser and Kenyon, 1992, 1996; Jiang and Sternberg, 1998). Precise control of *mab-5* expression is crucial for achieving wild-type development of at least two cell types, the V cells and the migratory Q neuroblasts. In the V5 lineage, *mab-5* is switched ON and OFF multiple times, and each change in expression regulates a different type of cell-fate

decision (Salser and Kenyon, 1996). The two Q cells are neuroblasts born in identical A/P positions but on opposite sides of the animal: QL on the left and QR on the right. In wild-type animals, after a short posterior migration, QL switches on *mab-5*, which, in turn, causes descendants of QL to migrate posteriorly (Salser and Kenyon, 1992). In contrast, *mab-5* remains off in QR and its descendants, and as a result the descendants of QR continue to migrate towards the anterior. Activation of a *heat-shock-mab-5* fusion gene specifically within anteriorly migrating QR descendants with a focused laser microbeam causes the cells to reverse direction and migrate posteriorly (Salser and Kenyon, 1992; Harris et al., 1996). Thus, *mab-5* acts as a switch to control the direction of Q descendant migration: in the absence of *mab-5* activity, the descendants of both Q cells migrate towards the anterior, and conversely, ectopic expression of *mab-5* in both cells causes the descendants of both cells to stay in the posterior.

A number of genes have been described that are specifically required for *mab-5* to be switched on in QL (Harris et al., 1996). One of these, *lin-17*, has been found to encode a homolog of *Drosophila frizzled* (Sawa et al., 1996). Members of the *frizzled* family of seven-transmembrane proteins can act as receptors for Wnt ligands (Bhanot et al., 1996), suggesting that the Wnt pathway might be used to control *mab-5* expression and, as a consequence, the direction of neuroblast migration, in the Q cell descendants (Harris et al., 1996).

The Wnt family of secreted signaling molecules provides positional information and establishes polarity in diverse tissues in many different organisms (reviewed by Cadigan and Nusse, 1997). Typically, Wnt signals activate a conserved signal transduction cascade that leads to translocation of β -catenin/Armadillo into the nucleus (Peifer et al., 1994). Once in the nucleus β -catenin/Armadillo acts in concert with Tcf/LEF-1 related transcription factors to activate expression of downstream genes such as *engrailed* and the Hox gene *Ubx* in *Drosophila*, and *siamois* in *Xenopus* (Brannon et al., 1997; van de Wetering et al., 1997; reviewed by Miller and Moon, 1996; Nusse, 1997). In the absence of Wnt signals, most of the β -catenin/Armadillo protein is found complexed with cadherins at adherens junctions. This is in part due to the Zeste-white 3 kinase, which, in the absence of Wnt signals, promotes rapid degradation of cytoplasmic β -catenin/Armadillo (Peifer et al., 1994). Activation of the Wnt pathway may cause nuclear translocation of Armadillo by inhibiting Zeste-white 3 kinase activity (Orsulic and Peifer, 1996; Pai et al., 1997). In *C. elegans*, a conserved Wnt pathway is used for establishment of embryonic polarity (Rocheleau et al., 1997; Thorpe et al., 1997), although in this pathway the role of the Tcf homolog appears to be reversed, since in this context it functions to repress signal transduction in the absence of Wnt signaling (Rocheleau et al., 1997; reviewed by Han, 1997). *Wnt* and *frizzled* homologs are also required for the determination of epidermal cell polarity in *C. elegans*, although in this case the downstream effectors are unknown (Herman et al., 1995; Sawa et al., 1996).

Knowledge of how both intricate spatio-temporal and broad regional patterns of Hox gene expression are achieved is critical for understanding A/P patterning. Here we show that the *egl-20* gene, known to be required for the specific activation of *mab-5* in QL, encodes a Wnt signaling molecule. In

addition, we show that two additional Wnt pathway genes control *mab-5* expression in the V cells and Q cells. One of these, *bar-1*, encodes a β -catenin/armadillo-like protein (Eisenmann et al., 1998) that functions to transduce Wnt signals and that, like *egl-20*, is required for activating *mab-5* in QL. The second, *pry-1*, is a negative regulator of the Wnt pathway. The *Pry-1* phenotype can be suppressed by mutations in *bar-1*, but not by mutations in *egl-20*, suggesting that *pry-1* normally functions downstream of the EGL-20/Wnt signal as a negative regulator of BAR-1/Armadillo activity. Together our findings show that a conserved Wnt pathway functions to specifically activate Hox gene expression in the QL cell, thereby controlling the migration of its descendants. Furthermore, mutations in *pry-1* cause dramatic, widespread ectopic expression of other *C. elegans* Hox genes, indicating that *pry-1* acts as a general repressor of Hox expression. Thus, our findings also suggest that inhibition of Wnt signaling plays an important role in restricting Hox gene expression to regional domains.

MATERIALS AND METHODS

General methods and strains

Strains were maintained using standard methods (Brenner, 1974; Wood, 1988). Unless otherwise noted, strains were maintained and analyzed at 25°C. The mutant alleles used are either described by this paper, described by Wood (1988), or referenced below.

Mutations used were: LGI: *pry-1(mu38)*, *pry-1(nc1)* (S. Takagi, personal communication), *mig-1(e1787)* (Desai et al., 1988), *lin-17(n671)* (Ferguson and Horvitz, 1985), *unc-101(m1)*, *glp-4(bn2)* (Strome et al., 1994), *lev-10(x17)*. LGII: *mig-14(mu71)* (Harris et al., 1996). LGIII: *mab-5(e2088)*. LGIV: *egl-20(n585)*, *egl-20(mu27)*, *egl-20(mu25)*, *egl-20(mu39)* (Trent et al., 1983; Harris et al., 1996), *him-8(e1489)*, *lin-22(n372)*. LGV: *him-5(e1490)*, *mulS3[mab-5-lacZ + pRF4(rol-6d)]* (Cowing and Kenyon, 1992; Salser and Kenyon, 1992), *mulS13 [egl-5-lacZ + pRF4(rol-6d)]* (Wang et al., 1993). LGX: *bar-1(ga80)* (Eisenmann et al., 1998); *bar-1(mu63)*, *bar-1(mu349)*, *unc-6(n102)*, *dpy-7(e88)*. The strain RW7000 (Williams et al., 1992) was used for STS mapping.

Isolation of mutants

pry-1(mu38) was isolated in a screen for mutations affecting *mab-5-LacZ* expression. *mab-5(e2088)*; *mulS3* animals were mutagenized with 25–50 mM ethymethanesulfonate (EMS; Sigma) using standard procedures (Wood, 1988). F2 worms were picked clonally to individual wells of 12-well cluster plates filled with NG agar medium. Staged populations of F4s were collected as late L1s and then stained with X-gal, as described below.

pry-1(nc1) was identified in an EMS screen (S. Takagi, personal communication). *bar-1(mu63)* arose spontaneously in a *pry-1(mu38)*; *mulS3* background. It was noticed because unlike *pry-1(mu38)* single mutants, animals also carrying *mu63* were active and healthy. *bar-1(mu349)* was identified in an EMS screen for mutations affecting the migration of the QL and QR descendants (Mary Sym, QueenLim Ch'ng and C. K., unpublished).

Genetic mapping

pry-1

STS mapping (Williams et al., 1992) was used to map *pry-1(mu38)* to the right arm of chromosome I, closely linked to the markers Tcbn2 and hP4. 1/105 homozygous *mu38* F2s acquired both STS markers and 1/105 acquired just hP4. Additional STS mapping was done by crossing an *unc-101(m1) pry-1(mu38)* double mutant strain to

RW7000. 0/5 Pry non-Unc recombinants acquired the markers, whereas 7/11 Unc non-Pry worms acquired the markers, suggesting that *pry-1* maps to the right of Tcbn2 and hP4. Three-factor mapping placed it between *glp-4* and *lev-10*: from *glp-4(bn2) lev-10(x17)/pry-1(mu38)* heterozygotes, 5/9 Lev non-Glp recombinants segregated Pry progeny.

bar-1

STS mapping (Williams et al., 1992) was used to map *bar-1(mu63)* to the middle of chromosome X, between the markers Stp103 and Stp156. Three-factor mapping placed it between *unc-6* and *dpy-7*: from *unc-6(n102) dpy-7(e88)/bar-1(mu63)* heterozygotes, 4/8 Dpy non-Uncs segregated Bar progeny.

pry-1 recessiveness

Males carrying an integrated GFP marker construct were crossed to *pry-1(mu38)*; *him-5(e1490)* or *pry-1(nc1)*; *him-8(e1489)* hermaphrodites. All glowing F1s (the cross progeny) were found to have the QR descendants in a wild-type position and to have normal alae/ray patterns.

Complementation tests

pry-1

pry-1(mu38)/+; GFP/+; *him-5(e1490)* males were crossed to *nc1*; *him-8(e1489)* hermaphrodites. Approximately one half of the glowing progeny had mis-positioned QR descendants (posterior of the vulva), and about one half of the glowing male progeny had no alae but instead had ectopic rays.

bar-1

To show that *mu63* and *mu226* were alleles of *bar-1*, *him-5(e1490)*; *mu63* or *him-5(e1490)*; *mu226* males were crossed to *unc-30(e191)*; *bar-1(ga80)* hermaphrodites. From each cross, all non-Unc hermaphrodite progeny had mutant QL descendants (anterior of ALM). To show that *mu349* was an allele of *bar-1*, male GFP carrying worms were crossed to *bar-1(mu226)* hermaphrodites. The resulting GFP/+; *bar-1(mu226)/0* males were crossed to *mu349* hermaphrodites. 6/6 glowing hermaphrodite progeny from that cross were found to have QL descendants in a mutant (anterior of ALM) position.

Double mutant construction

pry-1(mu38) double mutants with *mig-1(e1787)*, *mig-14(mu71)* and *egl-20(n585)* were all made essentially the same way. *mig-1*, *mig-14* or *egl-20* mutant males were crossed to *pry-1* mutant hermaphrodites and non-Pry F1s were picked. The *mig-1*, *mig-14* or *egl-20* mutant alleles were rendered homozygous first, by picking F2s that had QL descendants in the anterior. Subsequently *pry-1(mu38)* was rendered homozygous by picking F3s with QR descendants in the posterior. The presence of *mig-1*, *mig-14* and *egl-20* in the strains was confirmed by observing their HSN migration defects (Desai et al., 1988; Harris et al., 1996).

The *pry-1(mu38)*; *bar-1(mu349)* double mutant was made by crossing *pry-1(mu38)/+* males to *bar-1(mu349)* hermaphrodites. F2 *pry-1(mu38)* progeny were picked on the basis of their mutant QR descendant positions. Subsequently, *bar-1(mu349)* homozygotes were obtained by picking animals with mispositioned QL descendants. A complementation test was done to confirm the presence of *pry-1(mu38)*.

Sequencing *bar-1* alleles

To isolate total RNA, approximately 100 μ l of mixed stage *bar-1(mu63)* or *bar-1(mu349)* worms were rinsed three times in distilled water (dH₂O) and then frozen in liquid N₂. For each tube, 1 ml Trizol reagent (Gibco BRL) was added to the worm pellets and then vortexed for 10 minutes. After a 5 minute 25°C incubation followed by a 5 minute spin at full speed in an Eppendorf microfuge, the bottom layer

was removed to a fresh tube and then vortexed with 200 μ l chloroform for 15 seconds. After another spin (as above), the upper, aqueous phase was removed to a fresh tube and mixed with 500 μ l isopropanol. After 10 minutes at room temperature RNA was collected by a 10 minute spin (as above). The pellet was washed with 75% ethanol in DEPC-treated dH₂O, spun again for 5 minutes at 7,500 revs/minute, allowed to air dry for 20 minutes and resuspended in 100 μ l DEPC-treated H₂O. First strand synthesis was done using SuperScript II (Gibco BRL) and a dT20 primer. PCR with the Pwo enzyme (Boehringer) was used to amplify the cDNA in three overlapping fragments: (1) 830 bp from primer SL1 (5'-GGTTTAATTACCCAAGTTTGAG-3') to primer SP3C (5'-GGAGTCACATGAGTAATCGC-3'); (2) 1,134 bp from primer SP5B (5'-CAGGATAATGAGGTTGTGC-3') to SP3B (5'-ATACAACCTTCAGCAGGAGAC-3'); and (3) 1,311 bp from SP5A (5'-CAAGGAGTTTATGTGTGGC-3') to SP3A (5'-CCAATTCATGAACCCG-3'). Overhanging A residues were added using Taq polymerase (Perkin Elmer) and the fragments were cloned into pGEM-T (Promega) or pCR2.1 (Invitrogen). DNA was purified using a SNAP mini-prep kit (Invitrogen) and sequenced on an ABI sequencer by the UCSF Biomolecular Resource Center.

Cloning of *egl-20*

The *egl-20* gene was previously localized to the gene cluster on Linkage Group IV between the cloned genes *fem-3* and *mec-3* (Trent et al., 1983; Harris et al., 1996). Using restriction-fragment-length polymorphisms in the region between *fem-3* and *mec-3*, we mapped *egl-20* to an interval covered by the cosmids from C28D4 to ZZH8. We generated transgenic lines of these candidate cosmids by germline transformation (Mello et al., 1991) and scored the lines for their ability to rescue both the QL migration and egg-laying defects caused by the *egl-20(n585)* mutation. A single cosmid located in this interval, W07H7, rescued the *egl-20(n585)* mutant phenotypes. Subsequently, the cosmid W07H7 was found to be identical by restriction digestion pattern to the cosmid W08D2, which had been sequenced by the *C. elegans* Genome Sequencing Project. One of the predicted open reading frames (ORFs) on the cosmid W08D2 (W08D2.1) was homologous to the Wnt family of signaling molecules. A 6.3 kb HindIII fragment of the cosmid W08D2 (pJW12), containing the entire predicted *Wnt* gene, was subcloned into the LITMUS38 vector (New England Biolabs). Transgenic lines carrying pJW12 in an *egl-20(n585)* background exhibited wild-type egg-laying and QL migration. We subsequently obtained the *egl-20* cDNA by RT-PCR from mixed-stage N2 RNA. We first synthesized cDNA templates using an oligo(dT) primer, Q_T (Frohman, 1993). We then amplified the *egl-20* cDNA by using *egl-20* specific primers. We obtained a single 1.2 kb product, which was subcloned into the pCRII vector (Invitrogen) and sequenced.

Sequencing *egl-20* alleles

We PCR-amplified two regions of the predicted *Wnt* gene from four *egl-20* mutant alleles: *n585*, *mu27*, *mu25* and *mu39*. Exons 1-4 were amplified with JW1: 5'-CTTAACCAGGCAAATCGGAA-3' and JW5: 5'-CACACATAAGACAACACCTG-3'; exons 5-10 were amplified with JW3: 5'-CGTGTCTGTTATGAAATACGC-3' and JW4: 5'-TCTGTTTTGCTAGGTCCCG-3'. These amplified regions included the entire coding region and all intron/exon boundaries of the predicted *Wnt* gene. Fragments were cloned and sequenced, as described above, from two independent PCR reactions. *egl-20(mu27)* was isolated in a non-complementation screen using *egl-20(n585)*, as previously reported in Harris et al. (1996). Sequence of the *Wnt* gene from this allele revealed that the molecular lesion is identical to that of the *egl-20(n585)* allele. Thus this 'allele' was most likely a re-isolation of the original *egl-20(n585)* mutation.

β -galactosidase detection and immunostaining

For detection of β -galactosidase, a PAP pen (Research Products International) was used to draw hydrophobic lines on poly(lysine)-

coated slides, creating a single well (12 wells were created on each slide for mutant screens). 1.5 μ l of 25% glutaraldehyde (Sigma) was added to each well, followed by 15 μ l of worms that had been rinsed several times in dH₂O + 0.01% Triton X-100 (Sigma). After 3 minutes of fixation an 18 mm² coverslip was placed over the worms, excess liquid was removed by aspiration, and the slides were frozen on dry ice. After 5 or more minutes, coverslips were prised off with a razor blade and the frozen slides were dipped in room temperature acetone (Sigma) for 1 minute. Slides were air dried and then stained overnight using a variation of the β -galactosidase stain solution described by Fire et al. (1990), with 10 mM MgCl₂.

Anti-MAB-5 staining was done as described by Salser and Kenyon (1996).

Anti-LIN-39 staining and staining using the monoclonal antibody MH27 was done as described by Maloof and Kenyon (1998).

In all cases the nuclear stain DAPI was also included to assist in cellular identification.

RESULTS

pry-1 is required to keep *mab-5* off in QR

In the wild type, the QL cell migrates a short distance toward the posterior and then switches on expression of the Hox gene *mab-5*. QL's bilateral homolog QR migrates a short distance

anteriorly and does not switch on *mab-5*. How is *mab-5* switched on in the migrating QL neuroblast? Previously we found that four genes, *mig-14*, *egl-20*, *lin-17* and *mig-1* are required for this process (Harris et al., 1996). In animals carrying mutations in these genes, QL migrates normally towards the posterior but does not turn on *mab-5*. As a consequence, the descendants of QL migrate toward the anterior. To look for additional genes that might participate in this process, we decided to screen for the opposite phenotype in hopes of finding genes required to keep *mab-5* off in QR. To do this, we mutagenized a strain carrying a *mab-5-lacZ* reporter construct and then looked for changes in *lacZ* expression. One mutation, *mu38*, caused ectopic expression of the *mab-5* reporter in QR and its descendants. We subsequently used anti-MAB-5 antibodies to confirm that the endogenous *mab-5* gene was expressed ectopically (Fig. 1). *mu38* defines a new gene, *pry-1* (polyray); a second, slightly weaker allele, *nc1*, has recently been isolated in an independent screen (S. Takagi, personal communication). This allele exhibits the same spectrum of phenotypes observed in the *pry-1(mu38)* allele.

As expected from the ectopic *mab-5* expression, *pry-1(mu38)* causes the descendants of QR to stay in the posterior, just like the wild-type QL descendants (Table 1). To confirm that the posterior migration of the QR descendants was due to

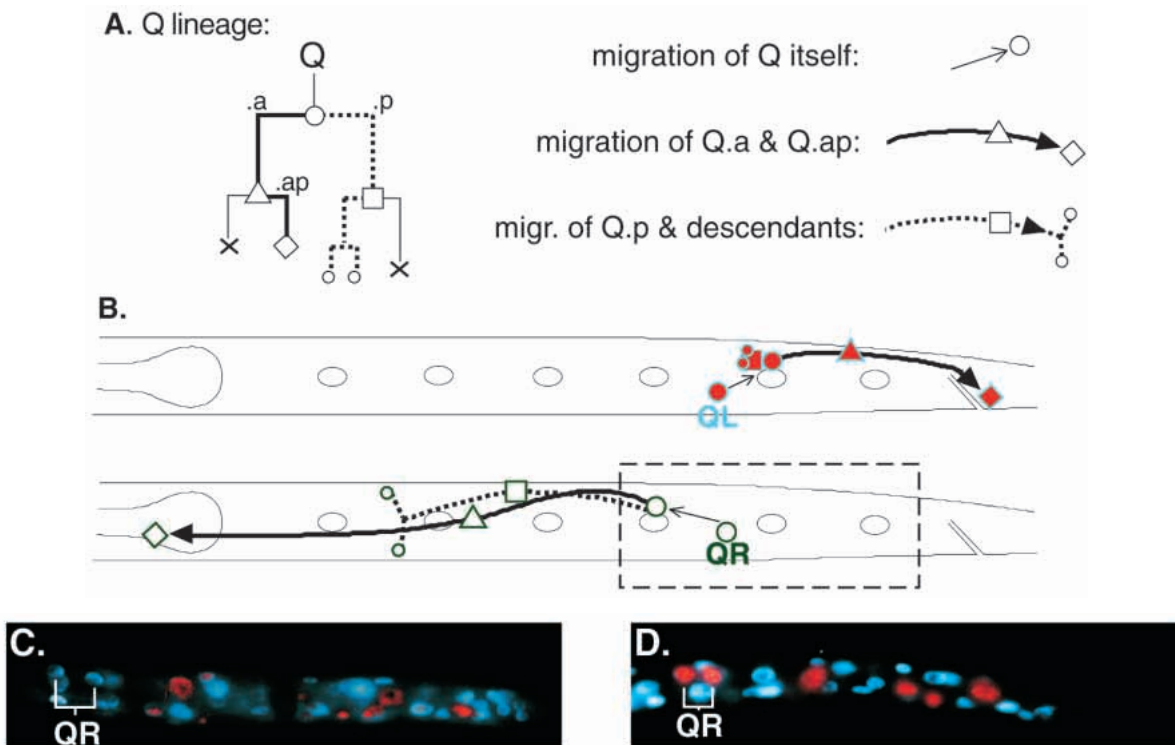


Fig. 1. *pry-1(mu38)* causes ectopic *mab-5* expression in QR. Anterior is to the left and ventral is down (in this and all subsequent figures). (A) Lineage diagram for the Q cells. QL and QR are bilateral homologs that migrate in opposite directions but have identical division patterns. X represents a cell death. (B) Schematic diagram showing the wild-type migrations of QL (blue; shown in top worm) and QR (green; shown in bottom worm). Symbols and migration lines match those in A. QL migrates towards the posterior and QR towards the anterior. *mab-5* (red) is switched on in QL shortly before it divides, but is not expressed in QR. After division the descendants of QL continue to express *mab-5* and stay in the posterior, undergoing additional rounds of division. The QR descendants continue to the anterior, also undergoing additional divisions. The dashed box indicates the approximate region shown in C and D. (C,D) False-color, composite images of animals stained with anti-MAB-5 antibodies (red) and DAPI (blue). Animals are approximately 3-4 hours old and are shown just after the first QR division. (C) Wild-type L1, showing that *mab-5* is not expressed in QR.a or QR.p. (D) *pry-1(mu38)* L1. Ectopic *mab-5* expression can be seen in QR.a and QR.p.

Table 1. *mab-5* expression in Q and final position of the Q descendants

Genotype	% QL descendants in posterior	% QR descendants in posterior	% QL expressing <i>mab-5</i>	% QR expressing <i>mab-5</i>
Wild type	100	0	100	0
<i>pry-1(mu38)</i>	100	81	100	80
<i>pry-1(nc1)</i>	100	40	n.d.	n.d.
<i>mab-5(e2088)</i>	0	0		
<i>pry-1(mu38); mab-5(e2088)</i>	0	0		
<i>bar-1(mu349)</i>	0	0	0	0
<i>pry-1(mu38); bar-1(mu349)</i>	0	0	0	0
<i>mig-14(mu71)</i>	0	0	0	0
<i>pry-1(mu38); mig-14(mu71)</i>	100	100	n.d.	n.d.
<i>egl-20(n585)</i>	0	0	0	0
<i>pry-1(mu38); egl-20(n585)</i>	88	71	63	70
<i>mig-1(e1787)</i>	15	0	28	0
<i>mig-1(e1787); pry-1(mu38)</i>	100	82	100	80

mig-14, *egl-20* and *mig-1* single mutant data from Harris et al. (1996).

20 or more animals were scored for *mab-5* expression except for the *pry-1*; *egl-20* double, for which ten animals were scored. 25 or more animals were scored for Q descendant final position.

For *mab-5* expression, animals were 3-5 hours old. QL and QR were not scored for *mab-5* expression unless they had migrated over V5 or V4, respectively. This phase of the migration, which is independent of *mab-5* and is not affected in these mutants, causes the QR descendants to be anteriorly displaced relative to the QL descendants. For this reason, the QR descendants were scored as being in the posterior when they were adjacent to or posterior to V3.p, whereas the QL descendants were scored as being in the posterior when they were posterior of V4.p.

n.d., not determined.

mab-5 activity, we made a *pry-1(mu38); mab-5(e2088lf)* double mutant and found that in this double mutant the Q cells migrated into the anterior (Table 1). Both alleles of *pry-1* were found to be recessive (see Materials and methods), suggesting that the mutations reduce gene activity and that wild-type *pry-1* activity functions to keep *mab-5* expression off in QR. In contrast to the large number of mutations that affect *mab-5* expression in QL, few mutations affecting QR have been described. Only one mutation has previously been described that, without affecting QR's anterior migration, causes ectopic activation of *mab-5* in QR: this is a gain-of-function promoter mutation in the *mab-5* gene itself (Hedgecock et al., 1987; Salser and Kenyon, 1992). Thus *pry-1* defines a new class of genes involved in regulating *mab-5* expression in the Q cells: genes that are required in wild-type animals to repress *mab-5* in QR.

***pry-1* functions downstream of *mig-14*, *egl-20* and *mig-1* in QL**

In *mig-14*, *egl-20*, *lin-17* and *mig-1* mutants, *mab-5* expression is not initiated in QL and as a result the descendants of QL migrate into the anterior (Harris et al., 1996). Since *pry-1(mu38)* causes ectopic *mab-5* expression in QR, we wondered if it would also allow *mab-5* to be expressed in QL in *mig-14*, *egl-20* or *mig-1* mutant animals. Therefore we constructed strains that were mutant for *pry-1* and either *mig-14*, *egl-20* or *mig-1* (we were unable to construct *lin-17 pry-1* double mutants because the double mutant was too unhealthy). We

found that in all three double mutants, *mab-5* expression was activated in both QL and QR, and subsequently descendants of both both QL and QR adopted a posterior-specific pattern of migration similar to that of cells in the wild-type QL lineage (Table 1). This demonstrates that *pry-1(mu38)* is epistatic to mutations in all three of these genes, and suggests that *pry-1* acts downstream of *mig-14*, *egl-20* and *mig-1* to negatively regulate *mab-5* expression. In other words, these findings imply that in wild-type animals, *mig-14*, *egl-20* and *mig-1* activate *mab-5* expression in QL by relieving *pry-1*-mediated repression of *mab-5*.

The *mu63* mutation suppresses the *Pry-1* phenotype

In addition to causing ectopic *mab-5* expression, the *pry-1(mu38)* mutation also causes worms to be unhealthy: *pry-1(mu38)* animals are scrawny, often herniated, and uncoordinated. The *mu63* mutation was discovered when healthy, active animals spontaneously appeared in a population of *pry-1(mu38)* animals, suggesting that *pry-1(mu38)* was being suppressed by a second mutation. To test whether or not suppression was due to an extragenic mutation, we outcrossed the suppressed strain to wild type. Approximately 1/4 of the F2 progeny from the outcross showed a *pry-1* phenotype, demonstrating that the suppressor was not linked to *pry-1* and therefore was extragenic.

***mu63* is an allele of *bar-1*, a β -catenin/armadillo related gene**

We mapped *mu63* to a small region of the X chromosome between the cloned genes *unc-6* and *dpy-7*. Interestingly, the gene *bar-1*, which encodes a β -catenin/Armadillo-like protein, mapped to the same interval (Eisenmann et al., 1998). Mutations in *bar-1* previously had been identified on the basis of a defect in vulval development suggestive of Hox gene misregulation. For this reason we hypothesized that *mu63* might be an allele of *bar-1*. Indeed, we found that *mu63* failed to complement *bar-1(ga80)*, supporting the idea that *mu63* is an allele of *bar-1*. To confirm this, we sequenced *bar-1* cDNA from *mu63* and found a missense mutation in the first *arm* repeat, which changed the conserved Leu-130 residue to Phe. Subsequently, a number of additional alleles of *bar-1* were identified in a screen for mutations affecting Q cell migration (Mary Sym, Queelim Ch'ng and C. K., unpublished). We found that one of these new alleles, *bar-1(mu349)*, causes Gln-147 (also in the first *arm* repeat) to be changed to a stop codon. *mu349* is therefore likely to be a strong loss-of-function or null allele.

To determine whether the ability of *bar-1* mutations to suppress *pry-1* was specific to the *mu63* allele, we constructed a *pry-1(mu38); bar-1(mu349)* double mutant strain. We found that this putative null allele of *bar-1* was able to suppress *pry-1(mu38)*, confirming that wild-type *bar-1* is necessary for the manifestation of the *Pry-1* mutant phenotype.

***bar-1* is required for ectopic *mab-5* expression in *pry-1(mu38)* mutants**

The BAR-1-like protein Armadillo is known to act as a transcriptional co-activator in *Drosophila* (van de Wetering et al., 1997), suggesting two possible ways that *bar-1* mutations might act to suppress *pry-1(mu38)*. First, wild-type *bar-1* could function as an activator to promote *mab-5* expression. In this

case, mutations in *bar-1* would suppress *pry-1(mu38)* by preventing ectopic *mab-5* expression. Alternatively, *bar-1* might act as a co-activator with *mab-5* to initiate expression of downstream genes. In this case, mutations in *bar-1* would not affect *mab-5* expression. To distinguish between these possibilities, we examined expression of *mab-5* in the Q cells of *pry-1(mu38)*; *bar-1(mu63)* and *pry-1(mu38)*; *bar-1(mu349)* strains. We found that *mab-5* was no longer ectopically expressed in either strain (Table 1 and data not shown). Therefore, we infer that *bar-1* mutations suppress the *Pry-1* phenotype by preventing ectopic *mab-5* expression.

***bar-1* is required to activate *mab-5* in QL**

Since *bar-1* is required for *mab-5* expression in *pry-1(mu38)* mutants, we wondered if it was also required for *mab-5* expression in QL in otherwise wild-type animals. We found that in *bar-1(mu63)* mutants, *mab-5* was expressed only weakly in QL, and that in the stronger *bar-1(mu349)* allele, *mab-5* was not expressed in QL at all (Table 1 and data not shown). In addition, mutations in *bar-1* caused the descendants of QL to migrate into the anterior (Table 1). Thus, *bar-1* functions like *mig-14*, *egl-20*, *lin-17* and *mig-1*, to activate *mab-5* expression in QL. However, unlike these genes, *bar-1* acts downstream of *pry-1*, since *bar-1* is still required for *mab-5* expression in the absence of wild-type *pry-1* function.

***egl-20* encodes a Wnt family member**

To further investigate how *mab-5* is switched on in QL, we cloned the upstream activator *egl-20* by positional mapping and transformation rescue (see Materials and methods). A single cosmid, W07H7, was found to rescue the *egl-20(n585)* egg-laying and QL descendant migration defects. This cosmid was located in a sequenced region of the genome, and was found to contain an open reading frame (ORF) predicted to encode a Wnt family member. We found that a 6.3 kb fragment containing this ORF (as the only complete ORF) also rescued *egl-20(n585)*. To confirm that *egl-20* encoded this Wnt family member, we sequenced the entire coding region and all intron/exon boundaries of the predicted *Wnt* gene from three mutant alleles of *egl-20*: *egl-20(n585)*, *egl-20(mu25)* and *egl-20(mu39)*. We identified mutations in highly conserved cysteine residues of the putative Wnt protein in all three alleles, indicating that the *egl-20* gene does encode this Wnt protein. In addition, we have recently found *egl-20* mutants with similar phenotypes that contain early stop and splice junction mutations (data not shown), suggesting that these mutations might eliminate gene activity.

Comparison of the predicted EGL-20 protein sequence with other members of the Wnt family (Fig. 2) shows that EGL-20 shares the greatest identity with mouse Wnt-7B (47% amino acid identity) and mouse Wnt-7A (35% amino acid identity). As in other Wnt proteins, there is a hydrophobic region at the N terminus that could serve as a signal sequence (von Heijne, 1986). In addition, the predicted EGL-20 protein has three potential sites for N-linked glycosylation and contains 22 conserved cysteine residues.

Previous work from our laboratory showed that *egl-20* and the *lin-17/frizzled* genes are required to activate *mab-5* expression in QL (Harris et al., 1996). As described above, in this study we have found that the β -catenin/armadillo-like gene *bar-1* is also required to turn on *mab-5* in QL and that

Table 2. Changes in V cell fate specification

Genotype	Alae/ray fates		% with postdeirid
	Extent of V cell-derived Alae	Average no. of anterior V cell-derived rays	
Wild type	V1-V5	0	100
<i>pry-1(mu38)</i>	None	22.2	25
<i>mab-5(e2088)</i>	V1-V6	0	100
<i>pry-1(mu38)</i> ; <i>mab-5(e2088)</i>	Weak V1-V6	0.6	100
<i>pry-1(mu38)</i> ; <i>bar-1(mu349)</i>	V1-V5	0	100

Ten or more animals were scored for each genotype. All strains included *him-5(e1490)* to increase the frequency of males.

Rays from anterior V cells were assayed in *pry-1(mu38)* by using the monoclonal antibody MH27 to outline the ray socket cell, in combination with DAPI staining to identify ray cell group nuclei (Salser and Kenyon, 1996). Only ray cell groups anterior to the anus/intestine junction were scored, allowing identification of V1-V4 derived, and some V5 derived, rays.

For all other genotypes, anterior rays were scored by looking for papillae (ray tips) associated with gaps in alae.

egl-20 encodes a Wnt molecule. Together these findings indicate that a Wnt signaling pathway, with components conserved from the Wnt signal to an Armadillo-related transactivator, functions to control the direction of Q cell migration by regulating *mab-5* expression. These results also strongly suggest that *bar-1* functions to transduce the Wnt signal, possibly in a manner analogous to its *Drosophila* homolog *armadillo*.

***pry-1* is required for proper V cell patterning**

Effects of the *egl-20* mutations are limited primarily to the Q cells and the cells in their immediate vicinity (Harris et al., 1996). In contrast, we found that *pry-1* affects patterning throughout the animal. For example, *pry-1* (*polyray*) is named for the most dramatic transformation caused by *pry-1* mutations, the formation of ectopic male sensory rays all along the A/P body axis. In wild-type males, *mab-5* expression is restricted to the descendants of V5 and V6, and specifies that they generate sensory rays. The descendants of V1-V4 do not express *mab-5* and, as a result, produce ridged cuticular structures known as alae (Fig. 3A,B). Strikingly, we found that in *pry-1* mutants none of the V cells made alae; instead, all the V cells generated ray-like structures and cell types (Fig. 3C-E; Table 2). These ectopic rays were dependent on wild-type *mab-5* activity; in *pry-1(mu38)*; *mab-5(e2088)* double mutants, almost all of the V cells gave rise to cells that made alae (Table 2).

pry-1(mu38) also affects a second V cell patterning decision: the formation of the postdeirid. The postdeirid is a sensory structure normally made by the descendants of V5.pa; however, ectopic *mab-5* expression in V5.pa prevents postdeirid formation (Salser and Kenyon, 1996). We examined *pry-1* mutants and found that they failed to make a postdeirid (Table 2). This defect also depends on *mab-5* activity, since it is corrected by *mab-5(-)* mutations (Table 2).

The dependence of the postdeirid and ray phenotypes on wild-type *mab-5* activity strongly suggested that *pry-1(mu38)* was causing ectopic *mab-5* expression in the V cells. To determine if this was the case, we examined *mab-5* expression in mutant *pry-1* animals. We found that *mab-5* was expressed

ectopically in anterior V cell lineages (Fig. 4A-D). In addition, we observed that *pry-1(mu38)* causes *mab-5* expression to begin too early in the V5 lineage (see below), starting in the V5.p cell instead of V5.pp. It is likely that this early *mab-5* expression causes the loss of the postdeirid. Thus, in wild-type

worms, *pry-1* is required to restrict *mab-5* expression spatially to the posterior V cells and temporally to a later portion of the V5 lineage so that the postdeirid and alae/ray fates can be specified correctly.

In addition to observing ectopic *mab-5* expression in the V

A.

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MQFFICLIIFLVLLVYGQYSPPSATYSTQQVPYNWLTlafVGSNDYLPRPHYHSTDRHFKELCRR
LDGLNPNQOALCAENPFSIPFVARGVREAIREFENKFKFERWNGSSRDEVTTETRHGKFQDILGKTL
RSANKEAAFLNAIMAAISIVHSITKCGNTGNLTFCGCDSKPGMQRVQAESDPSMSRDQFSWGGCSDN
VPHGIRYAKKFLDDWETAQFDKTKNVAHLVRRHNNFVGREAIQNIIRRCRCHGVSGSGEFKTCWLV
QMQKFSQVSDLLKKRYDHFVAVQVTRKATKRLRRKERTERKIPLRGNEMAYVHRSPSYCEKNLITAGI
LGTSGRECIHNSYSSESGLLCCGRGYNTRLEIRQTQCECKFVWCCVCKTCTEEVAVHTCK
    
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Fig. 2. *egl-20* encodes a Wnt homolog. (A) The predicted EGL-20 protein based on the 1225 bp *egl-20* cDNA sequence is shown above. Molecular lesions in three alleles of *egl-20* are indicated. The strong reduction-of-function allele, *n585*, was associated with a T-to-A transversion at nucleotide 295, changing a highly conserved cysteine at position 99 to a serine. The less severe allele, *mu25*, was associated with a G-to-A transition at nucleotide 329, changing a highly conserved cysteine at position 110 to tyrosine. Similarly, the hypomorphic allele, *mu39*, was associated with a G-to-A transition at nucleotide 497, which changed a conserved cysteine at position 166 to a tyrosine. The potential signal sequence (von Heinje, 1986) is underlined with a solid line, and three potential N-linked glycosylation sites are underlined with dotted lines. Conserved cysteines are highlighted. (B) Alignment of the EGL-20 protein sequence with mouse Wnt7a and Wnt7b (Gavin et al., 1990), *Drosophila* Wg (Rijsekijk et al., 1987) and *C. elegans* LIN-44 (Herman et al., 1995). EGL-20 shares closest homology to the mouse Wnt7b and Wnt7a. Identical and similar residues are indicated by black and gray boxes, respectively. Highly conserved cysteines are indicated by asterisks. The GenBank accession number for the *egl-20* gene is AF103732.

B.

EGL-20	1	<u>M</u> QFFICLIIFLVLLVYG--QYSPPSATYSTQQVPYNWLTlafVGSNDYLPRPHYHSTDRHFKELCRR
WNT-7B	1	MHRNFRKWFYVFLCFG--VLVYKLCALSS-----VVALGAN-----
WNT-7A	1	MTRKARRCLGHFLSLG--IVYLRIGGFSS-----VVALGAS-----
Wg	1	MDISYIFVLCMLALCSGGSSLSQVLEKQKSGRGRGSMWVGIVAGPEP-----
LIN-44	1	MRRAAPDFDFFQSTALSTFFLILCSLATNEIPTISGAPAGKIVQPPK-----
EGL-20	58	<u>E</u> HFKE <u>L</u> CRRLDGLNPF-----N--QOALCAENPFSIPFVARGVREAIREFENKFKFERWNGSSRDEVTTETRHGKFQDILGKTL
WNT-7B	36	-----IICNKIPGLAP-----R--QRAICQSRPDALIVIGEQAQMGIDRECEQHQFRFRGRWN
WNT-7A	36	-----IICNKIPGLAP-----R--QRAICQSRPDALIVIGEQAQMGIDRECEQHQFRFRGRWN
Wg	48	-----NNITFIMYMDPAIHSITLRRKQRRLVLRDNPGLVGLALVKGANLAIASECQHOFRRNRWN
LIN-44	47	-----NHLKQGCPSDLLHSRALR--SIQLACRTHPATVLSAFEGVQEGELQNCANRRLRFQQWD
EGL-20	110	<u>C</u> SSRDEVTTETRHGKFQDILGKTLRSANKEAAFLNAIMAAISIVHSITKCGNTGNLTFCGCDSKPGMQRVQAESDPSMSRDQFSWGGCSDN
WNT-7B	84	CSALGE-----KTVFGQELRVGSRREAAFTYAITAAGVAHAVTAAACSOGNLSNCGCD
WNT-7A	84	CSALGE-----RTVFGKELRVGSRREAAFTYAITAAGVAHAVTAAACSOGNLSNCGCD
Wg	104	CSTRNFS-----RGKNLFGKLVDRGCFRETSFIYAITSAAVTHSITARACSEGTIESCTCD
LIN-44	102	CSEAGN-----IMHDPPLLRQCFRESSLIWALSASAAGVAVATACAQGWIDDCACN
EGL-20	170	-SKPG--MQRVQAESDPSMSRDQFSWGGCSDNVPHGIRYAKKFLDDWETAQFDKTKNVAHLVRRHNNFVGREAIQNIIRRCRCHGVSGSGEFKTCWLV
WNT-7B	135	REKQG--YYNQAE-----WKWGGCSADVRYGIDFSRRFVDARE-----IKKNARR
WNT-7A	135	KEKQG--QYHWDE-----WKWGGCSADVRYGIDFSRRFVDARE-----IKKNARR
Wg	158	YSHQSRSPQANHQAGSVAGVRD--WEWGGCSNDIIGFKEFSREEVDTGE-----RGNLRRE
LIN-44	153	-NQMG-----QNE-----YFEGGCTHGVIHQGITASRKLTLTKVG-----AVNTLRE
EGL-20	227	<u>L</u> VRRHNNFVGREAIQNIIRRCRCHGVSGSGEFKTCWLVQMQKFSQVSDLLKKRYDHFVAVQVTRKATKRLRRKERTERKIPLRGNEMAYVHRSPSYCEKNLITAGI
WNT-7B	179	LMNLHNNFVGREAIQNIIRRCRCHGVSGSGCTTKTCWTLTPKFRFVGLHLLKKEKYN-AVQ
WNT-7A	179	LMNLHNNFVGREAIQNIIRRCRCHGVSGSGCTTKTCWTLTPKFRFVGLHLLKKEKYN-AVQ
Wg	212	KMNLHNNFVGREAIQNIIRRCRCHGVSGSGCTTKTCWTLTPKFRFVGLHLLKKEKYN-AVQ
LIN-44	192	KVEKHNLRKAGRLAIKKTLLISSCKCHGVSGSQQKTCWKRRTATLEHITDYLVEKYA-----
EGL-20	287	<u>V</u> EL--EDVT-----
WNT-7B	238	VEV--VRAS-----
WNT-7A	238	VEP--VRAS-----
Wg	271	VQVTNSLRAFNALAPVSPNAAGSNVGSNGLIIPQSGLVYGEERMLNDHMPDILLENS
LIN-44	247	-----
EGL-20	294	-----R--KATKR-----LRRKERTERKIPLRGNEMAYVHRSPSYCEKNLITAGI
WNT-7B	245	-----RL-RQP-----TFLRIKQLRSYQKPMETDLDLV
WNT-7A	245	-----RN-KRP-----TFLKIKKPLSYRKPMDTDLVY
Wg	331	HPI SKIHPNMPSPNSLPQAGQRGGRNRRRQGRKHNRHYFQLNPHNPEHKPPGSKDLVY
LIN-44	247	-----RAKLYTDDSVVKT--TDLTY
EGL-20	320	VHRSPSYCEKNLITAGILGTSGRFCIH--NSYSSSESGLLCCGRGYNTRLEIRQTQCECKF
WNT-7B	271	IEKSPNYCEDAATGSVGTQGRLCNR--TSPGADGCDTMCGRGYNTHQYTKVWQCNCKF
WNT-7A	271	IELSPNYCEDPVTGSVGTQGRACNK--TAPQASGCDLMCCGRGYNTHQYTKVWQCNCKF
Wg	390	LEPSPSFCENLRQGLVGTGHRQCMNE--TSLGVDGCGELMCCGRGYRDEVVVVERCACTF
LIN-44	265	LEASPDPVCKAK--SV--AGRVCAWRNETHTQGDGCDRLCCGNFESIRHEVVRVKCDCEP
EGL-20	378	<u>V</u> WCCEVCKCTCTEEVAVHTCK
WNT-7B	329	HWCCFVKCNCTSERTEVFTCK
WNT-7A	329	HWCCYVKCNCTSERTEMYTCK
Wg	448	HWCEVCKCTCCTKRTKKVIYTCV
LIN-44	319	VWCENLVCKDCTQHRWISTCNGTTPPKSLIF

cell lineages, we also saw ectopic *mab-5* expression in the anterior ventral cord neurons (Fig. 4E,F). This indicates that wild-type *pry-1* is important for limiting *mab-5* expression in other cell types.

As mentioned above, mutations in *bar-1* could suppress the ectopic *mab-5* expression in QR caused by *pry-1(mu38)*. We

found that *bar-1(mu63)* and *bar-1(mu349)* also suppressed the ectopic expression of *mab-5* in the V cells and ventral cord caused by *pry-1(mu38)* (Fig. 4G and data not shown). Consistent with this observation, the postdeirid and ray phenotypes of *pry-1* were fully suppressed in *pry-1; bar-1* double mutants (Table 2).

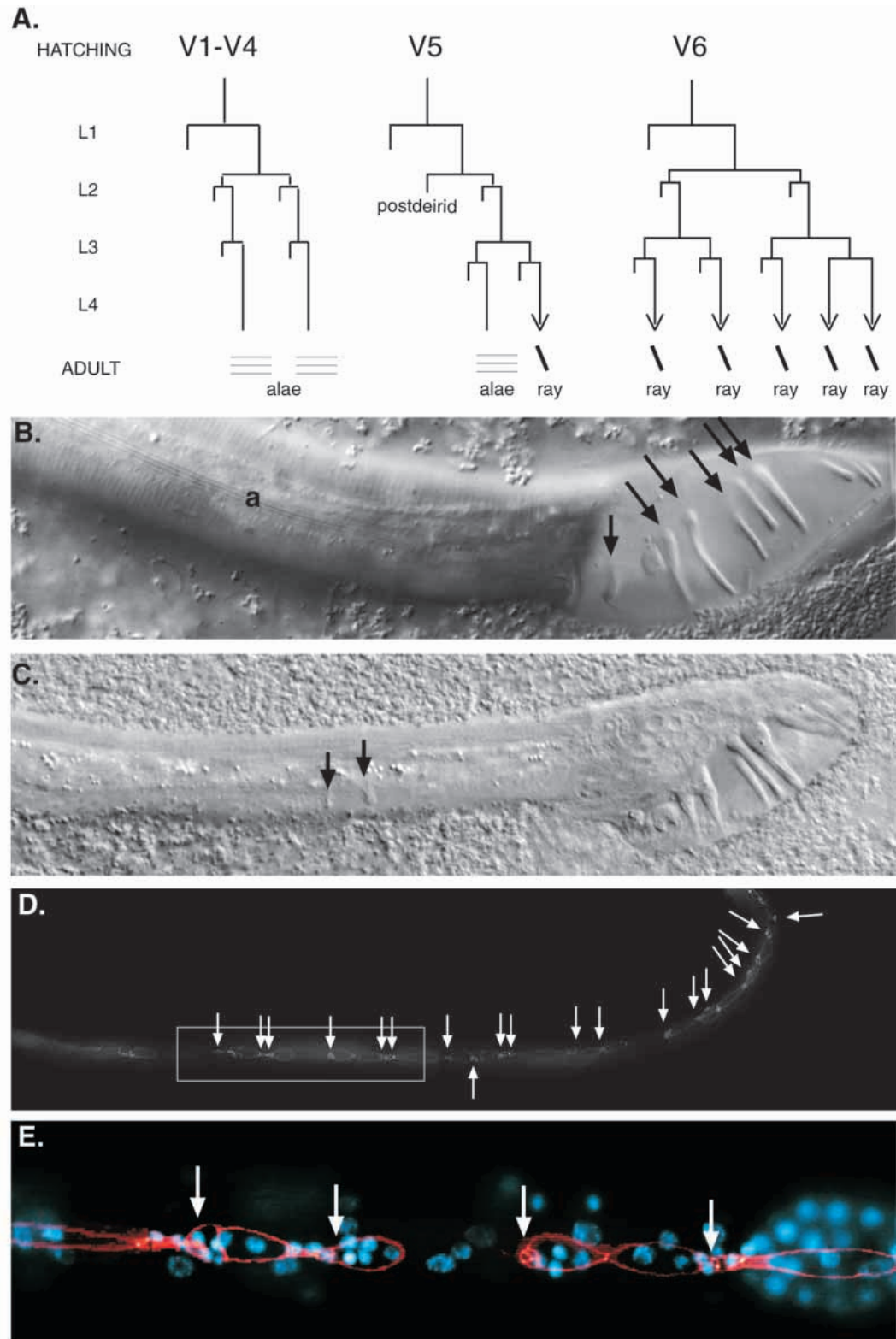


Fig. 3. The *pry-1(mu38)* mutation causes ectopic rays to be made. (A) Lineage diagram for the V cells in males. Anterior cells do not express *mab-5* and generate alae. V5 has a hybrid fate; anterior cells in the V5 lineage generate a postdeirid and alae; they do not express *mab-5*. Posterior cells in the lineage express *mab-5* and generate a ray. All cells in the V6 lineage express *mab-5* and generate rays. (B) Wild-type male tail showing rays (arrows) and alae (a). Nomarski image. (C) *pry-1(mu38)* male tail. The alae have been replaced with ectopic rays (arrows). Rays in the body do not extend out from the cuticle, but can be identified by their papillae, or ray tips. (D) L4, *pry-1(mu38)* male, stained with MH27 monoclonal antibody. At this stage, each of the 3 cells that make up the ray cell group are outlined by the antibody (arrows). (E) Enlargement of the region boxed in (D). False color composite image showing MH27 (red) and DAPI (blue). The ray cell groups can be identified both by MH27 outlines and by the clustered small nuclei (arrows).

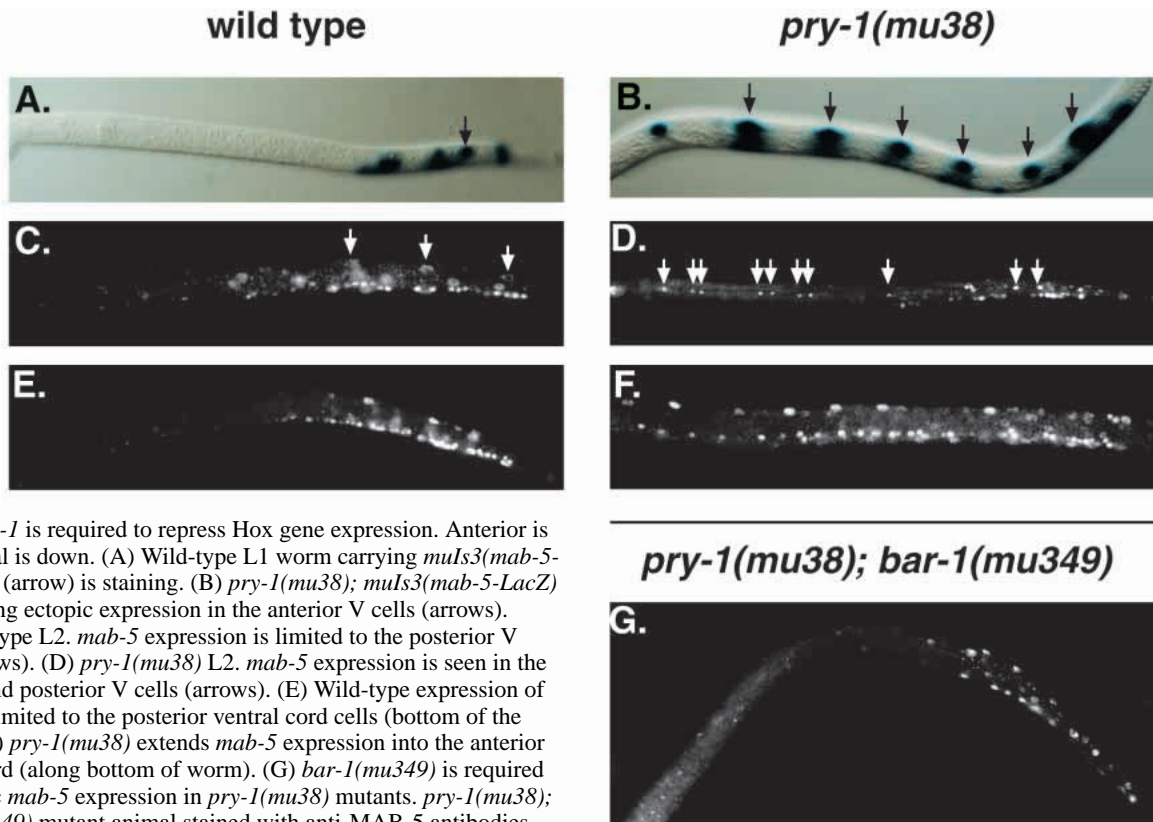


Fig. 4. *pry-1* is required to repress Hox gene expression. Anterior is left, ventral is down. (A) Wild-type L1 worm carrying *mulS3(mab-5-LacZ)*. V6 (arrow) is staining. (B) *pry-1(mu38); mulS3(mab-5-LacZ)* L1, showing ectopic expression in the anterior V cells (arrows). (C) Wild-type L2. *mab-5* expression is limited to the posterior V cells (arrows). (D) *pry-1(mu38)* L2. *mab-5* expression is seen in the anterior and posterior V cells (arrows). (E) Wild-type expression of *mab-5* is limited to the posterior ventral cord cells (bottom of the worm). (F) *pry-1(mu38)* extends *mab-5* expression into the anterior ventral cord (along bottom of worm). (G) *bar-1(mu349)* is required for ectopic *mab-5* expression in *pry-1(mu38)* mutants. *pry-1(mu38); bar-1(mu349)* mutant animal stained with anti-MAB-5 antibodies. Staining is limited to the posterior (compare to Fig. 4D,F).

Since the ectopic expression of *mab-5* seen in *pry-1* mutants required *bar-1* activity, we wondered whether *bar-1* played a role in wild-type V cell patterning. Surprisingly, when we examined expression of *mab-5* in *bar-1* single mutants, we found that *mab-5* expression was normal in the V cells. In addition, the V rays were made almost as frequently as in wild type: 64 out of 76 *bar-1(ga80); him-5(e1490)* males examined had all six V rays, as do wild type; 12 animals had five rays, one animal had four rays, and one had no rays. In comparison, 67 of 76 *him-5(e1490)* animals had all 6 rays, and nine had only 5 rays. This suggests that even though *bar-1* is downstream of *pry-1* in the V cells, either *bar-1* is not a major component of the machinery used to activate *mab-5* expression in wild-type V cells, or it is redundant with other activators of *mab-5*. Together these results suggest that although the downstream components of the Wnt signaling pathway are functional in the V cells to repress *mab-5* expression, Wnt signaling may not play a major role during wild-type development to activate *mab-5* expression in the V cell lineages.

We decided to investigate how *bar-1* interacts with another gene required to regulate *mab-5* expression in the V cells: *lin-22*. *lin-22* encodes a *hairy/enhancer of split* homolog that is required to specify the fates of the anterior V cells V1-V4 (Fixsen, 1985; Wrishnik and Kenyon, 1997). In *lin-22(-)* mutants, these cells adopt V5-like fates: each generally makes an ectopic postdeirid and two rays, and expresses *mab-5* late in development, in a pattern similar to that of wild-type V5 descendants. Since *bar-1(mu63)* can suppress the ectopic *mab-5* expression and the ectopic rays caused by *pry-1(mu38)*, we

wondered if it would also be able to suppress the *lin-22* mutant phenotype. We constructed *lin-22(n372); bar-1(mu63)* double mutants, and found that its ray and postdeirid phenotypes were indistinguishable from those of *lin-22(n372)* alone. This result demonstrates that *bar-1* mutations cannot suppress *lin-22* mutations, and, therefore that *bar-1* activity is not required for the *lin-22(-)* phenotype.

***pry-1* regulates expression of other Hox genes**

Because *pry-1* is important for limiting *mab-5* expression in a broad, general way – affecting the Q cells, V cells and ventral cord neurons – we wondered if it might also be involved in limiting expression of other Hox genes. To determine if this was the case, we examined the effect of *pry-1(mu38)* on the expression of the *Abd-B* homolog *egl-5* and the *Scr* homolog *lin-39*. *egl-5* is normally expressed in the tail of the worm where it specifies tail cell fates (Chisholm, 1991; Wang et al., 1993). We used an *egl-5-lacZ* fusion (Wang et al., 1993) to monitor *egl-5* expression, and found that *pry-1(mu38)* caused it to be expressed ectopically in anterior cells (Fig. 5A,B). Similarly, expression of *lin-39*, which normally specifies cell fates in the mid-body of the worm (Clark et al., 1993; Wang et al., 1993; Maloof and Kenyon, 1998), was expanded anteriorly (Fig. 5C,D). These results show that *pry-1* functions as a general repressor that restricts Hox gene expression to the correct domains.

To investigate whether or not *bar-1* was required in *pry-1(mu38)* animals for ectopic expression of Hox genes other than *mab-5*, we examined *lin-39* expression in the *pry-1(mu38); bar-1(mu349)* double mutant strain. We found that *bar-1(mu349)* was able to suppress the ectopic expression of

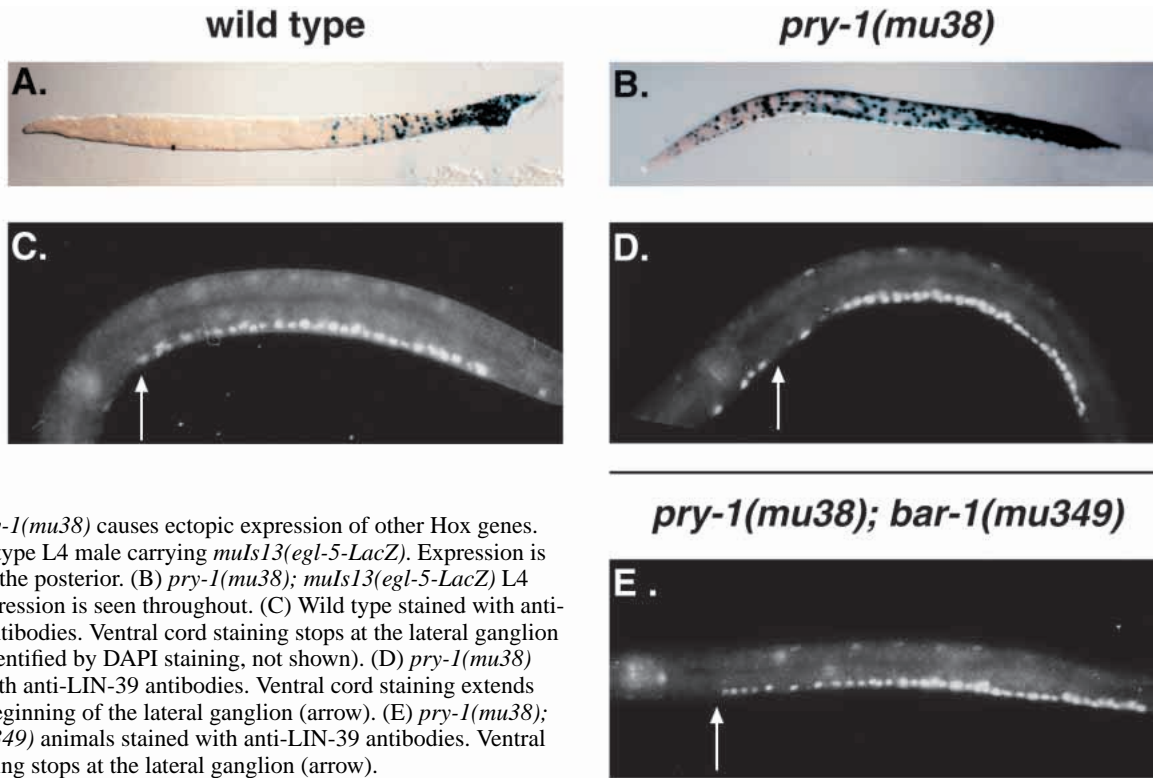


Fig. 5. *pry-1(mu38)* causes ectopic expression of other Hox genes. (A) Wild-type L4 male carrying *muIs13(egl-5-LacZ)*. Expression is limited to the posterior. (B) *pry-1(mu38); muIs13(egl-5-LacZ)* L4 male. Expression is seen throughout. (C) Wild type stained with anti-LIN-39 antibodies. Ventral cord staining stops at the lateral ganglion (arrow; identified by DAPI staining, not shown). (D) *pry-1(mu38)* stained with anti-LIN-39 antibodies. Ventral cord staining extends past the beginning of the lateral ganglion (arrow). (E) *pry-1(mu38); bar-1(mu349)* animals stained with anti-LIN-39 antibodies. Ventral cord staining stops at the lateral ganglion (arrow).

lin-39 normally caused by *pry-1(mu38)* (Fig. 5E). This result indicates that *bar-1* activity is required for expression of multiple Hox genes in *pry-1* mutants.

Initial spatial expression of the Hox genes is normal in *pry-1(mu38)*

During our studies we observed that young *pry-1(mu38)* worms show relatively normal patterns of *mab-5* and *egl-5* expression (Fig. 6A,B); ectopic expression does not begin until later in development. In addition, the spatial limits of *lin-39* expression are similar in wild type and in *pry-1(mu38)* mutants at hatching ($n=20$); apparently spatial misexpression of this gene also does

not begin until later. However, while the spatial limits of *lin-39* expression are correct at hatching, expression levels in anterior ventral cord neurons (within the normal domain of expression) are higher in *pry-1(mu38)* than in wild type (data not shown).

To further investigate the timing of ectopic *mab-5* expression in the V cells, we examined expression at 4 hour intervals, starting at hatching (Fig. 6C). We found that ectopic expression was first evident in the V5.p cell. Subsequently, ectopic *mab-5* expression appeared in most of the Vn.ppp cells. Thus it seems that in these cells, wild-type *pry-1* is not required to establish the initial anterior limits of expression, but rather is required to prevent ectopic expression of the Hox genes after

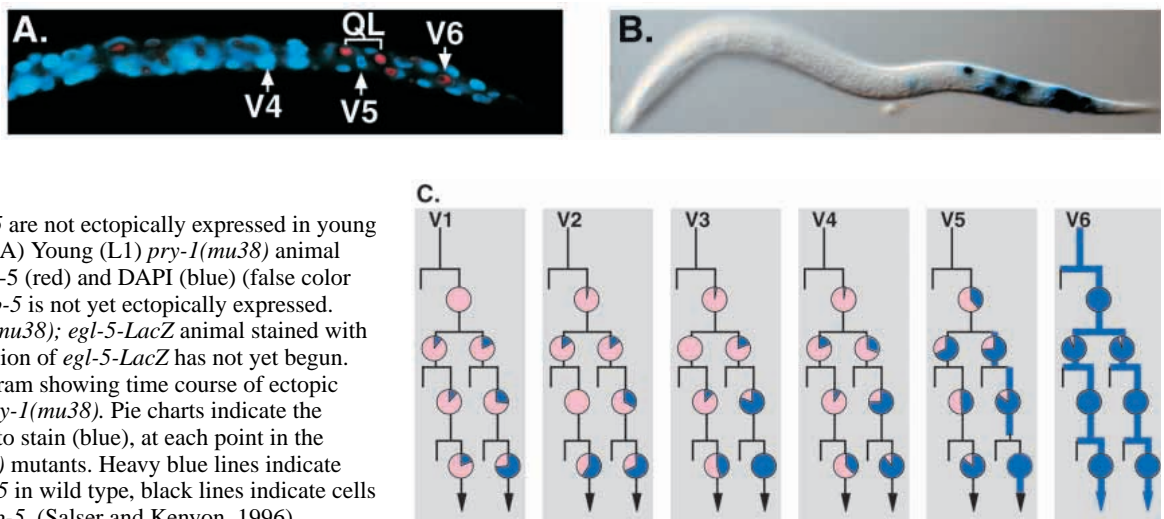


Fig. 6. *mab-5* and *egl-5* are not ectopically expressed in young *pry-1(mu38)* animals. (A) Young (L1) *pry-1(mu38)* animal stained with anti-MAB-5 (red) and DAPI (blue) (false color composite image). *mab-5* is not yet ectopically expressed. (B) Young (L1) *pry-1(mu38); egl-5-LacZ* animal stained with X-Gal. Ectopic expression of *egl-5-LacZ* has not yet begun. (C) V cell lineage diagram showing time course of ectopic *mab-5* expression in *pry-1(mu38)*. Pie charts indicate the fraction of cells found to stain (blue), at each point in the lineage, in *pry-1(mu38)* mutants. Heavy blue lines indicate cells that express *mab-5* in wild type, black lines indicate cells that do not express *mab-5*. (Salser and Kenyon, 1996).

the initial patterns are set. Thus, *pry-1* may perform a function analogous to the *Polycomb*-group genes of *Drosophila*.

We also investigated the onset of *mab-5* expression in QL and QR. For these experiments we determined developmental age by observing QL's position in fixed, stained animals (the migration of QL over V5 is not affected by *pry-1* mutations or by changes in *mab-5* expression). 'Young' animals were those in which QL had not yet migrated over V5 (approximately 1.5-2.5 hours old); 'older' animals were those in which QL had migrated at least 50% over V5 (approximately 2.5-3.5 hours old). In young wild-type animals no *mab-5* expression was seen in QL (or QR) ($n=10$; also S. Salser and C. K., unpublished observations). In older wild-type animals expression was seen in QL in 50% of the animals ($n=10$). Similarly, we found that in young *pry-1(mu38)* animals expression of *mab-5* was off in both QL and QR ($n=13$). In older *pry-1(mu38)* animals *mab-5* was expressed in QL 64% of the time and in QR 38% of the time ($n=47$). Thus, expression of *mab-5* in the Q cells begins in *pry-1* mutants at approximately the same stage that expression begins in wild-type animals, suggesting that there is temporal control of *mab-5* expression operating independently of *pry-1*.

DISCUSSION

In this study, we have investigated the regulation of Hox gene expression in *C. elegans*. Our results demonstrate that a Wnt signaling pathway regulates Hox gene expression in a number of cell types. We have found that the *egl-20* gene, which activates expression of the Hox gene *mab-5* in the QL neuroblast, encodes a Wnt signaling molecule and that *egl-20* is part of a conserved Wnt pathway that involves the *lin-17/frizzled* receptor homolog and the *bar-1/β-catenin/armadillo*-like gene. We also find that the *pry-1* gene functions in this pathway downstream of *egl-20/Wnt* to inhibit the ability of *bar-1* to transduce the Wnt signal. Our studies of these genes show that proper regulation of Wnt signaling is critical: activation of this pathway is required to promote Hox gene expression in selected cells in order to achieve proper patterning, and inhibition of this pathway is required to prevent widespread ectopic activation of Hox genes and the resulting homeotic transformations.

A conserved Wnt signaling system acts as an on/off switch for *mab-5* in the Q cells

Prior to this study, it was known that *lin-17*, which activates *mab-5* expression in QL (Harris et al., 1996), encodes a *frizzled* homolog (Sawa et al., 1996). In *Drosophila*, *frizzled* family members are known to participate in two different signaling pathways: the first one is known to be well conserved and includes an upstream Wnt signal and the downstream β -catenin/Armadillo and dTcf/Pangolin proteins. The second is a less well-defined pathway required for tissue polarity (reviewed by Adler, 1992). Our findings that *egl-20* encodes a Wnt signal and that the β -catenin/Armadillo-like protein BAR-1 is required to turn on *mab-5* in QL argues that the *C. elegans mig-14*, *egl-20*, *lin-17*, *mig-1*, *pry-1* and *bar-1* genes all participate in a conserved pathway analogous to the first described above. The *mig-14* and *mig-1* sequences have not yet been reported. However, another gene, *mig-5*, has been

reported to be a homolog of the Wnt pathway member *disheveled*, and to have a QL migration phenotype (Guo, 1995). It is not known whether this mutation affects the same aspect of Q migration, but it seems possible that it too functions in the Wnt pathway to activate expression of *mab-5* in QL.

Our epistasis results imply that *pry-1* functions downstream of *egl-20* and *mig-1* but upstream of *bar-1*. Together with the expression data, this suggests that in wild-type worms PRY-1 inhibits BAR-1 activity, thereby preventing Hox gene expression. Our genetic analysis is consistent with PRY-1 acting either downstream of or in parallel to BAR-1. PRY-1 protein could directly repress BAR-1 activity, or it could act at a more downstream point in the pathway; for example, by binding to the targets of BAR-1 and rendering them insensitive to BAR-1 activity (see below). Although the molecular identity of *pry-1* is not known, it could encode a *C. elegans* homolog of a Wnt pathway regulator such as *zeste-white 3* (Peifer et al., 1994; Siegfried et al., 1992), Axin (Zeng et al., 1997), or APC (Munemitsu et al., 1995; Polakis, 1997; Rubinfeld et al., 1996), or it could encode a novel protein. The fact that *egl-20*, *lin-17* and *mig-1* mutants share some phenotypes that *bar-1* mutants do not have (for example a defect in the migration of the HSN neuron (Harris et al., 1996; data not shown) raises the possibility that they may activate a second type of signaling pathway in other cells.

One of the most interesting aspects of Q cell migration is the fact that in spite of being born in bilaterally symmetric A/P positions, QL and QR and their descendants migrate in opposite directions. One critical difference between these cells is the expression of *mab-5* in QL but not in QR. This difference is necessary and sufficient for controlling the behavior of the Q descendants. Why is it that *mab-5* is switched on in QL but not in QR? In principle, this regulation could be exerted at the level of the Wnt signaling pathway, or, alternatively, QR might be intrinsically different from QL and simply be immune to Wnt signaling. The observation that *pry-1* is required to keep *mab-5* off in QR demonstrates that QR is sensitive to activation of the Wnt pathway and thus has all of the components downstream of *pry-1* necessary for activation of *mab-5*. Therefore, in wild-type animals, some step in the Wnt signaling pathway upstream of *pry-1* must be blocked in QR.

pry-1 functions as a general repressor of Hox gene activation

pry-1 is required in many tissues to keep Hox genes from being expressed outside of their normal domains of function along the A/P body axis. Interestingly, the initial expression patterns of both *mab-5* and *egl-5* in the epidermis are largely normal; dramatic ectopic expression is not seen until many hours after hatching. For this reason, *pry-1* (polyray) mutants phenotypically resemble the *Drosophila Polycomb*-group (PcG) mutants, which are characterized by exhibiting normal patterns of Hox gene expression early in development, but later exhibiting widespread Hox gene misexpression. The cloning of some of these genes has suggested that they repress Hox gene expression by affecting chromatin dynamics (reviewed by Pirota, 1997). Why might *pry-1* and PcG mutants have analogous phenotypes? The simplest interpretation of our findings is that the molecular mechanism of *pry-1* activity is different from that of the cloned PcG genes. This is because *pry-1* appears to be a member of a conserved Wnt signaling pathway,

since *pry-1* mutations can be suppressed by mutations in the β -catenin/armadillo-like gene *bar-1*. Since Armadillo is thought to be regulated by phosphorylation, which in turn affects protein stability, it would not be surprising if the *C. elegans* PRY-1 protein regulated BAR-1 in a similar fashion. Nevertheless, it is possible that there is a connection between Wnt signaling and the PcG genes. To explore this possibility it would be interesting to determine if mutations in the *Polycomb*-group genes can be suppressed by *armadillo* mutations in *Drosophila*.

The *Drosophila* Wnt homolog *wingless* (*wg*) is known to control transcription of *Ubx* in the developing midgut (van de Wetering et al., 1997). However, in contrast to the *C. elegans* pathway described here, the Wnt pathway in *Drosophila* is not known to play a large, organism-wide role in regulating Hox expression. There are several possible explanations for the apparent difference. One possibility is that the *Drosophila* Wnt pathway may be more important for regulating Hox genes than has been realized. The Hox misexpression phenotype may have been missed due to other more dramatic consequences of mutations in the Wnt pathway. If so, it is possible that some of the uncloned *Polycomb*-group members could in fact be regulators of Wnt signal transduction. Another possibility is that *pry-1*, like some known *Polycomb*-group members, affects chromatin stability. In this scenario one would imagine that PRY-1 activity somehow renders chromatin resistant to transcriptional activation by BAR-1/Armadillo. Finally, the differences in the importance of Wnt signaling for Hox gene expression may simply reflect evolutionary differences between worms and flies. For this reason it will be interesting to learn whether the Wnt pathway is a relatively minor or major regulator of Hox gene expression in other species.

***pry-1* may set the stage for *lin-22* activity in the V cells**

We found that the *bar-1(mu63)* mutation was not able to suppress the ectopic rays caused by *lin-22(n372)*. This finding indicates that *lin-22(+)* activity is still required to repress ray production in anterior V cells even in the absence of *bar-1*. One possibility is that wild-type *bar-1* activity promotes the formation of ectopic rays, at least in part, by inhibiting or bypassing *lin-22*-mediated ray repression. The pattern of ectopic *mab-5* expression differs in *lin-22* and *pry-1* mutants. In *lin-22* mutants, the V cells express *mab-5* in an ON-OFF pattern similar to that of wild-type V5 (Wrischnik and Kenyon, 1997), whereas in *pry-1* mutants, *mab-5* is expressed at an earlier time than normal and simply remains on. Our model, which would be analogous to the role of the *Polycomb* system in *Drosophila*, is that *lin-22(+)* activity can only commence repression of *mab-5* if Hox gene expression already off. In wild-type animals, *pry-1* activity keeps the Hox genes off by preventing *bar-1* from activating their expression. In *pry-1* mutants, this early repression fails, so *lin-22* cannot act. However, in *bar-1(-)* animals, *mab-5* remains off during early larval development so that *lin-22* is also able to commence repression of *mab-5*.

Cell-type specificity of *pry-1* and *bar-1* function

bar-1 and *pry-1* function quite differently in different cell types. Not only do the Hox genes under their control vary from tissue to tissue, but the precise roles that *bar-1* and *pry-1* play during wild-type development vary as well. *pry-1* represses the *Abd-B*

homolog *egl-5* in epidermal cells, the *Scr* homolog *lin-39* in ventral cord neurons, and the *Antp* homolog *mab-5* in the Q cells, the V cell descendants, and the ventral cord neurons. In the Q cells, *bar-1* and *pry-1* function as part of an ON/OFF switch that keeps *mab-5* expression off in QR and initiates *mab-5* expression in QL. In the V cells, however, their primary role seems to be to prevent *mab-5* expression in the anterior body region; *pry-1* mutations cause ectopic *mab-5* expression, but loss of *bar-1* has little or no effect on *mab-5* expression or V cell development. In contrast, *bar-1* has been found to be required in the Pn.p cells to maintain *lin-39* activation after expression has been initiated by other factors (Eisenmann et al., 1998).

Cells also change their responsiveness to *bar-1* and *pry-1* during development. For example, although *pry-1* mutations cause ectopic expression of *mab-5* in QR, this ectopic expression does not appear until the time that wild-type *mab-5* expression begins in QL, as if the Q cells are not competent to respond to activation of the Wnt pathway until that time. Similarly ectopic expression in the V5 lineage does not begin until late in the first larval stage. It seems that there are at least three phases to the regulation of *mab-5* in the V5 lineage: first *mab-5* is kept off by a *pry-1*-independent mechanism; later, at the time when expression begins in the *pry-1* mutant, *mab-5* is kept off by *pry-1*; and yet later *pry-1* repression is relieved (directly or indirectly), allowing *mab-5* expression to begin in wild type. Taken together these observations suggest that other spatially and temporally regulated factors interact combinatorially with *bar-1* and *pry-1* to modulate and specify their actions. Identifying these factors and understanding their interactions with *bar-1* and *pry-1* should provide interesting insights into the control of the Wnt pathway during development.

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