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egl-27 generates anteroposterior patterns of cell fusion in *C. elegans* by regulating *Hox* gene expression and Hox protein function

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SUMMARY

***Hox* genes pattern the fates of the ventral ectodermal Pn.p cells that lie along the anteroposterior (A/P) body axis of *C. elegans*. In these cells, the *Hox* genes are expressed in sequential overlapping domains where they control the ability of each Pn.p cell to fuse with the surrounding syncytial epidermis. The activities of Hox proteins are sex-specific in this tissue, resulting in sex-specific patterns of cell fusion: in hermaphrodites, the mid-body cells remain unfused, whereas in males, alternating domains of syncytial and unfused cells develop. We have found that the gene *egl-27*, which encodes a *C. elegans* homologue of a chromatin regulatory factor, specifies these patterns by regulating both *Hox* gene expression and Hox protein function. In *egl-27* mutants, the expression domains of *Hox* genes in these cells are shifted posteriorly, suggesting that *egl-27* influences A/P positional information. In addition, *egl-27* controls Hox protein function in the Pn.p cells in two ways: in hermaphrodites it inhibits MAB-5 activity, whereas in males it permits a combinatorial interaction between LIN-39 and MAB-5. Thus, by selectively modifying the activities of Hox proteins, *egl-27* elaborates a simple *Hox* expression pattern into complex patterns of cell fates. Taken together, these results implicate *egl-27* in the diversification of cell fates along the A/P axis and suggest that chromatin reorganization is necessary for controlling Hox gene expression and Hox protein function.**

Key words: *Caenorhabditis elegans*, *egl-27*, *Hox* genes, Pattern formation, Post-translational regulation, Sexual dimorphism, MTA1, NURD

INTRODUCTION

Homeotic selector (*Hox*) genes encode homeodomain transcription factors that specify regional identities along the A/P axis of many animals (see review by McGinnis and Krumlauf, 1992). In *C. elegans*, *Hox* genes achieve this by regulating a diverse array of cell fate decisions, including cell migration, proliferation, differentiation, apoptosis and fusion (reviewed by Kenyon et al., 1997). Genes of the *C. elegans Hox* cluster include *ceh-13*, *lin-39*, *mab-5* and *egl-5*, whose *Drosophila* homologues are *labial* (*lab*), *Sex combs reduced* (*Scr*), *Antennapedia* (*Antp*) and *Abdominal-B* (*Abd-B*) respectively (Clark et al., 1993; Costa et al., 1988; Wang et al., 1993). Two additional *Abd-B* homologues of unknown function have also been identified by the *C. elegans* genome project (see Ruvkun and Holbert, 1998). Like their homologues in insects and vertebrates, *lin-39*, *mab-5* and *egl-5* are expressed in sequential domains along the A/P axis where they generate position-specific pattern elements (reviewed by Kenyon et al., 1997).

Despite its small repertoire of *Hox* genes, *C. elegans* generates considerable cellular diversity along the A/P axis during development. Some of this arises from the choice of *Hox* gene expressed; for example, although the migratory QL and QR neuroblasts are bilateral homologues, the descendants of QR migrate anteriorly because they express *lin-39* whereas the descendants of QL migrate posteriorly because they express *mab-5* (Clark et al., 1993; Kenyon, 1986; Salser and Kenyon, 1992; Wang et al., 1993). Additional cellular diversity derives from the spatial and temporal variation in the expression of a single *Hox* gene in distinct cell lineages. For instance, the different lineages of the lateral ectodermal cells V5 and V6, which generate sensory rays in males, are specified by a single *Hox* gene, *mab-5* (Kenyon, 1986). In the V5 lineage, *mab-5* expression is switched on and off repeatedly to specify patterns of cell division, cell differentiation or cell morphology. In contrast, in the V6 lineage, *mab-5* is continually expressed and generates a pattern of cell fates that is distinct from that of V5 (Salser and Kenyon, 1996).

Although intricate *Hox* gene expression patterns make a significant contribution to the formation of the A/P pattern in *C. elegans*, they are not sufficient to generate all its regional diversity. A/P pattern is further diversified by specific alterations in the activities of Hox proteins in specific cells once they are expressed. For example, *mab-5* is expressed in the posterior Pn.p cells of both hermaphrodites and males, but is prevented from acting in the Pn.p cells of the hermaphrodite (Salser et al., 1993). In another case, both *lin-39* and *mab-5* are expressed in a subset of the Pn.aap cells, but *lin-39* prevents *mab-5* from acting in these cells (Salser et al., 1993). In a third

example, *lin-39* and *mab-5* act combinatorially in male Pn.p cells to specify a fate that differs from that produced by either alone (Clark et al., 1993; Salser et al., 1993; Wang et al., 1993). How the activities of these Hox proteins are controlled post-translationally with this kind of specificity is not known.

Post-translational controls of Hox protein function play a prominent role in pattern formation in the Pn.p cells. In this study, we have investigated the regulation of the *Hox* genes during the development of the Pn.p cells (P1.p to P12.p; Fig. 1A) that are located in a row along the ventral epidermis. The Pn.p cells are born at the end of the first larval stage; each Pn.p cell is the posterior daughter of a P cell. Soon after their birth, these cells either fuse with the epidermal syncytium known as hyp7, or remain unfused (Sulston and Horvitz, 1977). Pn.p cells that fuse adopt a syncytial fate, whereas many of the cells that remain unfused divide later in development to generate reproductive structures. In hermaphrodites, cells in the mid-body region remain unfused; these cells become the 'vulval equivalence group' and subsequently generate the vulva (Sulston and Horvitz, 1977). In males, cells that remain unfused in the posterior body region become the 'male equivalence group' and subsequently generate the hook and other copulatory structures (Sulston and Horvitz, 1977).

In wild-type hermaphrodites (Fig. 1B), the pattern of Pn.p cell fusion is governed by *lin-39*, the *Hox* gene that directs cell fates in the mid-body region (Clark et al., 1993; Wang et al., 1993). Pn.p cells in the mid-body that express *lin-39* remain unfused. Conversely, Pn.p cells that do not express *lin-39* fuse with hyp7, adopting a syncytial fate. In males, the pattern of Pn.p cell fusion is more elaborate (Fig. 1C). As in hermaphrodites, some of the Pn.p cells in males are kept unfused by *lin-39* (Fig. 1C) (Clark et al., 1993; Wang et al., 1993). In addition, certain other Pn.p cells in males are kept unfused by *mab-5*, the *Hox* gene that specifies cell fates in the posterior body region (Kenyon, 1986). Thus, in males, anterior Pn.p cells that do not express *lin-39* or *mab-5* adopt the syncytial fate, whereas more posterior Pn.p cells that express either *lin-39* in the mid-body or *mab-5* in the posterior remain unfused. Interestingly, in the region where *lin-39* and *mab-5* expression overlap, they act combinatorially, causing Pn.p cells expressing both *lin-39* and *mab-5* to adopt the syncytial fate (Clark et al., 1993; Salser et al., 1993; Wang et al., 1993); this contrasts with the individual activities of *lin-39* or *mab-5* that keep Pn.p cells unfused.

mab-5 is able to keep Pn.p cells unfused in males; however, it is unable to do so in hermaphrodites. This difference is not due to differences in the expression pattern of *mab-5* because MAB-5 protein distribution in the Pn.p cells is identical in both sexes at the time of the fusion decision (Salser et al., 1993) (compare the hatched backgrounds in 1B and 1C). Instead, MAB-5 protein function is somehow inhibited in the Pn.p cells of hermaphrodites but not males, generating the sexually dimorphic Pn.p fusion patterns (Sulston and Horvitz, 1977) (compare pattern of black and white circles in 1B and 1C).

Thus, establishing the male and hermaphrodite patterns of Pn.p cell fusion requires regulation of *Hox* genes at two levels. First, it requires that the *Hox* genes be expressed in the correct Pn.p cells. Second, it also requires that the function of Hox proteins be regulated after they are expressed. Thus, MAB-5 can be expressed but inhibited in hermaphrodites, and LIN-39 and MAB-5 proteins can act together to specify a new cell fate in males.

How is the sex- and cell-specific regulation achieved? In this study, we have found that the gene *egl-27* controls both the expression and function of *Hox* genes in the Pn.p cells. Our findings indicate that *egl-27* specifies the normal expression domains of the *Hox* genes *lin-39*, *mab-5* and *egl-5* in the Pn.p cells, suggesting that it plays a role in specifying A/P positional information in this tissue. In addition, *egl-27* is required for both types of post-translational Hox regulation that take place within this tissue; that is, the hermaphrodite-specific inhibition of MAB-5 function and the combinatorial interactions between LIN-39 and MAB-5 in the male. Without the post-translational controls on Hox function, *Hox* gene activity in the Pn.p cells is regulated only at the level of gene expression, and a much simpler pattern of cell fates is generated. Thus, by regulating the expression of *Hox* genes and by influencing the functions of Hox proteins after they are expressed, *egl-27* plays a key role in the generation of regional diversity in this tissue.

Recent studies have shown that *egl-27* encodes nuclear proteins homologous to MTA1, a component of NURD (nucleosome remodeling and histone deacetylation), a complex with chromatin remodeling and histone deacetylase activities (Herman et al., 1999; Solari et al., 1999; Xue et al., 1998). Thus, our results suggest that changes in chromatin structure are required for position-specific *Hox* gene expression as well as for cell- and sex-specific modulation of Hox protein function.

MATERIALS AND METHODS

General methods and strains

Strains were cultured using standard methods (Brenner, 1974; Wood, 1988). All strains were maintained at 20°C. Males were generated by the *him-5(e1490)* mutation, which causes spontaneous generation of males; this mutation does not affect larval development. The following alleles/transgenic constructs were used in this study:

LG II: *egl-27*(*mn553*)

LG III: *dpy-17*(*e164*), *mab-5*(*e2088*), *mab-5*(*e1751gf*), *lin-39*(*n1760*), *mab-5*(*e1239*)

LG IV: *him-5*(*e1490*), *muIs13*[*rol-6*(d), *egl-5::lacZ*]

mab-5(*e2088*) and *lin-39*(*n1760*) are genetic and protein null alleles (Clark et al., 1993; Costa et al., 1988; Maloof and Kenyon, 1998). A strong but non-null allele of *egl-27* by genetic and molecular criteria, *egl-27*(*mn553*), was used for this study; it harbors a premature stop codon in the largest transcript of *egl-27* but does not affect smaller transcripts from the *egl-27* locus (Herman et al., 1999). We were not able to analyze larval phenotypes when *egl-27* activity is further reduced because of embryonic lethality (Herman et al., 1999; Solari et al., 1999). Another allele, *egl-27*(*n170*), also causes hermaphrodite Pn.p fusion phenotypes similar to *egl-27*(*mn553*) (Q. C., S. Alper and C. K., unpublished results).

Genetics and strain construction

Standard methods were used for strain construction. Double mutants between *egl-27* and the *Hox* genes were generated by crossing homozygous *Hox* mutants or heterozygous *Hox* mutants over a marked chromosome into *egl-27(mn553)* or *egl-27(mn553); dpy-17(e164)* hermaphrodites. Some of these strains also contained the *him-5(e1490)* mutation. *dpy-17* is linked to the *Hox* cluster and served to genetically mark the absence of *Hox* mutations. Double mutants were isolated by picking animals that displayed Egl-27 phenotypes as well as the phenotypes of the respective *Hox* mutant or the absence of the *dpy-17* marker. In cases where the *dpy-17* marker was used, the putative

double mutant was confirmed by examining them for diagnostic phenotypes. Some of these double mutant isolates retained the *him-5(e1490)* mutation and were used to analyze the male phenotypes.

Immunofluorescence and β-galactosidase staining

MH27 immunofluorescence was used to determine the syncytial or unfused fate of the Pn.p cells (Kenyon, 1986). Immunofluorescence with monoclonal antibodies to the MH27 epitope, polyclonal antibodies to LIN-39 and MAB-5 as well as β-galactosidase staining were performed as described previously (Maloof and Kenyon, 1998; Maloof et al., 1999; Salser and Kenyon, 1996; Salser et al., 1993). In all cases, animals were also stained with DAPI to visualize and facilitate identification of cell nuclei. Staged populations of animals were examined at the end of L1 to the beginning of L2 as determined by the pattern of V cell descendants. Males were identified by the nuclear morphology of the B descendants in the tail.

Lineage analysis and microscopy

Larvae were mounted and observed using Nomarski/DIC optics; cell lineage analysis was performed as described in Sulston and Horvitz (1977). Images were collected from a Zeiss Axiophot microscope with a Photometrics Imagepoint CCD camera.

RESULTS

Mutations in the *C. elegans* gene *egl-27* were first isolated in a screen for egg-laying defective mutants (Trent et al., 1983). More recently, *egl-27* was found to encode nuclear protein isoforms similar to a protein encoded by *mta1* (Herman et al., 1999; Solari et al., 1999), whose expression is elevated in metastatic tumor cell lines and functions as a component of NURD, a human chromatin regulatory complex (Toh et al., 1994; Xue et al., 1998). *egl-27* appears to be widely expressed and is involved in the control of cell polarity, cell migration and embryonic morphogenesis (Herman et al., 1999; Solari et al., 1999). In this study, we have investigated the role of *egl-27* in generating patterns of cell fusion in the ventral epidermis during early larval development by examining changes in these patterns in animals bearing a strong reduction of function allele of *egl-27* (see Materials and Methods).

egl-27 is required for inhibition of MAB-5 protein activity in hermaphrodite Pn.p cells

Previous work showed that the wild-type Pn.p cell fusion pattern arises through the regulation of both *Hox* gene expression and Hox protein activity. In both wild-type males and hermaphrodites, *lin-39* is expressed in P(3-8).p in the mid-body and *mab-5* is expressed in an overlapping domain that spans P(7-11).p in the posterior (Fig. 1B, C) (Maloof and Kenyon, 1998; Salser et al., 1993). At the beginning of the second larval stage, only P(3-8).p in the mid-body remain unfused in wild-type hermaphrodites (Fig. 2A) (Sulston and Horvitz, 1977). The posterior Pn.p cells adopt the syncytial fate despite *mab-5* expression because the activity of MAB-5 protein is inhibited in hermaphrodite Pn.p cells (Salser et al., 1993).

These cell fusions can be monitored by immunofluorescence; unfused cells can be labeled with the adherens junction-specific monoclonal antibody MH27, whereas syncytial cells are not labeled (Kenyon, 1986). We examined the fates of the Pn.p cells in *egl-27* hermaphrodites by MH27 immunostaining and found that not only were P(3-8).p in the mid-body unfused, 40-50% of P(9-11).p cells in the posterior remained unfused as well (Fig. 2A).

Since the extra unfused Pn.p cells in *egl-27* hermaphrodites were located in the *mab-5* expression domain, these cells might be kept unfused because MAB-5 was activated inappropriately in the Pn.p cells of *egl-27* hermaphrodites. To test this, we expressed *mab-5* in all Pn.p cells using the *mab-5(e1751gf)* mutation, a promoter mutation in *mab-5* that results in ectopic *mab-5* expression in many cells (Salser and Kenyon, 1992; Salser et al., 1993), and asked if ectopic *mab-5* expression in P(1-2).p could keep these cells unfused in *egl-27* hermaphrodites. P(1-2).p were ideal for this purpose because they lie in the anterior and do not express either *lin-39* or *mab-5* in either wild-type or *egl-27* animals (Table 1); consequently, they always fuse with hyp7. In control *mab-5(e1751gf)* hermaphrodites, P(1-2).p still adopted the syncytial fate even though they expressed *mab-5* (Fig. 2B); in fact, the Pn.p cell fusion pattern of *mab-5(e1751gf)* hermaphrodites was indistinguishable from that of wild-type hermaphrodites. In contrast, P(1-2).p often remained unfused (approx. 60%) in *egl-27; mab-5(e1751gf)* hermaphrodites, demonstrating that these cells could respond to MAB-5 protein in this an *egl-27* mutant background (Fig. 2B).

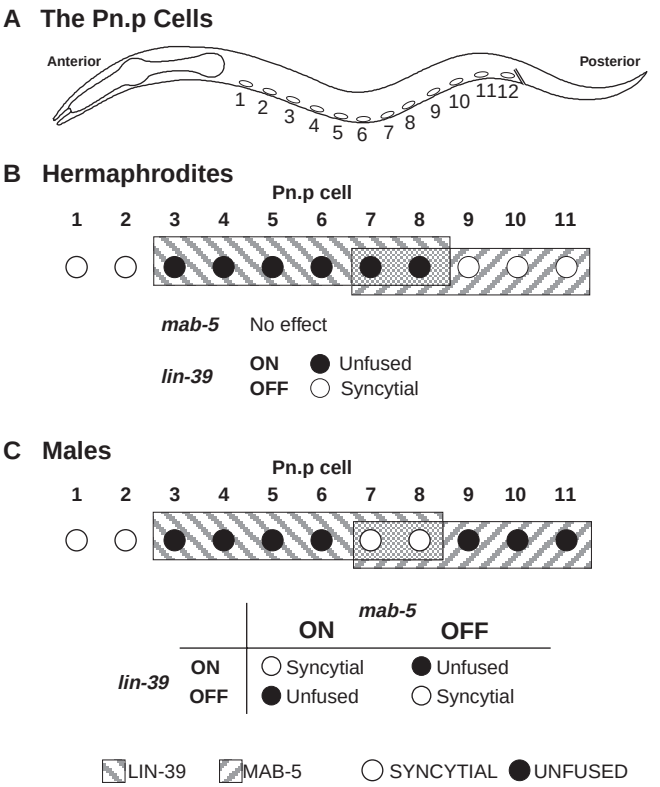

Fig. 1. *Hox* genes pattern Pn.p cell fates in *C. elegans*. (A) A drawing of a *C. elegans* larva showing the 12 ectodermal Pn.p cells (numbered 1-12) located in the ventral cord at the end of the first larval stage. (B) The pattern of Pn.p cell fusion and *Hox* gene expression in hermaphrodites. (C) Pattern of Pn.p cell fusion and *Hox* gene expression in males. See text for details. Hatched backgrounds indicate *lin-39* and *mab-5* expression domains; overlaps are shaded. White circles represent the syncytial fate and black circles represent the unfused fate.

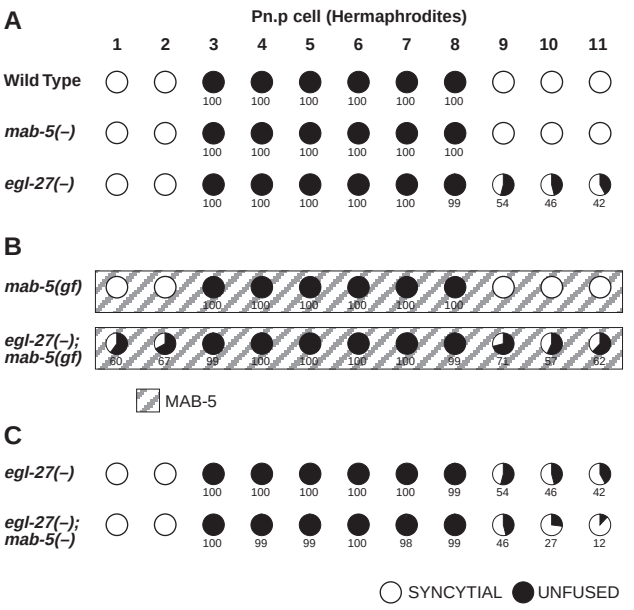

Fig. 2. MAB-5 is active in *egl-27* hermaphrodites. Pie charts show the frequency of Pn.p cells adopting the syncytial (white) or unfused (black) fate;. Numbers below the pie charts refer to the actual percentages of unfused fates; only percentages >0 are shown. (A) The Egl-27 phenotype in hermaphrodite Pn.p cells. (B) Effect of expressing *mab-5* (hatched background) in all Pn.p cells in hermaphrodites using *mab-5(gf)* in the absence (top) and presence (bottom) of an *egl-27* mutation. (C) Frequency of unfused Pn.p cells in *egl-27* and *egl-27; mab-5* hermaphrodites (data for *egl-27* hermaphrodites reproduced from A). The differences in P10.p and P11.p between these two strains are significant (P value = 0.01 and <0.0001 for P10.p and P11.p respectively, Fisher's Exact Test). Number and genotype of hermaphrodites examined: wild-type, n=90. *egl-27(mn553)*, n=85. *egl-27(mn553); mab-5(e2088)*, n=83. *mab-5(e2088); him-5(e1490)*, n=67. *mab-5(e1751gf)*, n=83. *egl-27(mn553); mab-5(e1751gf)*, n=92.

We confirmed that endogenous *mab-5* activity prevented Pn.p cell fusion in *egl-27* hermaphrodites by examining *egl-27; mab-5* double mutant hermaphrodites. If inappropriate MAB-5 activity contributed to unfused P(9-11).p cells, then removing *mab-5* would be expected to cause them to adopt the syncytial fate. In *egl-27; mab-5* hermaphrodites, the frequency of P(10-11).p cells adopting the syncytial fate in the posterior was significantly increased (compare *egl-27* to *egl-27; mab-5* animals in Fig. 2C), indicating that some of the P(10-11).p cells were kept unfused by inappropriate MAB-5 protein activity. P9.p, however, seemed to be unaffected by the removal of *mab-5* in the *egl-27* background. Surprisingly, some of the P(9-11).p cells in the posterior remained unfused in *egl-27; mab-5* double mutant hermaphrodites (Fig. 2C), indicating that another aspect of Pn.p cell fate regulation was also defective in *egl-27* mutants.

Wild-type *egl-27* represses *lin-39* expression in the posterior Pn.p cells

What other aspect of Pn.p cell fate might be disrupted in *egl-27* hermaphrodites? Since Pn.p cells are normally kept unfused by either *lin-39* or *mab-5*, the remaining unfused posterior Pn.p cells in *egl-27; mab-5* hermaphrodites could be kept unfused because of *lin-39* activity. However, *lin-39* is not normally expressed in these posterior Pn.p cells; thus, we examined *egl-27* mutants for changes in *lin-39* expression using polyclonal antibodies specific to LIN-39 protein (Maloof and Kenyon, 1998). In wild-type animals of both sexes, LIN-39 immunostaining is restricted to P(3-8).p in the mid-body region (Fig. 3A; Table 1) (Maloof and Kenyon, 1998). In *egl-27* hermaphrodites and males, LIN-39 immunostaining was observed in P(3-8).p; in addition, LIN-39 immunostaining also extended posteriorly to P(9-10).p in 20-30%

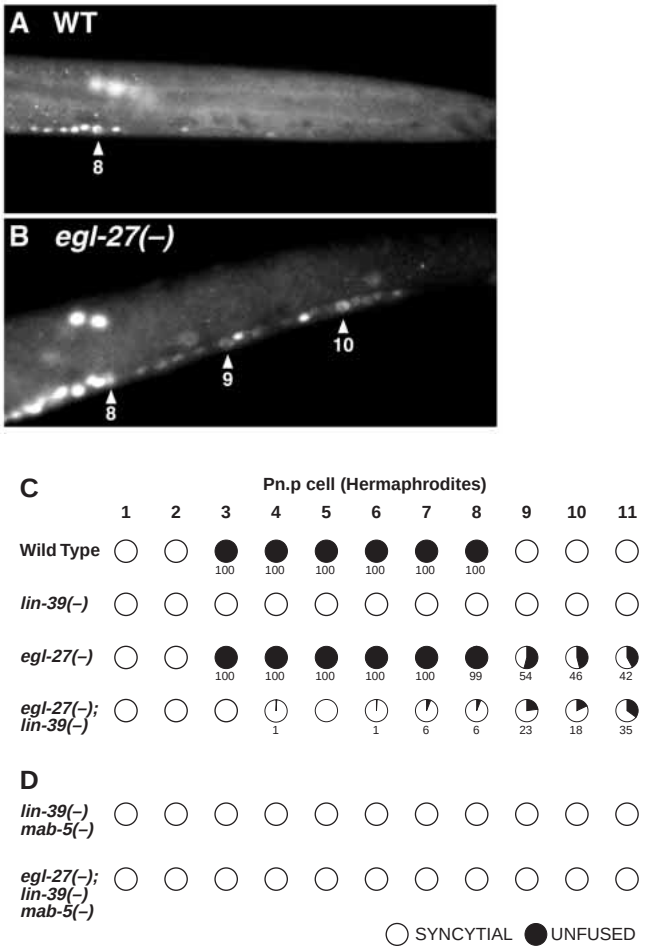

Fig. 3. *lin-39* is misexpressed in the posterior Pn.p cells in *egl-27* mutants. The expression of LIN-39 was determined by LIN-39 immunostaining. (A,B) LIN-39 immunostaining in the posterior region of a wild-type hermaphrodite (A) and an *egl-27(mn553)* hermaphrodite (B). Anterior is to the left. Arrowheads and numbers indicate the position of the Pn.p cells. See Table 1 for the quantification of cells staining. (C) The frequency of unfused Pn.p cells in *egl-27* hermaphrodites and *egl-27; lin-39* hermaphrodites; the difference between cell fusion frequencies are statistically significant (P value = 0.0001 and <0.0001 for P9.p and P10.p respectively, Fisher's Exact Test). (D) The Pn.p fusion phenotype in *lin-39 mab-5* and *egl-27; lin-39 mab-5* hermaphrodites. White, syncytial fate. Black, unfused fate. Actual percentage of unfused cells are indicated below each pie chart. The data for wild-type and *egl-27* hermaphrodites was reproduced from Fig. 2A. Number and genotypes of hermaphrodites examined: *egl-27(mn553); lin-39(n1760)*, n=82. *lin-39(n1760); him-5(e1490)*, n=73. *egl-27(mn553); lin-39(n1760) mab-5(e1239)*, n=86. *lin-39(n1760) mab-5(e1239); him-5(e1490)*, n=64.

Table 1 – Hox Gene Expression

Genotype	Pn.p cell staining (%)												*n*
	1	2	3	4	5	6	7	8	9	10	11	12	
anti-LIN-39 staining													
Wild type			100	100	100	100	100	100					32
him-5			100	100	100	100	100	100					43
egl-27; him-5			100	100	100	100	100	100	23	10			69
egl-27; mab-5(gf); him-5			100	100	100	100	100	100	26	4			27
anti-MAB-5 staining													
him-5							66	77	100	100	100		80
egl-27; him-5							5	40	73	77	78	15	78
egl-5::lacZ staining*													
him-5												82	384
egl-27; him-5												58	643

lin-39 and *mab-5* expression were examined in both sexes in these mutant strains. A *him-5* mutation was used to increase the frequency of males in these strains (see Materials and Methods). The pattern of expression in wild-type hermaphrodites and *him-5(e1490)* males are shown for comparision. In all strains examined, no detectable difference in the expression patterns of *lin-39* and *mab-5* was observed between the sexes; the numbers shown represent data combined from both sexes that were scored separately. *n*, number of animals scored. *These strains also contain *muIs13*, a chromosomal insertion bearing *egl-5::lacZ* and *rol-6(d)* (Wang et al., 1993).

of these animals (Fig. 3B; Table 1). Consistent with the misexpression of LIN-39 in the posterior, at a low frequency (4/102), P(9-10).aap cells in the posterior of *egl-27* males produced a serotonergic neuron (data not shown), a fate specified by *lin-39* in P(3-8).aap in the mid-body (Clark et al., 1993; Hunter and Kenyon, 1995; Wang et al., 1993).

To confirm that *lin-39* misexpression was responsible for the unfused cells in the posterior of *egl-27* hermaphrodites, we compared the Pn.p fusion patterns of *egl-27; lin-39* hermaphrodites to that of *egl-27* hermaphrodites. P(9-10).p in the posterior adopted the syncytial fate at significantly higher frequencies in *egl-27; lin-39* hermaphrodites (Fig. 3C), indicating that misexpression of *lin-39* in the posterior was partially responsible for keeping some of these Pn.p cells unfused.

lin-39 and *mab-5* were the only factors involved in keeping Pn.p cells unfused in *egl-27* hermaphrodites because in *egl-27; lin-39 mab-5* triple mutant hermaphrodites, all the Pn.p cells adopted the syncytial fate, as in *lin-39 mab-5* double mutant hermaphrodites (Clark et al., 1993; Wang et al., 1993) (Fig. 3D). Together, these results indicate that the Pn.p cells remained unfused in *egl-27* hermaphrodites solely because of ectopic activity of MAB-5 protein in the hermaphrodite and ectopic expression of *lin-39* in the posterior Pn.p cells.

egl-27 is required for the combinatorial interaction between LIN-39 and MAB-5 in male Pn.p cells

In males, the Pn.p cell fusions are governed combinatorially by both *lin-39* and *mab-5* to generate alternate regions of syncytial and unfused cells along the A/P axis (Fig. 1C) (Clark et al., 1993; Salser et al., 1993; Wang et al., 1993). The expression domains of *lin-39* and *mab-5* overlap in P(7-8).p and these two cells adopt the syncytial fate because LIN-39 and MAB-5 cancel out one another's activities within these cells (Salser et al., 1993). Since *lin-39* expression extended posteriorly in *egl-27* animals of both sexes, one might expect a larger overlap between the domains of *lin-39* and *mab-5* in *egl-27* animals. This would be expected to increase the frequency of posterior Pn.p cells in *egl-27* males adopting the syncytial fate. Contrary to this expectation, P(3-11).p in *egl-27* males remained unfused very frequently (>90%) (Fig. 4A), raising the possibility that where *lin-39* and *mab-5* expression overlapped, LIN-39 and MAB-5 activities were failing to cancel out in some of these cells, allowing Hox protein activity to prevent cell fusion.

This hypothesis was tested using the *mab-5(e1751gf)* mutation to express *mab-5* in all the Pn.p cells in *egl-27* males, thereby causing the Pn.p cells already expressing *lin-39* to also express *mab-5*. As previously shown by Salser et al. (1993), in males with the *mab-5(e1751gf)* mutation alone, P(1-2).p in the anterior and P(9-11).p in the posterior, expressed only *mab-5* and remained unfused whereas P(3-8).p in the mid-body expressed both *mab-5* and *lin-39* and adopted the syncytial fate, illustrating the mutual cancellation of these two Hox protein activities in these cells (Fig. 4B). In contrast, in *egl-27; mab-5(e1751gf)* males, P(3-8).p in the mid-body remained unfused at high frequency (>85%) even though they expressed both *lin-39* and *mab-5* (Fig. 4B). In these animals, LIN-39 was present in P(3-8).p as determined by anti-LIN-39 staining (Table 1); P(9-10).p in the posterior also expressed LIN-39 occasionally, due to the misexpression of *lin-39* caused by the *egl-27* mutation. The observation that P(1-2).p in the anterior were unfused in these double mutant males confirmed that the *mab-5(e1751gf)* mutation was capable of ectopically expressing *mab-5* in the Pn.p cells even in the *egl-27* background. We conclude that *egl-27* is required for LIN-39 and MAB-5 to interact combinatorially to neutralize each other's protein activities.

Wild-type *egl-27* activates *mab-5* expression in the posterior Pn.p cells

Although the defect in LIN-39/MAB-5 neutralization could account for most of the unfused Pn.p cells in *egl-27* males, this alone could not explain why P9.p, which lies outside of the normal overlap domain, occasionally adopted the syncytial fate in *egl-27* males (Fig. 4A). This phenotype might be due to *mab-5* expression defects in posterior Pn.p cells.

Indeed, in immunostaining experiments using polyclonal antibodies specific to MAB-5 (Salser and Kenyon, 1996), we found defects in *mab-5* expression in the Pn.p cells of *egl-27* animals. In wild-type males and hermaphrodites, we observed a graded pattern of MAB-5 staining that extended from P7.p (low expression) to P11.p (high expression) (Fig. 5B; Table 1), similar to that reported previously (Salser et al., 1993). In

contrast, in *egl-27* mutants of both sexes, MAB-5 immunostaining was reduced or absent in some of the Pn.p cells and ventral cord neurons (Fig. 5C; Table 1). This defect in *mab-5* expression was also consistent with the observation that the P(11-12).aaap cells in *egl-27* animals occasionally (2/14) failed to undergo apoptosis (data not shown) because this apoptotic fate requires *mab-5* (Kenyon, 1986).

To verify that this defect in *mab-5* expression was reflected in cell fate changes in the Pn.p cells, we compared the pattern of unfused cells in *lin-39* males to *egl-27; lin-39* males because removing *lin-39* reveals the contribution of *mab-5* to the unfused Pn.p cells (Fig. 5A) (Clark et al., 1993; Wang et al., 1993). Without *lin-39*, the only factor that keeps Pn.p cells unfused is *mab-5*; thus, only Pn.p cells that express *mab-5* will remain unfused. In *lin-39* males, P(7-11).p in the posterior remained unfused, corresponding to *mab-5* activity in these cells (Fig. 5A) (Clark et al., 1993; Wang et al., 1993). In *egl-27; lin-39* males, the frequency of unfused P(7-11).p cells in the posterior was reduced relative to those of *lin-39* males (Fig. 5A), consistent with the hypothesis that the functional domain of *mab-5* was reduced in *egl-27* mutants.

Thus, we infer that the defects in the pattern of Pn.p cell fusion in *egl-27* males were caused by changes in the expression domains of *lin-39* and *mab-5* as well as the inability of LIN-39 and MAB-5 protein to neutralize one another's activities where their expression domains overlap.

Wild-type *egl-27* activates *egl-5* expression in P12.p

P12.p is the most posterior Pn.p cell. Unlike P(1-11).p, it divides to give rise to two cells: one that adopts an epidermal fate and another that undergoes apoptosis (Fig. 6) (Sulston and Horvitz, 1977). To do this, P12.p requires the activity of *egl-5*, the *Hox* gene that patterns the body region posterior to the *mab-5* domain (Chisholm, 1991). In 2/7 *egl-27* animals, P12.p did not divide; instead, it adopted a fate similar to the more anterior Pn.p cells (Fig. 6), indicating that *egl-27* was also required for the P12.p fate. An *egl-5::lacZ* reporter was expressed less frequently in the P12.p cell of *egl-27* animals compared to wild-type controls (Table 1), suggesting that the P12.p phenotype in *egl-27* animals was due to an inability to express *egl-5*.

In the MAB-5 immunostaining experiments described above, we also observed that *mab-5* was ectopically expressed in 15% of P12.p cells in *egl-27* mutants (Table 1). This could be due to the defect in *egl-5* expression in P12.p since *egl-5* normally inhibits *mab-5* expression in P12.p (Salser et al., 1993).

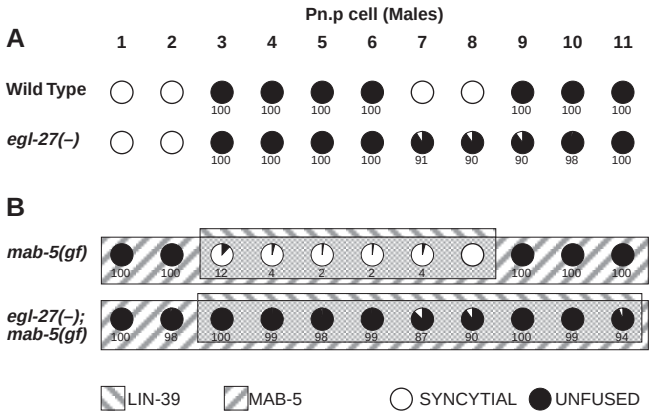

Fig. 4. *egl-27* is required for the combinatorial interaction between MAB-5 and LIN-39 in males. (A) The Pn.p cell fusion phenotype in *egl-27* males. (B) Effect of expressing *mab-5* in all Pn.p cells in males using *mab-5(gf)* in the absence (top) and presence (bottom) of an *egl-27* mutation. Black, unfused fate. White, syncytial fate. Hatched backgrounds indicate *lin-39* and *mab-5* expression domains; the overlaps are shaded. Actual percentage of unfused cells is indicated below each pie chart. Number and genotype of males scored: wild type, *n*=60. *egl-27(mn553)*, *n*=59. *mab-5(e1751gf)*, *n*=52. *egl-27(mn553); mab-5(e1751gf)*, *n*=56. In addition to the genotypes indicated, all these strains contain a *him-5(e1490)* mutation used to generate males.

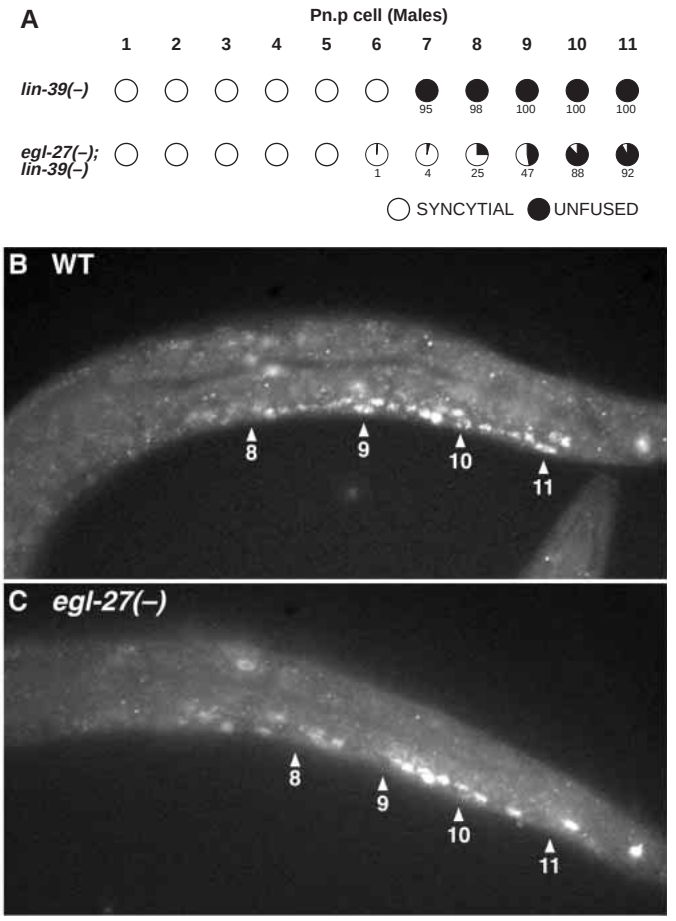

Fig. 5. *mab-5* expression is impaired in *egl-27* mutants. (A) The functional domain of *mab-5* as revealed by eliminating *lin-39* in the absence (top) or presence (bottom) of an *egl-27* mutation. The frequency of Pn.p cells adopting the syncytial (white) or unfused (black) fate are shown; actual percentage of unfused cells are indicated below each pie chart. Relevant genotypes are indicated. Number and genotype of males examined: *lin-39(n1760)*, *n*=65. *egl-27(mn553); lin-39(n1760)*, *n*=73. All strains here also contain a *him-5(e1490)* mutation used to generate males. (B) Posterior region of an *him-5* animal stained with anti-MAB-5 antibodies. (C) Posterior region of an *egl-27(mn553)* animal stained with anti-MAB-5 antibodies. Anterior is to the right. Pn.p cells are indicated by arrowheads and numbers. This staining is quantified in Table 1. Although MAB-5 staining in the P(7-8).p cells could be visualized under the microscope, it was faint and easily bleached, making it difficult to capture images of these cells exhibiting MAB-5 staining. MAB-5 expression in males and hermaphrodites of either genotype were indistinguishable.

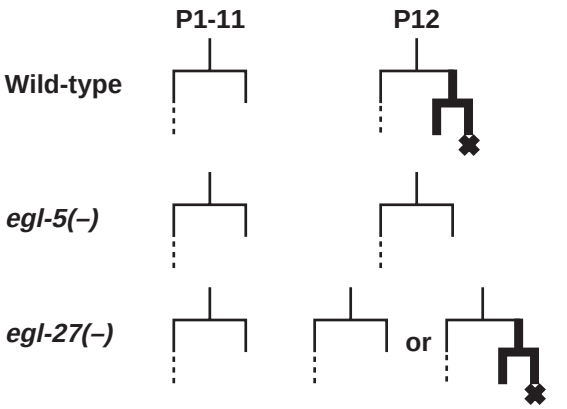

Fig. 6. *egl-27* mutants are defective in P12.p fate specification. The fate of the P cells in wild-type (top) and *egl-27* (bottom) hermaphrodites were observed with Nomarski/DIC microscopy. The bold lines indicate the *egl-5*-dependent fate of P.12.p. The phenotypes of *egl-5* mutants are reproduced from Chisholm (1991) for comparison. Normal P12.p lineages in two wild-type hermaphrodites were observed as a control. Seven lineages of P12 were performed on *egl-27* hermaphrodites. In 2/7 cases, P12.p failed to divide, adopting a fate similar to P1-11.p. In 4/7 cases P12.p adopted a wild-type fate. In 1/7 case, P12, the mother of P12.p, failed to descend into the ventral cord or divide.

DISCUSSION

In the ventral ectodermal Pn.p cells of *C. elegans*, a simple *Hox* gene expression pattern can, depending on the sex of the animal, give rise to either of two complex patterns of cell fates. This process requires both sex- and cell-specific controls on Hox protein activity. Our analyses of *egl-27* mutants implicate *egl-27* in both of these selective modifications of Hox protein function (Fig. 7A). In addition, we also found a posterior shift in the expression domains of three *Hox* genes in *egl-27* mutants (Fig. 7B), suggesting that *egl-27* influences the expression boundaries of these *Hox* genes (Fig. 7C). Taken together, the ability of *egl-27* to regulate *Hox* gene expression and Hox protein function in the ventral ectoderm imply a key role for *egl-27* in the diversification of cell fates along the A/P axis.

Recent studies have shown that EGL-27 is similar to MTA1, whose expression is elevated in metastatic cells. MTA1 and another related protein, MTA2, are components of complexes exhibiting chromatin remodeling and histone deacetylase activities (Xue et al., 1998; Zhang et al., 1998). This raises the possibility that the function of Hox transcription factors can be regulated by chromatin reorganization.

EGL-27 mediates two sex- and cell-specific controls of Hox protein function

In this study, we found that *egl-27* is required for both of the post-translational forms of Hox regulation that occur in the Pn.p cells. First, *egl-27* is required for the inhibition of MAB-5 activity in hermaphrodite Pn.p cells (Fig. 7A). In *C. elegans* hermaphrodites, the sex-determination pathway culminates in the activation of *tra-1* which acts cell-autonomously to specify hermaphrodite-specific cell fates (Hunter and Wood, 1990; Zarkower and Hodgkin, 1992). Since *tra-1* encodes zinc-finger transcription factor isoforms, Salser et al. (1993) postulated that *tra-1* might directly interfere with MAB-5's ability to bind a promoter or activate transcription. As there was no logical necessity for additional component(s) outside the sex-determination pathway for MAB-5 inhibition, we were surprised that this process also required *egl-27*. *egl-27* is not specifically involved in sex-determination because most of its other phenotypes occur in both sexes. Since MAB-5 inhibition involves both the sex-determination pathway and *egl-27*, *tra-1* (or another sex-specific factor) might interact directly or indirectly with *egl-27* to inhibit MAB-5 (Fig. 7A).

egl-27 is also required for the combinatorial interaction between LIN-39 and MAB-5 in male Pn.p cells (Fig. 7A). When multiple *Hox* genes function to specify a single body part, it is difficult to distinguish whether they do so by acting in distinct cells within that structure or by a combinatorial interaction within the same cells. In *C. elegans*, we were able to bypass this complication by examining the fate of single Pn.p cells that are controlled cell-autonomously by *mab-5* and *lin-39* (Clark et al., 1993; Kenyon, 1986).

How do LIN-39 and MAB-5 neutralize each other in this combinatorial interaction? This post-translational process is not regulated by nuclear access or protein degradation because it occurs when both LIN-39 and MAB-5 proteins are present in the nucleus of the same Pn.p cell (Maloof and Kenyon, 1998; Salser et al., 1993). Moreover, this neutralization has been shown to be insensitive to the relative levels of LIN-39 or MAB-5, arguing against the formation of inactive LIN-39/MAB-5

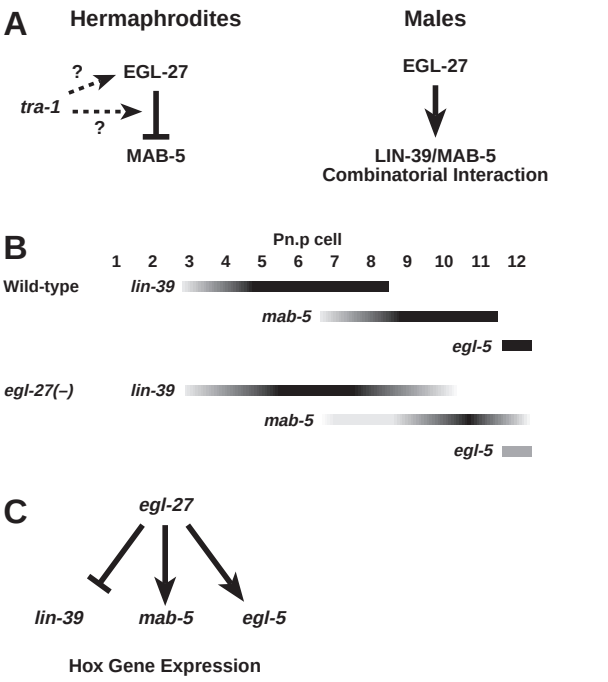

Fig. 7. *egl-27* controls *Hox* gene expression and Hox protein activity. (A) Sex- and cell-specific regulation of Hox protein activity. *egl-27* mediates MAB-5 inhibition in hermaphrodite Pn.p cells (left) and combinatorial interactions between LIN-39 and MAB-5 proteins in male Pn.p cells (right), possibly with a sex-specific factor such as *tra-1*. (B) Summary of the *lin-39*, *mab-5* and *egl-5* expression domains in wild-type and *egl-27* animals. Gray reflects a lower level or frequency of expression. (C) Regulation of *Hox* gene expression by *egl-27*.

heterodimers or competition between LIN-39 and MAB-5 for target promoter(s) or co-factor(s) (Salser et al., 1993). Instead, this suggests that the two proteins might act together on another target that is limiting, such as a promoter that could integrate their activities. Studies of other homeodomain proteins have suggested that Hox proteins can act combinatorially by direct interactions on target promoters. For example, in vitro cooperativity in DNA binding has been observed between purified homeodomain proteins such as UNC-86 and MEC-3 (Xue et al., 1993). However, the combinatorial interaction between LIN-39 and MAB-5 also requires EGL-27. It is interesting that another case of combinatorial control by homeodomain proteins also requires additional proteins: in diploid yeast, the combination of two homeodomain proteins, a1 and α2, mediates the repression of haploid-specific genes in a process that requires the transcriptional co-repressors SSN6 and TUP1 (Dranginis, 1990; Keleher et al., 1992; for a more extensive review, see Johnson, 1992).

Previous studies have shown that one mechanism of regulating Hox protein function involves cooperative DNA binding by Hox proteins and cofactors such as EXD (for review see Mann and Affolter, 1998). In addition, others have postulated that competition for co-factors or displacement from promoters could also inhibit Hox protein function (for example, see Andrew et al., 1994; McGinnis et al., 1998). The requirement for EGL-27, a putative chromatin reorganization factor, in controlling Hox protein function suggests that chromatin structure also influences Hox protein function. The involvement of EGL-27 in both types of Hox protein regulation in the Pn.p cells could be explained if EGL-27 activates multiple targets, one for each type of post-translational regulation. However, it is equally conceivable that these two controls on Hox protein activity operate in a fundamentally similar way. For example, EGL-27 (or other components of the NURD complex) might interact directly or indirectly with MAB-5 and/or LIN-39 on a promoter of a cell fusion target gene in a manner dependent on the activity of *tra-1* (or another sex-specific factor). Recruiting EGL-27 and the NURD complex to this promoter could direct its chromatin reorganization activities to this promoter and thus alter the transcriptional state of the target gene, resulting in different Pn.p fates.

Comparing *egl-27* to other regulators of Hox protein activity

Two of the best studied regulators of Hox protein function are *extradenticle* (*exd*) and *homothorax* (*hth*) in *Drosophila*. *exd* encodes a EXD/PBX class homeoprotein that increases the binding specificity and alters the transcriptional activities of Hox proteins (Chan et al., 1994; Peifer and Wieschaus, 1990; Pinsonneault et al., 1997; Rauskolb et al., 1993; van Dijk and Murre, 1994). *hth* encodes a TALE class homeoprotein that regulates the nuclear localization of EXD (Kurant et al., 1998; Pai et al., 1998; Rieckhof et al., 1997). These two genes regulate Hox function in a broad range of tissues. In contrast, several other genes encode spatial regulators of Hox protein function. For example, in *Drosophila, teashirt* and an isoform of *cap n' collar* are expressed in restricted spatial domains where they modify the function of *Scr* and *Deformed* respectively (Andrew et al., 1994; de Zulueta et al., 1994; Fasano et al., 1991; Mann and Abu, 1996; McGinnis et al., 1998; Mohler et al., 1995). *Drosophila* Hox proteins have also been shown to inhibit the activities of other Hox proteins (for example, see Gonzalez-Reyes and Morata, 1990), although the role of this inhibition during normal development is still obscure. In *C. elegans*, *lin-22* inhibits the response of the lateral ectodermal cells to MAB-5 in the anterior while *pal-1* potentiates it in the posterior (Salser and Kenyon, 1996). Thus, one function of these genes during A/P patterning is the position-specific modification of Hox protein function.

Unlike these position-specific regulators, *egl-27* does not provide spatial precision to the control of Hox protein function, as these controls seem to occur in both anterior and posterior Pn.p cells. Nor does *egl-27* appear to regulate Hox protein activity in a broad range of tissues, like *exd* or *hth*. Instead, these *egl-27*-dependent Hox protein controls appear to be highly cell-type specific: these post-translational Hox controls exerted by *egl-27* occur in the Pn.p cells but have not been observed in other cell types. In the hypodermal V and neuronal Q lineages, *mab-5* activity correlates with expression, indicating that, unlike the Pn.p cells, MAB-5 is not inhibited post-transcriptionally in these cells (Salser and Kenyon, 1992, 1996). Also, male Pn.aap cells expressing both *lin-39* and *mab-5* adopt the *lin-39* specific fate, suggesting that LIN-39 function overrides that of *mab-5* (Salser et al., 1993); this still occurs in *egl-27* mutants (data not shown). This kind of specificity may allow the Pn.p cells to be patterned independently of other cell types in the same region that also express the same *Hox* genes. The type of regulation performed by *egl-27* was also sex-specific: in hermaphrodites, *egl-27* inhibits MAB-5 function, whereas in males, it mediates the LIN-39/MAB-5 combinatorial interaction. This specificity thus generates sexual dimorphism and, by diversifying the cellular responses to Hox proteins, also allows *egl-27* to increase pattern complexity in the Pn.p cells along the A/P body axis.

Some Hox regulators specify Hox-independent fates in the same cells where they appear to control Hox protein function. For example, the fate of the anterior lateral ectodermal cells (V1-V4) in *C. elegans* require *lin-22* (Fixsen, 1985; Horvitz et al., 1983) but not the *Hox* genes *lin-39*, *mab-5* or *egl-5*. V1-V4 are normally refractory to MAB-5, but mutations in *lin-22* allow them to respond to MAB-5 (Salser and Kenyon, 1996). One explanation for this phenotype is that the mutant LIN-22 protein is no longer able to inhibit MAB-5. Alternatively, it is also possible that these cells can respond to other differentiation cues such as MAB-5 because they are no longer committed to the V1-V4 fate. Thus, it can be difficult to ascertain whether changes in the responsiveness of cells to Hox proteins are due to changes in the activities of Hox proteins themselves or to changes in the differentiated states of the cells in which Hox proteins find themselves. Such complications can be eliminated in the case of *egl-27* because the fate of each Pn.p cell was still a Hox-dependent binary fusion decision in *egl-27* animals; only the extent and effects of *Hox* gene expression were changed. Moreover, the division pattern and cell morphologies characteristic of the P lineages during the first and second larval stages were normal in *egl-27* mutants, except for occasional lineage defects in P11 and P12 (data not shown).

egl-27 specifies *Hox* gene expression domains in the ventral ectoderm

In this study, we also found that *egl-27* regulates the expression domains of three *Hox* genes in the posterior ventral ectoderm in highly specific ways: it represses *lin-39* expression but activates *mab-5* and *egl-5* expression (Fig. 7C). Thus, in *egl-27* mutants,

the expression domain of the mid-body *Hox* gene, *lin-39*, appeared to expand posteriorly at the expense of the posterior *Hox* gene, *mab-5* (Fig. 7B). We also observed corresponding fate changes in other cells of the ventral ectoderm in *egl-27* animals; some posterior Pn.aap and Pn.aaap cells seemed to adopt the fate of their more anterior homologues. Taken together, this suggests a broad role for *egl-27* in establishing *Hox* expression boundaries in the ventral ectoderm.

In wild-type animals, *lin-39* and *mab-5* do not regulate one another's expression in the Pn.p cells (Salser et al., 1993); this also appears to be the case in *egl-27* mutants. The ectopic expression of *lin-39* in *egl-27* animals was not due to the reduction in *mab-5* expression because it still occurred in *egl-27; mab-5(gf)* animals when *mab-5* was expressed in all Pn.p cells (Table 1). Nor was the reduction in the functional domain of *mab-5* due to expansion of *lin-39* because it was observed in *egl-27; lin-39* males (Fig. 5A). Thus, *egl-27* regulates *lin-39* and *mab-5* expression independently.

Currently, the mechanisms that establish *Hox* expression domains in *C. elegans* are still poorly understood. In *Drosophila*, position-specific *Hox* gene expression is controlled by gradients of morphogens within the embryonic syncytium. This mechanism does not appear to operate in *C. elegans*, where *Hox* gene expression in some cells seem to be controlled through lineal mechanisms. This has been shown for the *Hox* genes *mab-5* and *ceh-13* (Cowing and Kenyon, 1996; Wittmann et al., 1997) as well as for the *C. elegans even-skipped* homologue, *vab-7*, which patterns the posterior (Ahringer, 1996, 1997). In principle, the lineal mechanisms that direct the proper expression of these A/P-specific genes could operate via a tissue/cell polarity system (see Kenyon, 1994), such as the WNT signaling system that directs the asymmetric accumulation of the POP-1 transcription factor to specific daughter cells throughout many embryonic lineages (Lin et al., 1998, 1995; see Kenyon et al., 1997). In such a system, *egl-27* could alter chromatin structure in response to upstream polarity signals.

Although lineal mechanisms appear to be important for establishing *Hox* gene expression in *C. elegans*, there are reasons to think that A/P position may also play a role in this process in the Pn.p cells. The parents of the Pn.p cells, the P cells, are initially located in ventrolateral positions on the left and right sides; subsequently, they migrate into the ventral cord (Sulston and Horvitz, 1977). Most of these left-right pairs of P cells enter the ventral cord in a random fashion. Thus, the relative A/P positions adopted by each member of a bilateral pair of P cells are variable and not correlated with the side of origin. For instance, in a pair of P9/10 cells, the P cell that ends up in a more anterior position in the ventral cord becomes P9, whereas the P cell that ends up in the more posterior position becomes P10. Hence, in any individual animal, certain Pn.p cells could be derived from a P cell that originated from either the left or right side (Sulston and Horvitz, 1977). Previously, it was found that the expression levels of MAB-5 in the P9.p and P10.p cells correlated with their A/P position, which prompted Salser et al. (1993) to suggest that *Hox* gene expression in the Pn.p cells might be controlled by a cell extrinsic mechanism. This interpretation is supported by the observation that the effect of a mutation in *egl-27* tended to be more prominent in one cell of each pair of Pn.p cells and this also correlated with A/P position. For example, ectopic expression of *lin-39* was more frequently seen in P9.p than P10.p (Table 1). Similarly, in *egl-27; lin-39* males, the frequencies of the unfused fate increased from 4% in P7.p to 92% in P11.p with the intervening Pn.p cells adopting this fate at intermediate frequencies (Fig. 4C). Thus, we tend to favour the model that *egl-27* is part of a system of cell-extrinsic positional information that patterns the Pn.p cells. These A/P differences among the Pn.p cells could reflect requirements for different levels of *egl-27* activity along the A/P axis. Alternatively, *egl-27* activity levels could be similar in these cells but modulated by an A/P pre-pattern that influences that choice of *Hox* gene each Pn.p cell expresses.

egl-27, *Hox* genes and cell fate diversity

Hox genes are remarkably versatile; a single *Hox* gene can specify many cell fates over several distinct regions along the A/P axis. Some of this ability is derived from intricate variations in their expression patterns across distinct body regions. Other aspects of this versatility come from combinatorial interactions among *Hox* genes or other factors. In interactions between *lin-39* and *mab-5*, this strategy is extended by restricting specific interactions to specific cell types; for instance, in Pn.aap cells, LIN-39 is dominant over MAB-5, while in the Pn.p cells they neutralize each other in males (Salser et al., 1993). How do these cell-type-specific interactions arise? Since *Hox* genes regulate different cell fates decisions in different cell types, it was possible that all the specificity was encoded in the promoters of target genes. The finding that *egl-27* was required to mediate these interactions argues that the interaction between Hox proteins is not dictated solely by the target promoter or the type of cell fate decision, but also by mediators like EGL-27 that might be selectively activated to provide sex- and cell-specificity to the regulation of Hox protein activity.

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