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Interactions of EGF, Wnt and HOM-C genes specify the P12 neuroectoblast fate in *C. elegans*

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SUMMARY

We investigate how temporal and spatial interactions between multiple intercellular and intracellular factors specify the fate of a single cell in *Caenorhabditis elegans*. P12, which is a ventral cord neuroectoblast, divides postembryonically to generate neurons and a unique epidermal cell. Three classes of proteins are involved in the specification of P12 fate: the LIN-3/LET-23 epidermal growth factor signaling pathway, a Wnt protein LIN-44 and its candidate receptor LIN-17, and a homeotic gene product EGL-5. We show that LIN-3 is an inductive signal sufficient to promote the P12 fate, and the conserved EGF signaling pathway is utilized for P12 fate specification; *egl-5* is a downstream target of the *lin-3/let-23* pathway in specifying P12 fate; and LIN-44 and LIN-17 act synergistically with *lin-3* in the specification of the P12 fate. The Wnt pathway may function early in development to regulate the competence of the cells to respond to the LIN-3 inductive signal.

Key words: EGF, HOM-C, Wnt, Cell fate specification, Gene interactions, *Ceanorhabditis elegans*

INTRODUCTION

In certain developmental fields, cells are exposed to multiple extrinsic signaling molecules that combine with intrinsic factors to control cell proliferation, differentiation, and morphogenesis. Although we have an increasing understanding of how a single factor regulates cellular behaviors, we have limited understanding of how multiple factors work in concert to ensure cells take on their correct fates. Here we investigate how an epidermal growth factor (EGF)-like protein, a Wnt protein, and a homeotic cluster (HOM-C) transcription factor interact to specify P12 neuroectoblast fate in *Caenorhabditis elegans*.

The cells P11 and P12 are the most posterior pair of postembryonic ventral cord precursors (Sulston and Horvitz, 1977). These two cells both have the potential to express P12 fate during the early first larval (L1) stage before they enter the ventral cord (Sulston and White, 1980). When either cell is ablated by laser microsurgery, the remaining one takes on P12 fate. How is the P12 fate specified? Though no systematic studies on P11/P12 cell fate specification have been reported, previous investigations have accumulated data indicating that several classes of genes are involved in P11/P12 cell fate specification. These include genes of the *lin-3/let-23* pathway, which is the *C. elegans* EGF signaling pathway, a Wnt family gene *lin-44*, and a HOM-C gene *egl-5*.

These three classes of genes have been primarily studied for reasons other than P11/P12 cell fate specification. The *lin-3/let-23* signaling pathway, which is similar to the EGF signaling pathway of mammals (Fantl et al., 1993), has been studied extensively in *C. elegans* vulval development (reviewed by Sundaram and Han, 1996; Kornfeld, 1997). *lin-3* encodes a membrane-spanning protein with a single extracellular EGF domain that is similar in structure to members of the EGF family of growth factors (Hill and Sternberg, 1992). *let-23* encodes a receptor tyrosine kinase of the EGF receptor family (Aroian et al., 1990). LIN-3 functions as an intercellular signal and its activity is both necessary and sufficient for vulval induction (Ferguson and Horvitz, 1985; Hill and Sternberg, 1992; Katz et al., 1995), while LET-23 is the likely receptor for the LIN-3 signal. LET-23 transduces LIN-3 activity via an SH2/SH3 adaptor protein SEM-5 (Clark et al., 1992a), which in turn activates the downstream RAS protein LET-60 (Han and Sternberg, 1990). *lin-15* defines a general inhibitory pathway that negatively regulates *let-23* activity and prevents cells undergoing vulval differentiation (Ferguson and Horvitz, 1985; Ferguson et al., 1987; Herman and Hedgecock, 1990; Clark et al., 1994; Huang et al., 1994). The *lin-3/let-23* signaling pathway is also utilized to specify certain cell fates in male spicule development (Chamberlin and Sternberg, 1994).

The *C. elegans* gene *lin-44* is a member of the Wnt family genes (Herman et al., 1995). Wnt family genes encode secreted glycoproteins and are implicated in intercellular signaling to control cell fate specification and cell proliferation in many organisms (reviewed by Nüsse and Varmus, 1992; Moon et al., 1997). In *C. elegans*, while the major defects in *lin-44* mutants appear to be the reversed polarities of certain asymmetric cell divisions in the tail region, they are also defective in the specification of P12 fate (Herman and Horvitz, 1994). Both in

situ hybridization and the analysis of reporter gene constructs indicated that *lin-44* is expressed in epidermal cells at the tip of the tail and posterior to the cells affected by *lin-44* mutation (Herman et al., 1995). Mosaic analysis demonstrated that *lin-44* acts cell nonautonomously and *lin-44* function is required in the cells in which *lin-44* expression is observed. *lin-17* encodes a protein with seven putative transmembrane domains, homologous to the *Drosophila* Frizzled protein (Sawa et al., 1996). *lin-17* regulates several asymmetric cell divisions and cell fates which are also controlled by *lin-44* (Sternberg and Horvitz, 1988), therefore has been proposed to be a receptor for the Wnt protein LIN-44 (Sawa et al., 1996).

HOM-C genes are conserved throughout metazoan evolution. These HOM-C transcription factors establish position-specific fates along body axes (Lewis, 1978; reviewed by Krumlauf, 1994). HOM-C genes can act in multiple cell types and at multiple time during development (reviewed by Lawrence and Morata, 1994; Salser and Kenyon, 1994). They are regulated by multiple intercellular and intracellular molecules (Immergluck et al., 1990). They also regulate diverse targets to control position-specific cell fates (Mastick et al., 1995). In *C. elegans* a cluster of four HOM-C genes has been identified. Along the anteroposterior body axis it consists of *lin-39*, *mab-5*, and *egl-5*, respective homologues of the *Drosophila* genes *Sex combs reduced/deformed/proboscipedia*, *Antennapedia*, and *Abdominal-B*, with a labial homologue, *ceh-13*, being between *lin-39* and *mab-5* (reviewed by Salser et al., 1994). *lin-39* activity is required in the central body region for vulval differentiation and patterning, while *mab-5* and *egl-5* are involved in cell fate specification of the tail region (Kenyon 1986; Chisholm, 1991; Clark et al., 1993; Salser et al., 1993; Clandinin et al., 1997; Maloof and Kenyon, 1998).

We are particularly interested in these three classes of genes because mutations in all these genes show P11/P12 cell fate specification defects. We sought to determine how these intrinsic and extrinsic factors act together to specify a certain cell fate. We first demonstrate that LIN-3 is an inductive signal for the P12 fate, and the *lin-3/let-23* pathway functions to specify P12 fate in a similar way to its function during vulval induction. Then we examine whether the HOM-C gene, *egl-5*, sets up the competence of the cells to the LIN-3 inductive signal by establishing a pre-pattern, or if it plays an instructive role in P11/P12 cell fate specification. Our results support the latter possibility, that *egl-5* is a downstream target of the *lin-3/let-23* pathway and its activity is required in executing P12 fate. Finally we explore the interactions between the *lin-3/let-23* and the Wnt signaling pathways. Our results suggest that *lin-44* and *lin-3* may act at different times during development corresponding to two genetically parallel pathways, and the Wnt pathway may regulate the competence of the P11/P12 cells to respond to the LIN-3 signal.

MATERIALS AND METHODS

General methods

Routine culturing, maintenance and genetic manipulations of *C. elegans* strains were performed according to standard procedures (Brenner, 1974). N2 (Bristol) strain was used as wild type (Brenner, 1974). The following strains were used.

LG I: *lin-17(n671)* (Ferguson and Horvitz, 1985), *lin-44(n1792)* (Herman and Horvitz, 1994),

LG II: *let-23(sy97)* (Aroian and Sternberg, 1991),

LG III: *egl-5(n945)* (Chisholm, 1991),

LG IV: *dpy-20(e1282)* (Brenner, 1974), *lin-3(n378)* (Ferguson and Horvitz, 1985), *lin-3(n1059)* (Ferguson and Horvitz, 1985), *let-60(sy93)* (Han et al., 1990),

LG X: *lin-15(e1763)* (Ferguson and Horvitz, 1985), *sem-5(n2019)* (Clark et al., 1992a),

Transgenic strains:

PS1427: *unc-31(e169); syIs6[unc-31(+), pRH51(hsLIN-3EGF) 50 ng/μl]* (Katz et al., 1995),

PS2467: *dpy-20(e1282); syEx178[dpy-20(+), pLJ5(hsEGL-5) 20 ng/μl],*

PS2347: *dpy-20(e1282); muIs13[rol-6(su1006), egl-5-lacZ] him-5(e1490)* (Wang et al., 1993).

Construction of *hsp16.1-egl-5* transgene

The *hsp16.1-egl-5* construct, pLJ5, was constructed in the same way as previously described constructs *hs-mab-5* and *hs-lin-39* (Salser and Kenyon, 1992; Hunter and Kenyon, 1995). Briefly, primers overlapping sequences of the start and stop codons were used to amplify the coding sequence of *egl-5* from an *egl-5* cDNA clone by PCR. The amplification products were sequenced and ligated into the *Hpa*I site of the *hsp16.1* gene (Russnak and Candido, 1985). This construct generates a fusion protein with the first 44 amino acids of *hsp16.1* gene product and the full length EGL-5 protein.

Construction of transgenic strains

Transgenic animals were generated by microinjection of DNA into the mitotic germline of hermaphrodites according to the method of Mello et al. (1991). *syEx178* was obtained by microinjection of pLJ5 plasmid at 20 ng/μl, pMH86 (dpy-20(+)) at 15 ng/μl, and carrier DNA (pBlueScript) at 120 ng/μl into *dpy-20(e1282)* mutant animals. To test the function of the construct, the transgenic line *syEx178* was crossed into *egl-5(n945); dpy-20(e1282)* and *let-23(sy97) unc-4(e120)/mnC1(dpy-10 unc-52); dpy-20(e1282)* strains. The non-Dpy phenotype indicates the presence of the transgene.

syIs6 was obtained by X-ray integration of the *syEx23* strain, which expresses the EGF domain of LIN-3 under the heat shock promoter and has been described by Katz et al. (1995).

muIs13 is an integrated strain containing the *egl-5-lacZ* transgene (Wang et al., 1993), kindly provided by Craig Hunter. For ease of scoring, *dpy-20(e1282)* mutation was crossed into the strain to suppress the Roller phenotype caused by the transformation marker.

syIs6; dpy-20(e1282); muIs13 him-5(e1490) strain was constructed by crossing *syIs6; dpy-20(e1282); him-5(e1490)* males with *muIs13 him-5(e1490)* hermaphrodites. Animals homozygous for *syIs6* and *muIs13* displayed 100% multivulva phenotype under heat shock condition and had 100% β-galactosidase-positive staining.

The assay for β-galactosidase activity was performed as described previously (Fire et al., 1990).

Heat shock of transgenic animals

Cohorts of adult hermaphrodites were placed on standard worm culture plates to collect eggs. The hermaphrodites were transferred to a fresh plates every hour. The age of the worms at heat shock was determined as hours after egg-lay. Different strains hatch at slightly different times (±2 hours after egg-lay), and times were normalized to hatching. Animals were heat shocked in a 33°C water bath for 30 minutes or 60 minutes as indicated in each figure legend. For the experiment involving EGL-5 overexpression in a *let-23(sy97)* mutant background, eggs were collected by cutting egg-laying defective adult hermaphrodites into halves and then heat shocking at certain developmental times.

Anatomical and cell lineage analysis

L3 or early-mid L4 hermaphrodites were examined with Nomarski optics. P11 and P12 fates were determined according to the distinct nuclear morphologies and positions of P11.p and P12.pa cells. Cell

lineages were followed at 20°C as described by Sulston and Horvitz (1977).

RESULTS

P11/P12 development

In *C. elegans* there are twelve ventral cord precursor cells, P1-P12, numbered from anterior to posterior along the body axis. These cells divide postembryonically to generate cells of the ventral nervous system, as well as the vulva (Sulston and Horvitz, 1977). P11/P12 are the most posterior pair of the ventral cord precursors. At hatching, the cells AB.plapappa (left side) and AB.prapappa (right side) are disposed laterally (Sulston et al., 1983). In hermaphrodites, they start to migrate ventrally several hours after hatching and enter the ventral cord about 8-9 hours after hatching at 20°C (Fig. 1A). The left cell migrates anteriorly and becomes P11, whereas the right cell migrates posteriorly and becomes P12. Two hours later they both divide once. The anterior daughters, P11.a and P12.a are neuroblasts that will divide for three more rounds to generate several ventral cord neurons. These neurons are morphologically indistinguishable under Nomarski optics. The posterior daughter of P11, P11.p, does not divide but rather fuses with the large epidermal syncytium hyp7. P12.p divides once more about 1 hour prior to L1 molt to generate P12.pa, which becomes a unique epidermal cell, hyp12, and P12.pp, which undergoes cell death. P11.p and P12.pa can be distinguished by their different nuclear morphologies and positions observed with Nomarski optics (Fig. 1B). Sulston and White (1980) demonstrated that prior to

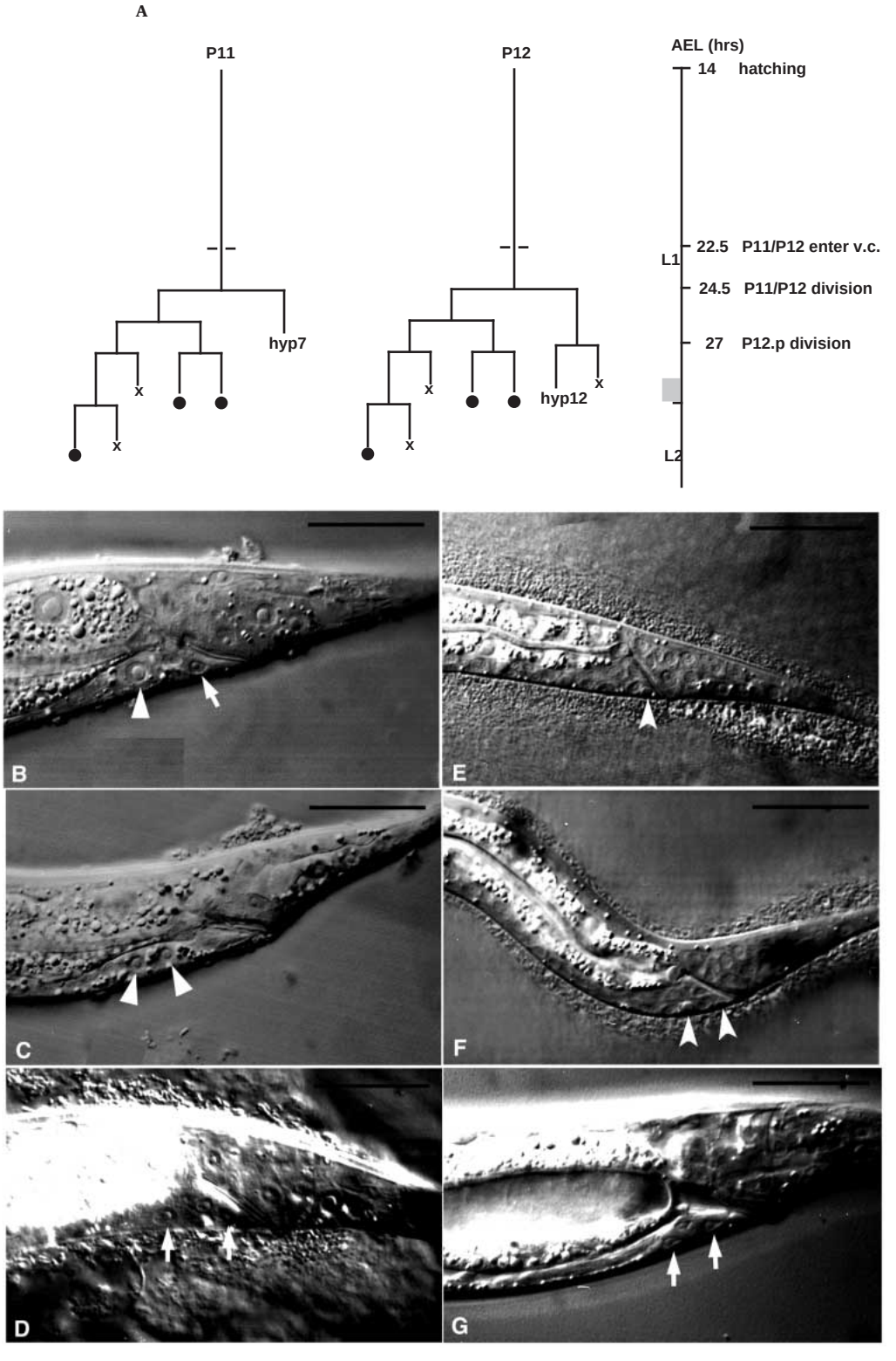

Fig. 1. P11/P12 phenotype observed in Nomarski photomicrographs. (A) Wild-type P11/P12 lineages at 20°C (Sulston and Horvitz, 1977). Terminal fates are: •, ventral cord neuron; x, programmed cell death. Timing is indicated as AEL, denoting hours after egg-lay, v.c. denotes ventral cord. (B) Wild type, (C) *let60(sy93dn)*, (D) *syIs6*, (E) wild-type animal, P12.pp undergoes cell death, indicated by a concave arrowhead. (F) P11 and P12 lineages are followed after heat shock treatment of *syIs6* strain (*n*=4). P11.p divides as does P12.p, and both P11.pp and P12.pp die. (G) *syEx178*. In B-D and G, arrowhead indicates P11.p, arrow points to P12.pa. Scale bars, 20 μm.

migration both P11 and P12 cells are able to express the P12 fate; and if only a single cell is present, it will adopt the P12-like fate. Therefore P12 represents a primary fate, while P11 is a secondary fate.

Mutants of several *lin-3/let-23* pathway components have defects in P11/P12 cell fate specification

Mutants of several *lin-3/let-23* pathway components have been reported to display defects in P11/P12 cell fate specification. Loss-of-function alleles of *let-23* showed a loss of the cell P12.pa with concommitant duplication of P11.p in the hermaphrodite tail (Fixsen et al., 1985; Aroian and Sternberg, 1991). Lineage analysis in males indicated that this defect likely represents a transformation of P12 to P11 fate, as the anterior branch is also affected (Fixsen et al., 1985). Mutations at the *lin-15* locus, which encodes negative regulators of the *lin-3/let-23* pathway, have the opposite defect, P11 to P12 cell fate transformation (Fixsen et al., 1985). Other components, *sem-5* and *let-60*, which encode a SH2/SH3 domain protein (Clark et al., 1992a) and a RAS protein (Han and Sternberg, 1990), respectively, are also involved in P11/P12 cell fate specification (Clark et al., 1992b). We examined hermaphrodites homozygous for a reduction-of-function allele of *sem-5*, *n2019*, and found a 31% penetrant P12 to P11 cell fate transformation (Table 1A). A dominant-negative allele of *let-60*, *sy93*, also displays a 45% penetrant P12 to P11 fate transformation (Fig. 1C, Table 1A).

Although mutations in these *lin-3/let-23* pathway components result in a P11/P12 defect, the penetrance of this phenotype is incomplete. We propose two possible models. The *lin-3/let-23* pathway functions in additional capacities including vulval induction, male spicule development, hermaphrodite fertility, and L1 lethality (Aroian and Sternberg, 1991). Although functions such as vulval induction, male spicule development, and hermaphrodite fertility can be genetically separated (Aroian et al., 1994; Lesa and Sternberg, 1997), mutations with P11/P12 defect share a common L1 lethality phenotype. It is possible that the P11/P12 defect and the lethality phenotype require similar aspects of gene function (e.g., expression during the L1 stage), therefore the dead larvae with P11/P12 defect are excluded from scoring. Alternatively, there might exist another pathway acting in conjunction with the *lin-3/let-23* pathway for P11/P12 fate specification.

Given the fact that these *lin-3/let-23* pathway components are involved in P11/P12 cell fate specification, we asked if the genetic interactions among these genes are conserved. During vulval development, *lin-15* defines a general inhibitory pathway which negatively regulates *let-23* activity. As *let-23* and *lin-15* also have an opposite effect on P11/P12, we tested if *lin-15* acts at the level of *let-23*. We constructed a strain defective in both genes. 30% of *let-23; lin-15* double mutants display a P12 to P11 cell fate transformation (Table 1A), resembling the mutation in *let-23* alone. Thus *let-23* is epistatic to *lin-15*, reminiscent of *lin-15* negative regulation of *let-23* in vulval induction.

LIN-3 is an inductive signal sufficient for P12 fate

Since none of the existing *lin-3* mutants exhibits any defect in P11/P12 cell fate specification and null alleles of *lin-3* are L1 lethal, it has been difficult to determine if *lin-3* plays a role in P11/P12 cell fate specification. We circumvented this problem by testing whether overexpression of LIN-3 would affect P11

Table 1.

A. *let-23* pathway is involved in P11/P12 cell fate specification.

Genotype*	*n*†	% P11→P12‡	% P12→P11§
N2 (wild type)	50	0	0
let-23(lf)	50	0	44
lin-15(lf)	74	54	0
let-23(lf); lin-15(lf)	49	0	33
sem-5(lf)	45	0	31
let-60(dn)	51	0	45

B. *egl-5* is epistatic to the lin-*3/let-23* pathway in P12 fate specification.

Genotype*	*n*†	% P11→P12‡	% wild type	% P12→P11§
egl-5(lf)	63	0	0	100
egl-5(lf); lin-15(lf)	41	0	0	100
egl-5(lf); let-23(lf)	25	0	0	100
syIs6, non-hs	50	0	100	0
egl-5(lf); syIs6 , non-hs	50	0	0	100
egl-5(lf); syIs6¶*, hs*	37	0	0	100
let-23(lf)	50	0**	56††	44‡‡
let-23(lf); syEx178, hs	21	15**	75††	10‡‡

non-hs, animals were not treated with heat shock; hs, animals were heat shocked.

*Alleles used are: *let-23(sy97)*, a severe reduction-of-function allele; *lin-15(e1763)*, a severe reduction-of-function allele, *sem-5(n2019)*, a reduction-of-function allele; *let-60(sy93)*, a dominant-negative allele; *egl-5(n945)*, a severe reduction-of-function. *syIs6* is a transgenic strain bearing the *hsLIN-3EGF* construct, *syEx178* is a transgenic strain bearing the *hsEGL-5* construct.

†Number of animals examined.

‡Percentage of animals with two P12-like cells. Animals were scored under Nomarski at L3-L4 stage, cell fate transformation is determined by nuclear morphology and position.

§Percentage of animals with two P11-like cells.

¶Animals were heat shocked during embryogenesis and L1 stage in 33°C water bath for 30 minutes.

***P*=0.0223, Fisher's Exact Test

††*P*=0.7935, Fisher's Exact Test

‡‡*P*=0.0057, Fisher's Exact Test

and P12. A transgenic strain bearing a construct that expresses the EGF domain of LIN-3 under the control of a heat shock promoter/enhancer was used for this experiment. This transgene, the 63 amino acid EGF domain of LIN-3 driven by the inducible and tissue general *hsp16-41* heat shock promoter (Stringham et al., 1992), has been demonstrated to be functional and sufficient to induce vulval development (Katz et al., 1995). The transgenic strain *syIs6* carries a high dose of the *hslin-3EGF* plasmid integrated in the genome. When these transgenic animals are grown at 25°C, the basal activity of LIN-3EGF can induce a multivulva phenotype, whereas the P11 and P12 cells remain wild type. We carried out a time course study to induce the expression of LIN-3EGF during embryogenesis and the L1 stage by a pulse of heat shock treatment at certain developmental times (Fig. 2A). We first observed that overexpression of LIN-3EGF does lead to a duplication of the cell P12.pa with a concommitant loss of P11.p in the hermaphrodite tail (Fig. 1D). We interpret this as a gain-of-function *lin-3* phenotype by analogy with results from the vulva and the spicules (Katz et al., 1995; Chamberlin and Sternberg, 1994). We confirmed this phenotype as being the result of the P11 to P12 cell fate transformation by following the P11/P12 lineages in hermaphrodites after heat shock (n=4). As shown in Fig. 1F, P11.p behaves like P12.p: it divides an extra round, and P11.pp undergoes cell death as does P12.pp. Also, the P11 to P12 cell fate transformation occurs when overexpression of LIN-3EGF is induced at late embryogenesis; it peaks to almost 100% when induced within several hours after hatching. Later, the effect of LIN-3EGF tapers off; overexpression of LIN-3EGF does not have any effect on P11/P12 cell fate after the P11/P12 cells enter the ventral cord. This timing is consistent with the ablation results of Sulston and White (1980) who found that the P11 cell loses its competence to become P12 after it enters the ventral cord. We conclude that LIN-3 is sufficient to induce the P12 fate and infer that LIN-3 is an inductive signal for the P12 fate.

egl-5 acts in a common pathway with the *lin-3/let-23* pathway in P12 fate specification

The *C. elegans* HOM-C gene *egl-5* is a homolog of the *Drosophila Abdominal-B* gene and is important for patterning the tail region of *C. elegans* (Chisholm, 1991; Wang et al., 1993). Loss-of-function mutations in *egl-5* cause a P12 to P11 cell fate transformation, indicating that *egl-5* activity is required for the P12 fate (Kenyon,

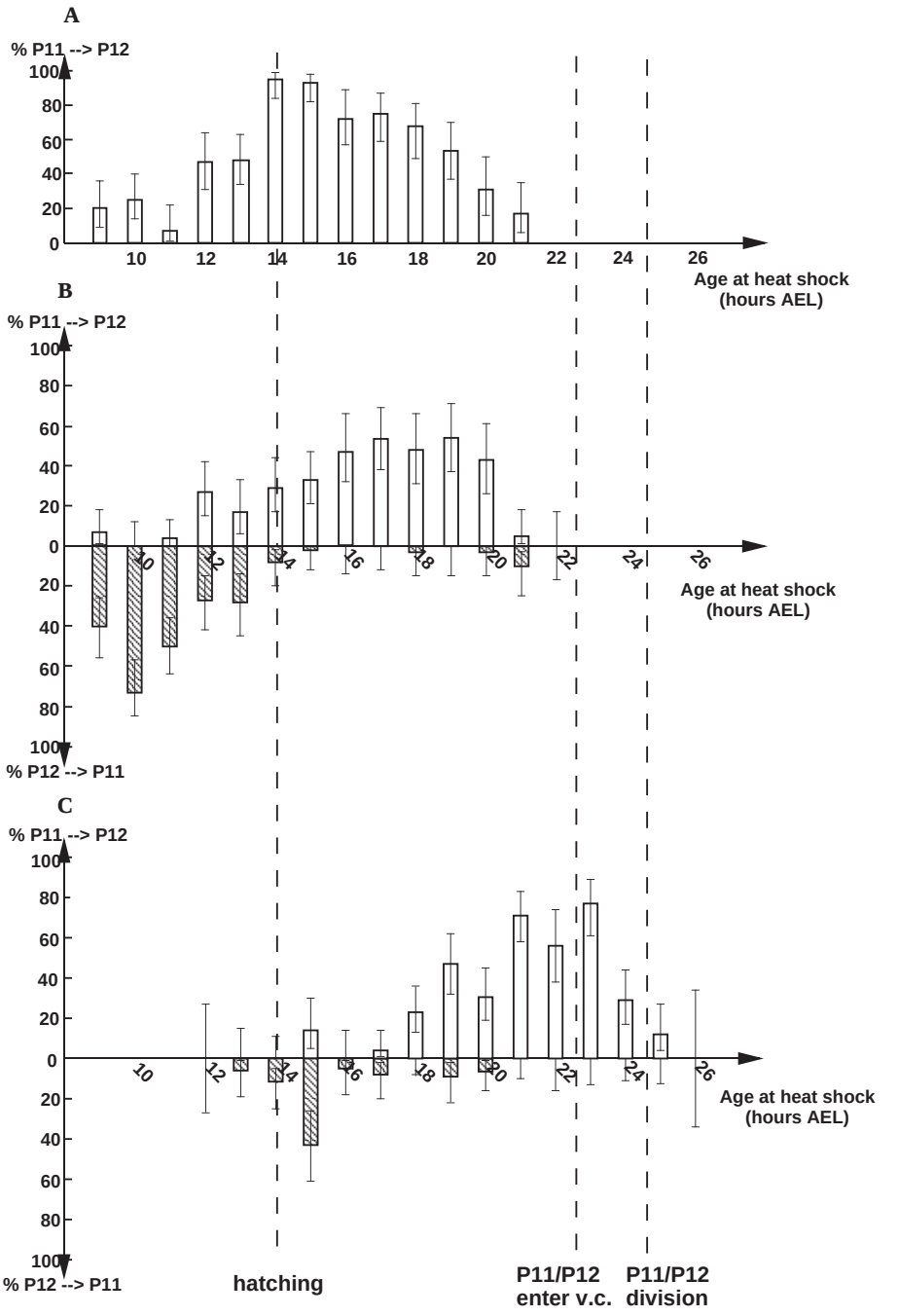

Fig. 2. Time course of the effect of overexpression of LIN-3EGF and EGL-5 on P11/P12 fate specification. (A) *syIs6(hsLIN-3EGF).* (B) *syIs6(hsLIN-3EGF)*; *lin-44(n1792).* (C) *syEx178(hsEGL-5).* Eggs were collected every hour on fresh plates and heat shocked at certain times after egg-lay as indicated on the abscissa, AEL denotes after-egg-lay. The heat shock conditions were 33°C, 30 minutes for A and B, and 33°C, 60 minutes for C. Open bars indicate percentage of P11 to P12 transformation; hatched bars indicate P12 to P11 transformation. Error bars are 95% confidence limits based upon the binomial distribution. Dashed lines indicate the times of hatching, P11/P12 cells entering the ventral cord, and the first division of P11/P12 cells, which are about 14, 22.5, and 27 hours after egg-lay respectively.

1986; Chisholm, 1991). We examined the epistasis relationship between *egl-5* and mutations of the *lin-3/let-23* pathway. Doubly mutant strains were constructed bearing an *egl-5* mutation and several *lin-3/let-23* pathway components. *egl-5(n945)* mutants display a 100% penetrance of P12 to P11 transformation. All the double mutants behave like mutation in *egl-5* alone (Table 1B). For example, *lin-15(lf)* animals display a 54% penetrance of P11 to P12 fate transformation, while all *egl-5(lf); lin-15(lf)* double mutants show a P12 to P11 fate transformation. Moreover, when overexpression of LIN-3EGF is induced in *egl-5* mutant animals, its effect on P11/P12 is completely blocked by *egl-5* mutation (Table 1B). Thus *egl-5* acts in a common pathway with the *lin-3/let-23* pathway in P12 fate specification.

egl-5 plays an instructive rather than permissive role in P12 fate specification

Previous studies have shown that HOM-C genes can specify the competence of cells to respond to an intercellular signal, thus establishing a pre-pattern in a developmental field (Clandinin et al., 1997). They themselves can also be regulated by intercellular signaling molecules (e.g., Immergluck et al., 1990; Maloof and Kenyon, 1998). *egl-5* might play a permissive role in P12 fate specification by setting up the competence of the cell to respond to the LIN-3 inductive signal, or *egl-5* might be an instructive factor for P12 fate specification. To distinguish these hypotheses, we first tested if overexpression of EGL-5 has an effect on P11/P12 cell fate specification. We engineered the full length *egl-5* cDNA (Salser and Kenyon, 1992) under the control of the *hsp16.1* promoter. Transgenic animals bearing this pLJ5 construct as an extrachromosomal array, *syEx178*, were generated. Upon heat shock treatment, the *hsp16.1* promoter should drive EGL-5 expression ubiquitously in somatic tissues (Russnak and Candido, 1985). When *syEx178* was introduced into *egl-5(n945)* mutants, expression of the transgene was able to rescue the P11/P12 mutant phenotype (data not shown). Overexpression of EGL-5 has a dominant sterile phenotype (L. Jiang, unpublished observation), making it impossible to examine the rescue of the egg-laying behavior. To analyze the effect of EGL-5 in wild-type animals, a pulse of EGL-5 expression was induced in the transgenic strain during late embryogenesis and L1 stage (Fig. 2C). Overexpression of EGL-5 in wild-type animals does lead to a P11 to P12 fate transformation (Fig. 1G). The most effective time to cause a P11 to P12 fate transformation is when overexpression of EGL-5 is induced around the time of the entry of P11/P12 into the ventral cord. This is later than the time of maximal effect of LIN-3EGF overexpression, which is within several hours after hatching. This temporal difference in responsiveness is consistent with our observation that *egl-5* is downstream of the *lin-3/let-23* pathway. It is also consistent with the observation that *egl-5* is not expressed in P cells during the early L1 stage (Wang et al., 1993).

We then asked if overexpression of EGL-5 could suppress the P11/P12 defect of *let-23* mutants. If *egl-5* is a permissive factor for P12 fate, no rescue of the P11/P12 defect would be expected; whereas if *egl-5* is an instructive factor, overexpression of EGL-5 should be able to rescue the P11/P12 defect caused by *let-23* mutation, and may additionally result in a P11 to P12 cell fate transformation. 44% of *let-23(sy97)* animals show a P12 to P11 transformation and 56% are wild type. When overexpression of EGL-5 is induced in transgenic *let-23(sy97)* animals bearing *syEx178*, the percentage of animals with a P12 to P11 transformation is lowered to 10% (P=0.0057, Fisher's Exact Test), while the percentage of wild-type animals is increased to 75%, the remaining 15% animals display a P11 to P12 cell fate transformation (P=0.0223) (Table 1B). This result suggests that overexpression of EGL-5 suppresses the P11/P12 defect of *let-23* mutants, as predicted by the instructive model. Therefore *egl-5* plays an active role in P12 fate specification and acts downstream of *let-23*.

The effect of overexpression of EGL-5 in a *let-23* deficient background is not as prominent as it is in a wild-type background. This may be attributed to technical limitation: *let-23(sy97)* worms are egg-laying defective so that mixed stage animals were collected for the heat shock experiments and timing may have been imprecise. Another possibility is that there exists a feedback loop between *egl-5* and the *lin-3/let-23* pathway.

egl-5 is a downstream target of the *lin-3/let-23* pathway in specifying P12 fate

We next tested whether *egl-5* is a downstream target of the *lin-3/let-23* pathway for P12 fate specification. Expression of an *egl-5-lacZ* reporter gene is detected in the cell P12.pa. As overexpression of LIN-3EGF leads to a P11 to P12 cell fate transformation, we examined if the transformed P11.pa cell is

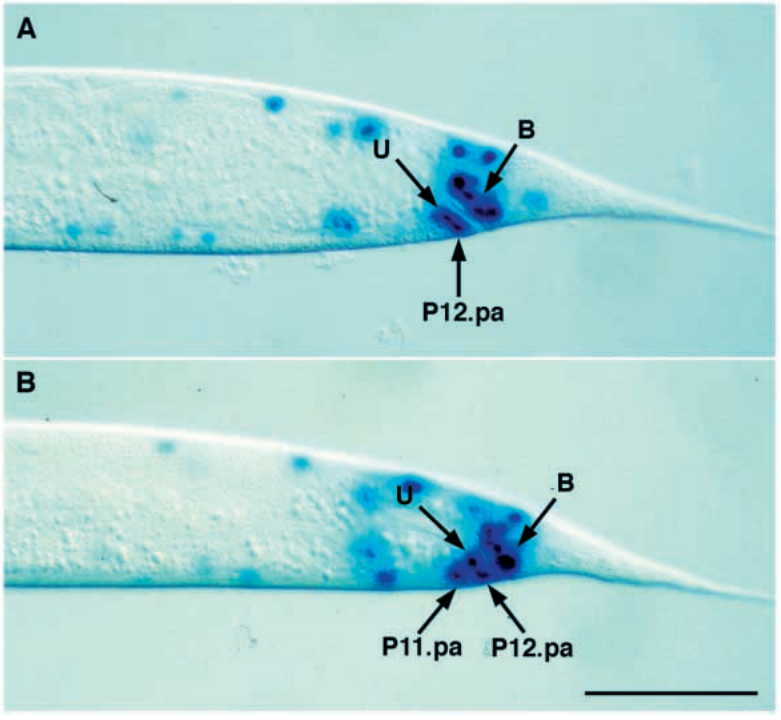

Fig. 3. LIN-3 can induce *egl-5* transcription in P12 lineage. (A) In wild-type animals, *egl-5-lacZ* expression is detected in P12.pa cell. (B) When overexpression of LIN-3EGF induces P11 to P12 transformation, *egl-5-lacZ* expression is detected in both P11.pa and P12.pa cells. Scale bar, 20 μm.

able to express the *egl-5-lacZ* marker. A strain bearing both the *hs-lin-3EGF* and the *egl-5-lacZ* transgenes was constructed. When overexpression of LIN-3EGF is induced at the proper time based on results of Fig. 2A, which is 15-17 hours after eggs were laid, a P11 to P12 cell fate transformation occurs in about 70% animals. As shown in Fig. 3, *egl-5-lacZ* expression is turned on in both the normal P12.pa and the transformed P11.pa cells. This observation indicates that *egl-5* expression in P12.pa can be turned on upon activation of the *lin-3/let-23* pathway.

Does *lin-44* act via *lin-3*?

lin-44, a gene encoding a Wnt family protein (Herman et al., 1995), is a member of a third class of genes that affect P11/P12 cell fate specification (Herman and Horvitz, 1994). 16% of *lin-44(n1792)* mutant animals show a P12 to P11 cell fate transformation. We wondered how the two signaling molecules, LIN-3 and LIN-44, act together to specify P12 fate. One possibility is that *lin-44* regulates *lin-3* expression by controlling the identity of the cell that normally expresses LIN-3 in the tail region. There are several reasons for this hypothesis. First, several cells in the tail region are defective in *lin-44* mutants. These include B, F, U, and T cells, which are posterior to the P11/P12 cells and anterior to *lin-44* expressing cells hyp8-11 (Herman et al., 1995). Second, analysis of *lin-3-lacZ* reporter constructs suggests that a potential source of LIN-3 signal exists in the tail region posterior to the P11/P12 cells (R. Hill, C. Chang and P. Sternberg, unpublished observation). Third, it has been suggested that Wnt genes are involved primarily in short-range cell interactions (van den Heuvel et al., 1989; Vincent and Lawrence, 1994). P11 and P12 lie anterior to the cells affected by *lin-44*, and may not be in direct contact with the *lin-44* expressing cells. Fourth, *lin-44* expression is turned on during embryogenesis, which is prior to *lin-3* expression in the tail.

If *lin-44* only regulates *lin-3* expression, overexpression of LIN-3EGF should be able to rescue the P11/P12 defect in *lin-44* mutants and have the same effect as it does in wild-type background. To test this hypothesis we crossed the *syIs6* strain into *lin-44(n1792)* mutant background and conducted heat shock experiments. As shown in Fig. 2B, overexpression of LIN-3EGF at early L1 stage rescues the P12 to P11 transformation caused by loss of *lin-44* function and results in a P11 to P12 transformation. However, the effect of LIN-3EGF overexpression is not as robust as it is in a wild-type background. In a *lin-44* mutant background, overexpression of LIN-3EGF only results in a maximum 50% P11 to P12 transformation, whereas in a wild-type background the transformation is almost 100% at the same stage. This difference is not expected if *lin-3* expression is solely regulated by *lin-44* in the tail region. However, we cannot rule out the possibility that the cells that provide functional LIN-3 might be altered in *lin-44* mutants, and thus overexpression of LIN-3EGF in *lin-44* mutants might not be as effective as it is in wild type.

To further test the hypothesis that *lin-44* regulates *lin-3*, we did the following two experiments. First, we examined the expression pattern of a *lin-3-lacZ* reporter gene in a *lin-44* mutant background. Transgenic animals bearing a *lin-3-lacZ* reporter construct which contains 12 kb of *lin-3* genomic sequence have been generated (R. Hill, C. Chang, and P. Sternberg, unpublished observations). *lin-3-lacZ* is expressed in the hermaphrodite tail region in newly hatched larvae. We believe the expression resides in the cells K, K′, and the daughters of K, which are posterior to the P11/P12 cells. When the transgenic strain is crossed into the *lin-44(n1792)* mutant background, we did not observe any change in the level of expression nor of the position of the cells (n=45).

Second, we examined the P11/P12 phenotype in *lin-44; lin-15* double mutant animals. *lin-15* is a negative regulator of the *lin-3/let-23* pathway and acts at the level of *let-23* in vulval development (Huang et al., 1994). *lin-15(e1763)* mutant animals display a 54% penetrance of P11 to P12 fate transformation (Table 1A). However *lin-15* acts in a signal-dependent manner as loss of LIN-3 activity removes its effect on P11 and P12 cells. *lin-3(n378/n1059)* mutants do not display a P11/P12 defect (Table 2), neither do *lin-3(n378/n1059); lin-15(e1763)* double mutant animals (n=45). If *lin-44* solely regulates *lin-3* activity, we would expect a similar result. Yet double mutant animals defective in both *lin-44* and *lin-15* show a mixed cell fate transformation, 10% P12 to P11 fate transformation and 35% P11 to P12 fate transformation (n=116). This cannot result from a quantitative effect since the *lin-44* genotype has a more severe effect on P12 than the *lin-3* genotype used. This additive effect argues against the hypothesis that *lin-44* affects P11/P12 fate by regulating *lin-3* activity. An additional *lin-44* function is required for proper P11/P12 fate specification.

lin-3 and *lin-44* act synergistically in P12 fate specification

We next tested the interactions between *lin-3* and *lin-44* by examining the P11/P12 defect in a strain defective in both genes. Heterozygotes of a *lin-3* reduction-of-function allele, *n378*, in *trans* to a null allele, *n1059*, do not display the P11/P12 defect, but have almost no vulval induction and severely defective spicules. However, *n378/n1059* enhances the phenotype of *lin-44(n1792)* from 16% P12 to P11 transformation to 78% (P<0.0001) (Table 2). Although it does not rule out a sequential model where *lin-3* acts downstream of *lin-44*, the strong synergy between *lin-3* and *lin-44* is consistent with the two signals acting in parallel. To confirm the interaction between *lin-3* and *lin-44*, we tested if synergy exists between *lin-3* and *lin-17* mutations. *lin-17*, which encodes a putative seven-transmembrane protein similar to the *Drosophila* Frizzled protein, has been suggested to be a receptor for the LIN-44 protein (Sawa et al., 1996). *lin-17(n671)* animals have a penetrance of 29% P12 to P11 fate transformation (Table 2). Double mutant animals defective in both *lin-17* and *lin-44* display a similar defect to *lin-17(n671)* alone, showing 28% P12 to P11 fate transformation (P=1) (Table 2). This lack of synergy supports the hypothesis that LIN-44 signal acts through the receptor LIN-17 for P11/P12 fate specification. We contructed a strain defective in both *lin-17* and *lin-3*. 82% of *lin-17; lin-3* double mutants show P12 to P11 cell fate transformation, resembling *lin-44; lin-3* double mutants (P=0.544), while different from *lin-17* single mutants (P<0.0001). Therefore, similar synergistic interactions exist between *lin-3* and *lin-17* as between *lin-3* and *lin-44*. A synergistic interaction is also found between mutations of *let-23*, the EGF receptor for LIN-3 signal, and the Wnt signal LIN-44 (Table 2). Our data support the hypothesis that both of the two signaling pathways, *lin-3* and *lin-44*, are required for the P12 fate specification.

Table 2. Synergistic interactions between the lin-3/let-23 and the Wnt signaling pathways

Genotype	*n*	% P12→P11
*lin-3(n378/n1059)**	24	0
let-23(sy97)	50	44
lin-44(n1792)	244	16‡
lin-17(n671)	41	29§,¶
lin-17(n671) lin-44(n1792)	54	28§
lin-44(n1792); lin-3(n378/n1059)†	159	78‡
lin-17(n671); lin-3(n378/n1059)†	114	82¶
lin-44(n1792); let-23(sy97)	91	93**

*A strain of genotype + *let-312(s1234)lin-3(n378)* + *unc-22(s7)/unc-24(e138)+lin-3(n1059)dpy-20(e1282)* + was used to examine the P11/P12 phenotype at L3-L4 stage. Homozygotes of *lin-3(n1059)* are L1 lethal. Homozygotes of *lin-3(n378)* control display no P11/P12 defect.

†Full genotype of *lin-3* region is + *lin-3(n378) let-59(s49)* + *unc-22(s7)/unc-24(e138) lin-3(n1059)* + *dpy-20(e1282)* +. As homozygotes of either *lin-3(n1059)* or *let-59(s49)* are L1 lethal, only transheterozygous animals bearing l*in-3(n378/n1059)* would be viable therefore scored at L3-L4 stage.

‡$P<0.0001$, Fisher's Exact Test

§$P=1$, Fisher's Exact Test

¶$P<0.0001$, Fisher's Exact Test

**$P<0.0001$, Fisher's Exact Test, compared with additive effect which would be 53%.

Bi-directional P11/P12 cell fate transformation

During the course of our experiments, we found the surprising phenomenon that in two cases, overexpression of the same protein, EGL-5 or LIN-3, can have opposite effect on P11/P12 fate depending on the time of induction. Overexpression of EGL-5 induced in newly hatched larvae causes a P12 to P11 fate transformation (Fig. 2C), which contradicts its role in specifying P12 fate. Overexpression of LIN-3EGF during late embryogenesis potentiates the P12 to P11 cell fate transformation in a *lin-44(n1792)* background (Fig. 2B). *n1792* animals only exhibits 16% penetrance of the P12 to P11 fate transformation. When overexpression of LIN-3EGF is induced within several hours before hatching in a *lin-44* mutant background, the P12 to P11 fate transformation phenotype is increased to 70%. This bi-phasic profile does not occur when overexpression of LIN-3EGF is induced in a wild-type background (Fig. 2A) nor when *lin-44(n1792)* animals are treated with heat shock at the same stage (data not shown). This surprising phenomenon suggests to us two possibilities. First, the onset of *egl-5* expression might be crucial for proper P12 fate specification. Second, the unique effect of LIN-3 overexpression in *lin-44* mutant background suggests a requirement of *lin-44* function during late embryogenesis for P12 fate specification.

DISCUSSION

By studying the specification of the P11 and P12 cell fates in *C. elegans*, we address the question how a cell integrates multiple intrinsic and extrinsic factors to take on its correct fate. We considered three classes of genes: EGF signaling pathway genes, Wnt signaling pathway genes, and a HOM-C gene, which are involved in the P12 neuroectoblast fate specification. Our results support three conclusions. First, LIN-3 is an inductive signal for P12 fate and the *lin-3/let-23* EGF signaling pathway is required for P12 fate specification. Second, the HOM-C gene *egl-5* is a downstream target of the *lin-3/let-23* pathway in executing P12 fate. Third, the Wnt gene *lin-44* acts in parallel with the LIN-3 signal but at a different developmental time, and the Wnt pathway may regulate the competence of cells to respond to the LIN-3 inductive signal.

The conserved EGF signaling pathway is used to organize cell fate specification in the *C. elegans* tail region

Previous studies have indicated that mutations in several genes of the *lin-3/let-23* pathway displayed P11/P12 cell fate specification defects (Fixsen et al., 1985; Aroian and Sternberg, 1991; Clark et al., 1992b). The genes *let-23*, *lin-15*, *sem-5*, and *let-60* are all involved in P11/P12 cell fate specification. We provide evidence that LIN-3 is an inductive signal for P12 fate. First, overexpression of the EGF domain of LIN-3 leads to P11 to P12 cell fate transformation. Second, loss-of-function mutations of *lin-3* decrease P12 fate specification to the reverse transformation in a sensitized *lin-44* mutant background. This is the first demonstration that mutation of *lin-3* does have an effect on P12 fate specification, and therefore the effect of LIN-3EGF overexpression is not simply mimicking the effect of another EGF-like protein. Our

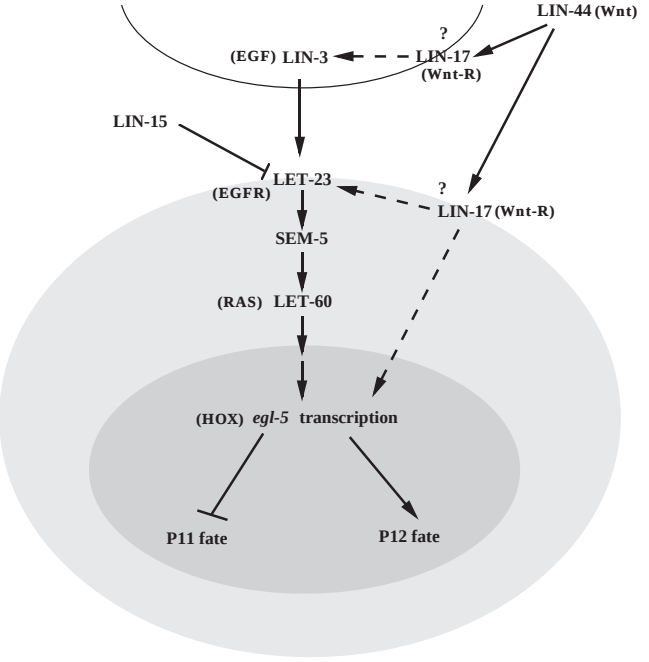

Fig. 4. Model for P12 fate specification. Inductive signal LIN-3 activates LET-23 receptor, which activates LET-60 RAS activity and turns on *egl-5* expression. Expression of *egl-5* at proper time specifies the cell to take on P12 fate. *lin-15* negatively regulates *let-23* activity and prevents the cell becoming P12. Wnt pathway may function at early developmental stage, LIN-44 signal acts via receptor LIN-17 to set up the competence of P11 and P12 cells to respond to the inductive signal and be able to express P12 fate. Integration of Wnt signal to the *lin-3/let-23* EGF signaling pathway may be at the level of LIN-3 signal, LET-23 receptor or *egl-5* transcription.

epistasis analysis suggests that the *lin-3/let-23* signaling pathway specifies P12 fate in the same fashion as it does for vulval induction. The same *lin-3/let-23* pathway has also been shown to organize *C. elegans* male spicule development (Chamberlin and Sternberg, 1994). Thus a conserved EGF signaling pathway is used at different times and in different cell types during development to organize cell fate specification.

During *C. elegans* hermaphrodite vulval development, a signal from the anchor cell (AC) in the gonad is required to induce three AC-proximal vulval precursor cells (VPC) to take on vulval fate, whereas the three distal VPCs produce nonspecialized epidermis (reviewed by Horvitz and Sternberg, 1991). This AC signal is an EGF-like protein encoded by the gene *lin-3* (Hill and Sternberg, 1992). *lin-3* expression revealed by a *lin-3-lacZ* reporter is detected in the AC at proper time for vulval induction (Hill and Sternberg, 1992). In male spicule development, *lin-3* mutants display defects similar to those observed in animals with F and U cells ablated (Chamberlin and Sternberg, 1993, 1994), suggesting that F and U cells may be the source of LIN-3 signal. The expression pattern of *lin-3* has not been precisely determined since the genomic clone used to determine AC expression does not contain the entire coding region (J. Liu, P. Tzou, R. Hill, and P. Sternberg, unpublished). What is the LIN-3 signal source in the hermaphrodite tail region for P12 fate specification? By analogy to vulva and spicule development, the LIN-3 signal source should be posterior to the P11 and P12 cells to promote the posterior fate, P12. Transgenic animals bearing a *lin-3-lacZ* reporter gene with additional genomic sequence have been generated (R. Hill, C. Chang and P. Sternberg, unpublished observation). Indeed we found that *lin-3-lacZ* is expressed in the hermaphrodite tail region in newly hatched larvae, in the cells K, K′, and the daughters of K, just posterior to the P11/P12 cells. To verify that the reporter gene expression truly reflects LIN-3 activity, we need to identify the source of the inductive signal for the P12 fate. Ablation experiments have been conducted to identify the source (Sulston and White, 1980; R. Hill, personal communication; L. Jiang, unpublished observation). We eliminated cells, including repD, K, K′, F and U, or combinations of these cells, in the rectal region by a laser microbeam to examine if the P12 fate would be disrupted. As the cells in the rectal region are essential for animal survival and growth, and the development of animals with ablated cells arrests before we could score the cell fates, such ablation attempts have been unsuccessful.

HOM-C gene *egl-5* is regulated by the EGF signaling pathway

HOM-C genes function in multiple cell types and at multiple times during development. Their expression patterns are finely tuned by diverse intercellular and intracellular interactions, which are important for the functions of HOM-C genes. *C. elegans* HOM-C genes, *lin-39*, *mab-5* and *egl-5*, are turned on early during embryogenesis in a position-specific manner from anterior to posterior along body axis (Wang et al., 1993). Their dynamic expression patterns and the regulation of their expression patterns have not been studied in detail until recently (Harris et al., 1996; Salser and Kenyon, 1996; Maloof and Kenyon, 1998). Here we have studied the interaction between the HOM-C gene, *egl-5*, and the *lin-3/let-23* EGF signaling pathway in the specification of P11/P12 cell fates and demonstrated that *egl-5* is a downstream target of the *lin-3/let-23* intercellular signaling pathway in specifying the P12 fate. *egl-5* expression is turned on in P12 lineage upon activation of the *lin-3/let-23* pathway, and it is necessary for P12 fate specification.

Interactions between HOM-C genes and the *lin-3/let-23* pathway have also been examined in *C. elegans* vulval development. Genetic analysis revealed two functions of *lin-39* during vulval development (Clark et al., 1993; Clandinin et al., 1997; Maloof and Kenyon, 1998): early LIN-39 activity specifies VPCs by creating a permissive condition for vulval differentiation; later LIN-39 activity regulates the response of VPCs to the *lin-3/let-23* pathway in the specification of a subset of VPCs as vulval tissue. Epistasis analysis has suggested that for the late role LIN-39 either regulates essential effectors of the response to inductive signal or itself is a target of the *lin-3/let-23* signaling pathway. Our molecular genetic analysis provides evidence that in the case of P12 fate specification, the HOM-C gene *egl-5* is a downstream target of the *lin-3/let-23* inductive pathway. If *lin-39* is indeed a target of the *lin-3/let-23* pathway during vulval development, as the data of Clandinin et al. (1997) and Maloof and Kenyon (1998) suggest, HOM-C genes may thus provide cell-specific responses to the same signaling pathway by being distinct targets.

lin-44 and *lin-3* act synergistically

Interactions between Wnt and growth signaling pathways can be either synergistic or antagonistic. Co-operative interactions between Wnt genes and members of the fibroblast growth factor (FGF) family have been identified during *Xenopus* embryonic development (Christian et al., 1992) and mammalian oncogenesis (Shackleford et al., 1993; Pan et al., 1995). Recently antagonistic interactions between *wingless* and EGFR signaling pathways have also been demonstrated during *Drosophila* larval cutical patterning (Szüts et al., 1997). Here we observed synergistic interactions between a Wnt protein, LIN-44, and the EGF-like protein LIN-3 in *C. elegans* P12 neuroectoblast fate specification. Moreover, this synergistic interaction also exists between the hypothesized Wnt receptor LIN-17 and LIN-3.

How do these two signaling pathways function in concert to specify P12 fate? We favor the model that both pathways are required for proper P12 fate specification and they act at different developmental times. First, LIN-3 overexpression experiments indicate that LIN-3 signal is required in early L1 before P11/P12 enter the ventral cord to induce P12 fate. Second, *lin-44* expression is turned on during embryogenesis, much earlier than the time of P11/P12 induction (Herman et al., 1995). Third, the unique effect of overexpression of LIN-3EGF during late embryogenesis in *lin-44* mutant suggests that *lin-44* function may be important in the early phase of P12 fate specification. It is possible that the Wnt pathway regulates the competence of the cells to respond to the LIN-3 inductive signal. But the Wnt signal alone is not sufficient to promote P12 fate, as overexpression of LIN-44 in wild-type animals has no effect on P11/P12 cell fate specification (L. Jiang and M. Herman, unpublished). These two pathways may also interact at multiple levels, for example, the Wnt pathway can regulate both the signaling cell and the responding cell. We set out to test the simple possibility that *lin-44* affects P12 fate by

regulating *lin-3* expression in the tail region. Results from three sets of experiments do not support this hypothesis. First, overexpression of LIN-3EGF can not completely bypass the requirement for *lin-44* function. A *lin-44* mutant decreased the response of P11 cell to LIN-3EGF overexpression (Fig. 2A,B). In addition, overexpression of LIN-3EGF during late embryogenesis caused a unique reverse cell fate transformation in a *lin-44* mutant background (Fig. 2B). Second, *lin-44* mutation did not affect *lin-3* expression as revealed by a *lin-3-lacZ* reporter gene. Third, decrease of *lin-3* activity had a more severe effect on P11/P12 fates than decrease of *lin-44* activity in a sensitized genetic background. Although our data do not favor this simple hypothesis, we can not completely rule out the possibility that *lin-44* somehow affects the property of the *lin-3* expressing cell in the tail region or the Wnt pathway may regulate LIN-3 protein processing. Further elucidation of how and where these two signaling pathways intersect requires additional genetic and molecular studies of both pathways.

Temporal regulation of gene interactions

Temporal control of gene activity is crucial for proper gene function during development. During our study of P12 neuroectoblast fate specification, we observed an unexpected phenomenon, in that overexpression of the same protein can result in opposite P11/P12 cell fate transformation depending on the time of induction. When overexpression of EGL-5 is induced in newly hatched larvae, it causes a P12 to P11 fate transformation (Fig. 2C), which contradicts its role in specifying P12 fate. This observation leads us to propose that the timing of *egl-5* expression is critical for P12 fate specification. To specify P12 fate, *egl-5* expression has to be turned on around the time of the first division of P11/P12 cells. If *egl-5* expression is turned on earlier, P12 fate would not be specified correctly. Consistent with this, in wild-type animals *egl-5-lacZ* expression is not detected in P12 cell at the early L1 stage (Wang et al., 1993). Similar ON-OFF behavior occurs in the activity of another HOM-C gene, *mab-5* (Salser and Kenyon, 1996). *mab-5* expression switching on and off in a lineage has been demonstrated to be important to control cell proliferation, differentiation and morphogenesis during development (Salser and Kenyon, 1996). In the case of LIN-3, when overexpression of LIN-3EGF is induced during late embryogenesis in a *lin-44(n1792)* background, it potentiates the P12 to P11 fate transformation caused by a *lin-44* mutation (Fig. 2B). This effect depends on both overexpression of LIN-3EGF and lack of LIN-44 activity. As activation of the *lin-3/let-23* pathway can turn on *egl-5* expression at the time of P12 fate induction, it might be that lack of LIN-44 activity enables *egl-5* expression to be turned on by LIN-3EGF earlier than usual, thus disrupting P12 fate specification. The timing difference in these two cases is consistent with this hypothesis as the effect of LIN-3 overexpression is earlier than that of EGL-5 overexpression. Another example of the bi-directional P11/P12 fate transformation is found in *mab-5* mutants (Kenyon, 1986). Loss-of-function mutants of *mab-5* show P11 to P12 as well as P12 to P11 fate transformations. As mutual repression has been suggested between HOM-C genes (Salser and Kenyon, 1993), temporal and spatial expression of *egl-5* might be abnormal in *mab-5* mutants, therefore resulting in cell fate transformation in both directions. A direct test of this hypothesis will require detailed temporal and spatial analysis of *egl-5* expression pattern in different genetic backgrounds.

Model for P12 fate specification

We propose the following model for P12 neuroectoblast fate specification (Fig. 4). In newly hatched larvae, LIN-44 signal acts via receptor LIN-17 to set up the competence of P11 and P12 cells to respond to the inductive signal and be able to express P12 fate. *egl-5* expression is kept off in both P11 and P12 cells. Later, an inductive signal LIN-3 coming from the posterior region activates LET-23 receptor activity in the posterior cell of the P11/P12 pair. Activation of the *lin-3/let-23* pathway turns on *egl-5* expression, which specifies the posterior cell to take on P12 fate. *lin-15* negatively regulates *let-23* activity and prevents the anterior cell becoming P12. Information from the Wnt signaling pathway may be integrated into the *lin-3/let-23* EGF signaling pathway at the level of LIN-3 signal, LET-23 receptor or *egl-5* transcription. Thus the temporal and spatial co-ordination and interactions between the Wnt signal, EGF signal and HOM-C transcription factor are important for P12 fate specification.

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