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Anterior organization of the *Caenorhabditis elegans* embryo by the *labial*-like *Hox* gene *ceh-13*

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SUMMARY

The *Caenorhabditis elegans lin-39, mab-5* and *egl-5 Hox* genes specify cell fates along the anterior-posterior body axis of the nematode during postembryonic development, but little is known about *Hox* gene functions during embryogenesis. Here, we show that the *C. elegans labial*-like gene *ceh-13* is expressed in cells of many different tissues and lineages and that the rostral boundary of its expression domain is anterior to those of the other *Hox* genes. By transposon-mediated mutagenesis, we isolated a zygotic recessive *ceh-13* loss-of-function allele, *sw1*, that exhibits an embryonic sublethal phenotype. Lineage analyses and immunostainings revealed defects in the organization of the anterior lateral epidermis and anterior body wall muscle cells. The epidermal and mesodermal identity of these cells, however, is correctly specified. *ceh-13*(*sw1*) mutant embryos also show fusion and adhesion defects in ectodermal cells. This suggests that *ceh-13* plays a role in the anterior organization of the *C. elegans* embryo and is involved in the regulation of cell affinities.

Key words: *Caenorhabditis elegans*, *Hox* gene, *labial* class, Morphogenesis, Pattern formation, Cell affinity

INTRODUCTION

The *Hox* genes, which encode homeodomain-containing transcription factors, are involved in the specification of body plans of multicellular organisms (reviews, Kenyon et al., 1997; Lawrence and Morata, 1994; McGinnis and Krumlauf, 1992). In both insects and vertebrates, these genes are organized into a conserved chromosomal cluster from which they are segmentally expressed in a temporal and spatial order, termed colinearity, along the anterior-posterior (A-P) body axis of the embryo (Duboule and Morata, 1994). Lack of expression or ectopic expression of *Hox* genes can cause the change of one segment identity to another, a phenomenon referred to as homeotic transformation.

The *C. elegans Hox* genes, *lin-39*, *ceh-13*, *mab-5* and *egl-5*, are not tightly clustered, unlike the *Hox* genes in *Drosophila* and vertebrates (Bürglin and Ruvkun, 1993). However, functional conservation has been demonstrated between *lin-39* and the *Drosophila Hox* gene *Scr*, as well as between *mab-5* and *Drosophila Antp* in larvae and adult worms (Hunter and Kenyon, 1995). In addition, region-specific expression of the genes *lin-39*, *mab-5* and *egl-5* has been observed in embryos and larvae. Unlike their insect and vertebrate counterparts, however, the *lin-39*, *mab-5* and *egl-5* genes do not appear to be essential for embryogenesis. Instead, mutants defective for these genes show abnormal postembryonic development and sexual maturation (Chisholm, 1991; Chow and Emmons, 1994; Clark et al., 1993; Wang et al., 1993) Analyses of these genes have demonstrated that they are necessary to specify cell fates along the A-P axis of the worm, as well as to act as a developmental switch that controls cell migration (op. cit.; Salser and Kenyon, 1992).

CEH-13 possesses structural features typical of the *labial* or HOX1 class of proteins including characteristic residues inside and outside the homeodomain (Schaller et al., 1990; Sharkey et al., 1997). In insects and chordates, genes of the *labial* class have been shown to have multiple functions in the anterior part of the embryo (Carpenter et al., 1993; Dollé et al., 1993; Gavalas et al., 1998; Goddard et al., 1996; Hirth et al., 1998; Mark et al., 1993; Merrill et al., 1989; Studer et al., 1996).

Here we show that CEH-13 is present in many different cell types and its rostral boundary of expression is located anteriorly to those of the other *C. elegans Hox* genes. Furthermore, we describe the phenotypic analysis of *ceh-13*(*sw1*) mutants during *C. elegans* embryogenesis. Using a 'four-dimensional' (4D) recording system and immunocytochemistry, we demonstrate that *ceh-13* mutants show a disorganization of epidermal and mesodermal cells as well as adhesion defects in the anterior part of the embryo. These results suggest that the *ceh-13* gene is required to organize the anterior part of the *C. elegans* embryo.

MATERIALS AND METHODS

General methods and strains

C. elegans strains were cultured using standard conditions (Brenner, 1974). Wild-type worms correspond to Bristol, strain N2. The following mutations and rearrangements were used in this study: (LGI) *mut-2*(*r459*); (LGIII) *dpy-17*(*e164*), *ceh-13*(*pk20::Tc1*), *ceh-13*(*sw1*) (this study), *unc-32*(*e189*), *dpy-19*(*n1347*), *qC1* [(*dpy-19*(*e1259ts*) *glp-1*(*q339*)], *nDf16*; (LGIV) *lin-39::lacZ* (*muIs6*) (kindly provided by C. Hunter and C. Kenyon), *unc-119::gfp* (kindly provided by M. Maduro); (LGV) *him-5* (*e1490*).

Generation of an anti-CEH-13 rabbit polyclonal antibody

A 476 bp long *Nsi*I-*Xmn*I *ceh-13* fragment from Bar12C (see below) was cloned into the vector pQEB (pQE3.100ΔBamNsi) and expressed in the *E. coli* M15 strain. This clone encodes a 12.6 kDa 6His-CEH-13 fusion protein containing the amino acids 97 to 202 of CEH-13. Rabbit polyclonal antibodies were raised against the PQE3.100ΔBamNsi protein. Anti-CEH-13 antibodies were affinity-purified using standard methods (Harlow and Lane, 1988).

Immunofluorescence

Embryos were immunostained as reported by Miller and Shakes (1995). *ceh-13* immunolocalization was performed as described by Wittmann et al. (1997) or as follows. Embryos were collected by cutting gravid hermaphrodites in water and transferring them to poly-L-lysine slides. After having removed most of the liquid, coverslips were applied by light squashing. The slides were frozen on dry ice for 10 minutes, the coverslip flicked off and the slides put in –20°C acetone for 4 minutes. The tissues were rehydrated through an acetone series, placed in phosphate-buffered saline (PBS) for 1 minute and washed in PBS with 0.1% Tween20 (PBST) for 15 minutes. Larvae were processed for immunostaining in 1-2% paraformaldehyde as described by Finney and Ruvkun (1990). Incubation with primary antibodies was performed O/N at 4°C in antibody buffer A (Finney and Ruvkun, 1990) that contained goat serum (DAKO X907; ≥ 1:2000 dilution). Primary antibodies were used at the following working dilutions: anti-CEH-13, from 1:40 to 1:100 and monoclonal anti-β-galactosidase (Sigma G8021) at 1:1500. The other antibodies used were the MH27 antibody (Francis and Waterston, 1985), α-LIN-26 antibody (Labouesse et al., 1996), NE2-1B4 antibody (Hutter and Schnabel, 1994) and the mAb5-6 antibody (Miller et al., 1983).

CEH-13-positive cells were determined either by doubly immunostaining worms with anti-CEH-13 antibodies and well-characterized markers (see above and UL1 strain expressing a *pes-1::lacZ* construct (Hope, 1994)) or by in vivo observation of the *ceh-13::gfp* reporter in FR317 embryos (Wittmann et al., 1997, data not shown). Figs 5-7 were obtained by using a Bio-Rad MRC1024 confocal microscope. Fig. 6 was processed using IMARIS software.

Isolation of a deletion mutation of *ceh-13*

The isolation of a *ceh-13* (*pk20::Tc1*) *dpy-19*(*n1347*)*III*; *mut-2*(*r459*)*I* mutant, in which the endogenous transposon Tc1 was inserted into the first intron of *ceh-13*, has been described previously (Zwaal et al., 1993). A bidirectional deletion derivative, *ceh-13*(*sw1*), was isolated as described by Zwaal et al. (1993) from the insertion mutant. The *ceh-13*-specific primers 1998 (5′-cgcgtctcattggtcgattgg-3′) and 1999 (5′-ctcttgatcggatggtgtctc-3′), located upstream of the Tc1 insertion site and 3106 (5′-ttgttcgatgagaacatgggtc-3′) and 3107 (5′-tacccgcttagaagtcgagctc-3′) located downstream of the insertion site, about 2.9 kb apart, were used to screen for the 1.55 kb deletion derivative (*sw1*). The deletion junctions were determined by sequencing the PCR product and Southern analysis confirmed that part of the *ceh-13* was deleted. The *sw1* allele was backcrossed ten times to wild type and balanced with *qC1* (Austin and Kimble, 1989). Linked chromosome III mutations were crossed off by selecting for 2 closely linked markers, *dpy-17*(*e164*) and *unc-32*(*e189*).

Northern analysis with total RNA from balanced heterozygous (*ceh-13*(*sw1*)/*qC1*) animals using a DIG-labelled complete *ceh-13* cDNA probe (Bar25B) revealed only one band corresponding to the wild-type transcript (data not shown).

Germline transformation, cDNA cloning and RNA interference

The semidominant *rol-6*(*su1006*) roller marker (plasmid pRF4 at 44 ng/μl) and the cosmid PD1 (20 ng/μl), which contains the *ceh-13* gene and a second predicted ORF showing sequence similarity to an acetylcholine receptor (The *C. elegans* Sequencing Consortium, 1998) were coinjected as described by Mello and Fire (1995). A *ceh-13*(*sw1*)-rescued F_2 population, *ceh-13*(*sw1*);*swEx504*[*PD1, rol-6*(*su1006*)], was defined by picking F_2 rolling animals that segregated only rolling and dead animals. The *ceh-13*(*sw1*) homozygous escapers grew so slowly that they were not taken into account.

Three cDNA clones (Bar12C, Bar23C and Bar25B) were isolated by screening a mixed-stage cDNA library (B. Barsteadt) with a subclone of the genomic clone λgceh-134 (Schaller et al., 1990). Antisense or sense RNAs corresponding to the full *ceh-13* cDNA insert, Bar25B, were produced using an in vitro transcription kit (Promega). RNAi experiments were carried out as described by Fire et al. (1998). RNA was injected at a concentration of 1 up to 5 mg/ml. Approximately 50% (944 out of 1844 progeny) of the progeny produced by injected hermaphrodites phenocopied the Ceh-13 phenotype.

Lineage analyses

Wild-type, *dpy-17*(*e164*) *ceh-13*(*sw1*), *ceh-13*(*sw1*) *unc-32*(*e189*) and *ceh-13*(*sw1*) embryos were recorded with a ‘four-dimensional’ video recording system until embryos began to move (Schnabel et al., 1997). We followed all or most of the cells in two wild-type and four Ceh-13 embryos until the bean stage. Using the SIMI Biocell software (Schnabel et al., 1997), we marked cell positions at various time points (175, 240 and 330 minutes) in order to visualize and compare reconstructed 3D embryos (data not shown, except for Fig. 4A,B).

RESULTS

CEH-13 is expressed more anteriorly than the *lin-39 Hox* gene and in many different cell types

The CEH-13 protein shows structural features typical for *labial* class of proteins, including the organization of exons and introns and the conservation of the homeodomain, as well as the presence of a conserved hexapeptide (see http://zoops1.unifr.ch/Nematode/labial.html for references and alignments and Fig. 1A). Sequence comparisons (Bürglin, 1994; Sharkey et al., 1997) also confirm that *ceh-13* is the *C. elegans labial* ortholog. Moreover, the genomic sequence of *C. elegans* is now essentially complete (The *C. elegans* Sequencing Consortium, 1998), and *ceh-13* is significantly more similar to *labial* than any other *C. elegans* homeobox-containing gene. In order to study its expression pattern, we raised rabbit polyclonal antibodies against recombinant CEH-13 (see Materials and Methods). On a western blot, purified anti-CEH-13 antibodies recognized a single band of the expected molecular mass (23 kDa) in a mixed stage wild-type N2 extract (data not shown). Furthermore, the anti-CEH-13 antibodies did not stain *ceh-13*(*sw1)* embryos, although these embryos were permeabilized as demonstrated by other antibodies (data not shown). This indicates that anti-CEH-13 antibodies are specific.

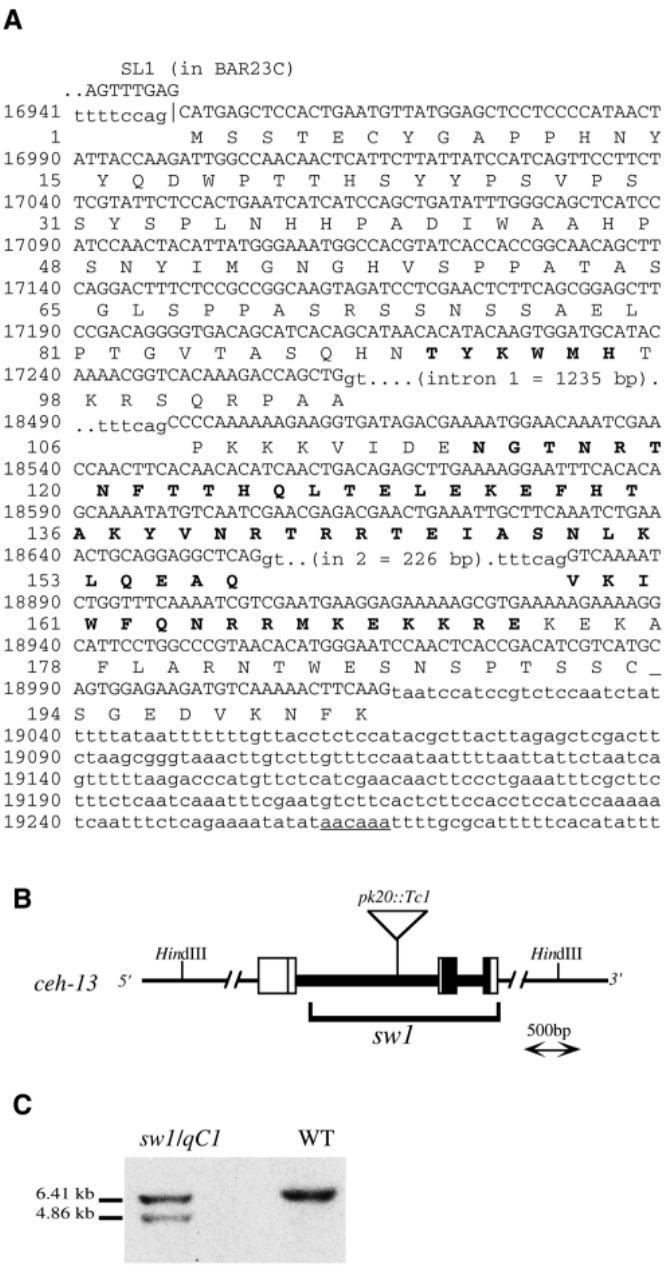

Fig. 1. Sequence and deletion derivative of *ceh-13*. (A) Nucleotide and deduced amino acid sequence of *ceh-13* (EMBL accession number: X17077). Numbers to the left refer to nucleotide positions in the reference cosmid R13A5 and to amino acid positions in CEH-13. The SL1 *trans*-spliced leader sequence found at the 5′ end of the BAR23C *ceh-13* cDNA clone is indicated. The hexapeptide and the homeodomain are bold faced. The putative polyadenylation signal is underlined. (B) Genomic structure of *ceh-13*. Exons are indicated by boxes, introns by thick lines, the Tc1 element by a triangle. The conserved hexapeptide sequence is indicated by a vertical black line in the first exon, the homeobox (black boxes) spans exons 2 and 3. The 1.55 kb deletion (*sw1*) is indicated by the bar beneath the genomic structure. (C) Southern blot analysis of wild-type (WT) and balanced heterozygous *ceh-13* (*sw1*)/*qC1* animals. DNA was digested with *Hin*d III and hybridized with a radioactively labelled *ceh-13* cDNA probe. The length of the fragments is indicated to the left of the panel.

As previously shown, *ceh-13* is first expressed at the onset of gastrulation in the posterior daughters of the intestinal precursor cell E (Ep) and posterior daughters of AB descendants ABxxx (ABxxxp) (Wittmann et al., 1997). The *ceh-13* endodermal expression is maintained in all Ep descendants for at least two more cell divisions before fading out during morphogenesis. No staining in the intestine could be detected in larval and adult stages (data not shown). During embryogenesis, in addition to the E lineage, CEH-13 is detected in many different cell types of AB, MS and D lineages. At the comma stage, CEH-13 is strongly expressed in the lateral hypodermal (epidermal) cells H2 and V1 (Fig. 2A) and in cells of the prospective ventral nerve cord (Fig. 2D). Signals in anterior dorsal hypodermal cells as well as in some anterior body wall muscle cells are also observed (Fig. 2A-D).

lin-39, *mab-5* and *egl-5* have been shown to be expressed in successive domains along the anterior-posterior body axis of 1.5-fold-stage embryos (Wang et al., 1993). At this stage, CEH-13 is mainly expressed anterior to the expression domain of *lin-39* (Fig. 2K-M). In L1 larvae, the expression of CEH-13 in the different ventral nerve cord cells, lateral and dorsal hypodermal cells shows the same anterior boundary as in the comma-stage embryos, while the most anterior H2 lineage appears much more weakly stained (Fig. 2I). Thus, throughout development, the CEH-13 rostral boundary of expression is located more anteriorly than the domains of expression of the other members of the *Hox* cluster. However, CEH-13-expressing cells are not limited to the anterior part of *C. elegans*, but are found all along the body axis (Fig. 2), and in the male tail (data not shown).

Isolation of a *ceh-13* deletion derivative

Since no known mutation could be associated with the *ceh-13* gene, we isolated a deletion derivative by a transposon-mediated reverse genetic strategy (Zwaal et al., 1993; see Materials and Methods). This deletion removes most of the first intron, the whole second and third exons, comprising the conserved homeobox and 22 bases of the 3′UTR (Fig. 1B,C).

ceh-13 (*sw1*) homozygous animals showed a lethal phenotype (see below) which could be rescued by germline transformation (Mello and Fire, 1995) with the genomic *ceh-13* sequence carried on cosmid PD1. In addition, we were able to phenocopy the *ceh-13* phenotype (data not shown) by injecting in vitro-synthesized *ceh-13* RNA into the gonad of wild-type hermaphrodites (Fire et al., 1998). These experiments confirm that the lethality of *ceh-13*(*sw1*) homozygous animals is due to the truncation of the *ceh-13* gene.

To rule out the possibility that the presence of the first exon of *ceh-13* in the *sw1* allele could give rise to a partially functional truncated product, we performed a northern blot analysis on total RNA from balanced heterozygous (*ceh-13*(*sw1*)/*qC1*) animals. The only transcript detected was that corresponding to the wild-type *ceh-13* RNA (data not shown). Moreover, the phenotype of hemizygous animals carrying the *sw1* allele in *trans* to the *nDf16* deficiency was similar to that of homozygous *ceh-13*(*sw1*) animals. These molecular and genetic data suggest that *ceh-13*(*sw1*) represents a null allele.

ceh-13 mutants show morphogenetic defects

Homozygous *ceh-13*(*sw1*) animals exhibit a recessive Vab

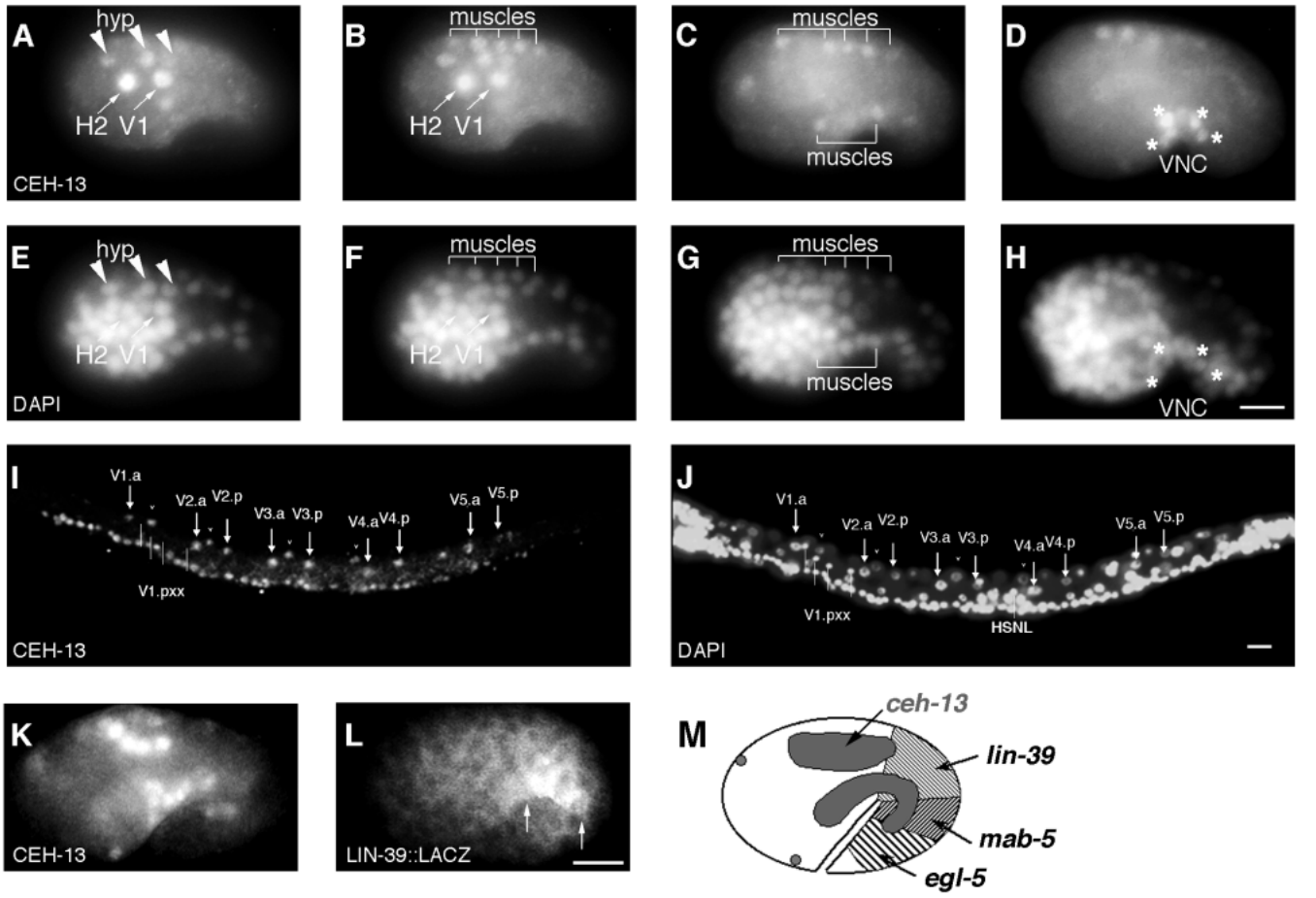

Fig. 2. *ceh-13* expression. (A-D) Four different focal planes of a comma-stage embryo stained with the anti-CEH-13 antibody; (E-H) corresponding DAPI stainings. Strong CEH-13 staining is detected in the lateral hypodermal cells H2 and V1 and in cells of the prospective ventral nerve cord (asterisks, VNC). CEH-13 expression in anterior dorsal hypodermal cells (arrowheads, hyp) and in anterior body wall muscle cells is indicated. Expression is also found in some unidentified cells located in the anterior part of the embryo. (I) CEH-13 distribution in a 15-16 hour L1 larva; (J) corresponding DAPI staining. V1 to V6 are lateral hypodermal cells, Vx/Vx.x are descendants of these cells; a, anterior; p, posterior (left view). V1.pxx cells are not fluorescing in the L1 larva. In contrast, all V1 to V4 descendants express *ceh-13* in L2 larvae (data not shown). (K-M) Embryonic expression pattern of the *C. elegans Hox* genes. Immunostainings of CEH-13 (K) and LIN-39::LACZ (L) in 1.5-fold embryos. Arrows indicate the borders of the LIN-39::LACZ expression domain. (M) Schematic drawing showing the expression domains of the different *C. elegans Hox* genes (adapted from Wang et al., 1993). Anterior, left. Bar, 10 μm.

(variable abnormal morphology) phenotype characterized by incompletely penetrant zygotic lethality. On average, about 97% of the *ceh-13*(*sw1*) homozygous animals arrest during embryogenesis or at early larval stages (Table 1). Rare *ceh-13*(*sw1*) homozygotes that survive to adulthood (approximately 3%) show less severe morphogenetic defects than the arrested Ceh-13 animals, but are smaller (Fig. 3E) and develop as much as 6 times slower than wild-type animals.

In wild-type *C. elegans*, when most of the embryonic cell divisions are completed, changes of cell shape and adhesion transform the ellipsoidal embryo into a cylindrical worm through the elongation process. *ceh-13*(*sw1*) mutants appear to be morphologically normal until the beginning of elongation. At this stage, mutant embryos start to elongate variably to some extent before again retracting. This results in very short animals with anterior, and occasionally more posterior, protuberances (Fig. 3B,D). In the most extreme mutant phenotypes, the hypodermis ruptures and the inner cells spill out of the embryo. In addition, *ceh-13*(*sw1*) mutants exhibit movement defects. Soon after the onset of elongation, wild-type embryos begin to twitch inside the eggshell; this twitching movement increases when animals reach the two-fold stage. Ceh-13 animals, by contrast, either continue to twitch weakly or even remain paralyzed. Interestingly, the *ceh-13*(*sw1*) escapers move forwards but are unable to move backwards properly. This latter defect suggests that some *ceh-13* activity is required in neurons since the same muscles are used for forward and backward movements. The putative affected neurons have not yet been identified.

ceh-13 mutant embryos show adhesion defects but no lineage transformation

To determine whether the morphogenetic defects in *ceh-13*(*sw1*) mutants could be caused by lineage transformation, we performed lineage analyses on four mutant embryos by using a ‘four-dimensional’ (4D) microscope and analysis software

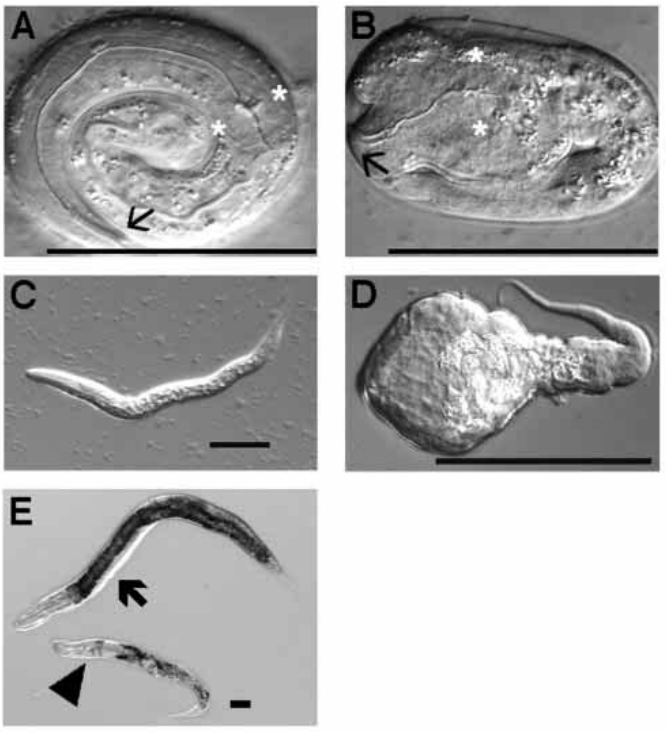

Fig. 3. Phenotype of *ceh-13* mutants. (A) A 3-fold wild-type embryo; only the anterior two thirds of the embryo are in focus. (B) a *ceh-13*(*sw1*) mutant embryo at the end of embryogenesis. The arrows in A and B point to the mouth; asterisks show the second bulb of the pharynx. Note the anterior dorsal protuberance in the *ceh-13* mutant embryo. (C) Wild-type and (D) *ceh-13*(*sw1*) first-stage (L1) larvae. The *ceh-13* mutant L1 larva has an abnormal anterior morphology and severe locomotion defects. (E) Adult wild-type (arrow) and adult *ceh-13* escaper (arrowhead). Anterior, left. Bar, 50 μm.

(Schnabel et al., 1997). No significant alterations in the division pattern up to the bean stage were noticed in the *ceh-13*(*sw1*) embryos compared to the naturally occurring variability observed in wild-type embryos (Schnabel et al., 1997). Furthermore, as determined by morphological criteria, the identity of all cells appeared to be correctly specified in the mutants. However, we could observe subtle deviations in the positions of nuclei when reconstructed wild-type and *ceh-13* mutant embryos were compared at the premorphogenetic stage (Fig. 4A,B).

Moreover, in three out of four lineaged *ceh-13* mutant embryos, individual cells lost contact with the rest of the embryo at the comma stage. In two cases, neuronal precursor cells detached from the embryos (Fig. 4C,D). Adhesion defects of neuronal cells were further confirmed by crossing *ceh-13*(*sw1*) mutants with an integrated *unc-119::gfp* fusion (DP132 strain) that is expressed in most of the neurons or neuronal precursor cells (Maduro and Pilgrim, 1995) and following GFP expression in the resulting animals. In 10 of 17 embryos, detaching GFP-expressing neuronal cells were observed (Fig. 4F). No adhesion defects were detected in any of the 31 observed embryos from the DP132 strain. In another *ceh-13*(*sw1*) mutant embryo, we identified two hypodermal descendants that partially lost contact with the embryo, leading to a rupture in the anterior hypodermis as the elongation proceeded (Fig. 4E). Altogether, these observations suggest that, while *ceh-13* has no effect on the specification of the cell lineage, it is required for proper cell-cell adhesion in the embryo.

Anterior epidermal and mesodermal cells are mislocalized in *ceh-13* mutant embryos

In wild-type embryos, immediately after ventral body enclosure, the first fusions of dorsal hypodermal cells are detected anteriorly and posteriorly to the deirid sensillae (Fig. 5A,C; arrowhead), to form the hyp7 syncytium (Podbilewicz and White, 1994). At the same time, constrictions of circumferential filament bundles in the hypodermis squeeze the embryo into a long, thin worm (Costa et al., 1998; Priess and Hirsh, 1986). In addition to the hypodermis, it has been shown that body wall muscles also play a crucial role in this morphogenetic transformation (Goh and Bogaert, 1991; Hresko et al., 1994; Williams and Waterston, 1994).

In order to understand the basis of the severe morphogenetic defects in *ceh-13*(*sw1*) homozygotes, we analyzed the organization of the hypodermal and muscle cells in mutant animals. By using the monoclonal antibody MH27, which stains the hypodermal adherens junctions (Francis and Waterston, 1985), and the polyclonal anti-LIN-26 antibody, which is specific for all non-neuronal ectodermal nuclei (Labouesse et al., 1996), we could show that the fate of all hypodermal cells was correctly specified (Fig. 5D-F). This result is in agreement with our lineage analyses. Furthermore, double staining of *ceh-13*(*sw1*) mutant embryos with MH27 and NE2-1B4, a monoclonal antibody that specifically recognizes a filamentous antigen in the lateral hypodermal or seam cells (Hutter and Schnabel, 1994), confirmed that the terminal fate of the 20 seam cells was correctly executed (Fig. 6B,C). However, our lineage analyses and immunostainings revealed that cells were mislocalized in *ceh-13*(*sw1*) mutant embryos. In wild-type embryos, the seam cells form lateral rows of ten cells, H0, H1, H2, V1-6 and T, on each side of the body along the anterior-posterior axis of the wild-type embryo (Figs 5A-D, 6A). In *ceh-13*(*sw1*) mutant embryos, from the bean stage onwards, a mislocalization of V1, and in most cases also of H1 and H2, was observed (n>100) (Figs 5E-H, 6B,C). These defects correlate with the strong expression of *ceh-13* in H2 and V1 (Fig. 2A). Moreover, at least one hypodermal cell remained unfused with the dorsal hypodermal syncytium in *ceh-13*(*sw1*) mutants (Fig. 5E,G; arrows). In two-fold-stage mutant embryos, this cell was still unfused (data not shown). This defective pattern of fusion is a typical feature of *ceh-13*(*sw1*) mutant embryos.

Since we found *ceh-13* expression in embryonic body wall muscles (Fig. 2B,C), we also analyzed the organization of these mesodermal cells in mutant embryos. During the elongation process, the body wall muscle cells are spread longitudinally beneath the hypodermis in two dorsal and two ventral rows, or quadrants (Fig. 7A). Immunofluorescence staining of *ceh-13*(*sw1*) mutant embryos with the monoclonal antibody anti-myosin heavy chain A, mAb5-6 (Miller et al., 1983), indicates that terminal mesodermal fate is achieved (Fig. 8B). The anterior body wall muscles are, however, disorganized in *ceh-13*(*sw1*) mutant embryos (Fig. 8B; arrow). We also observed that, at the bean stage in *ceh-13*(*sw1*) mutant embryos, on which lineage analyses were performed, muscle cells of the D lineage were mislocalized in the region surrounding the seam

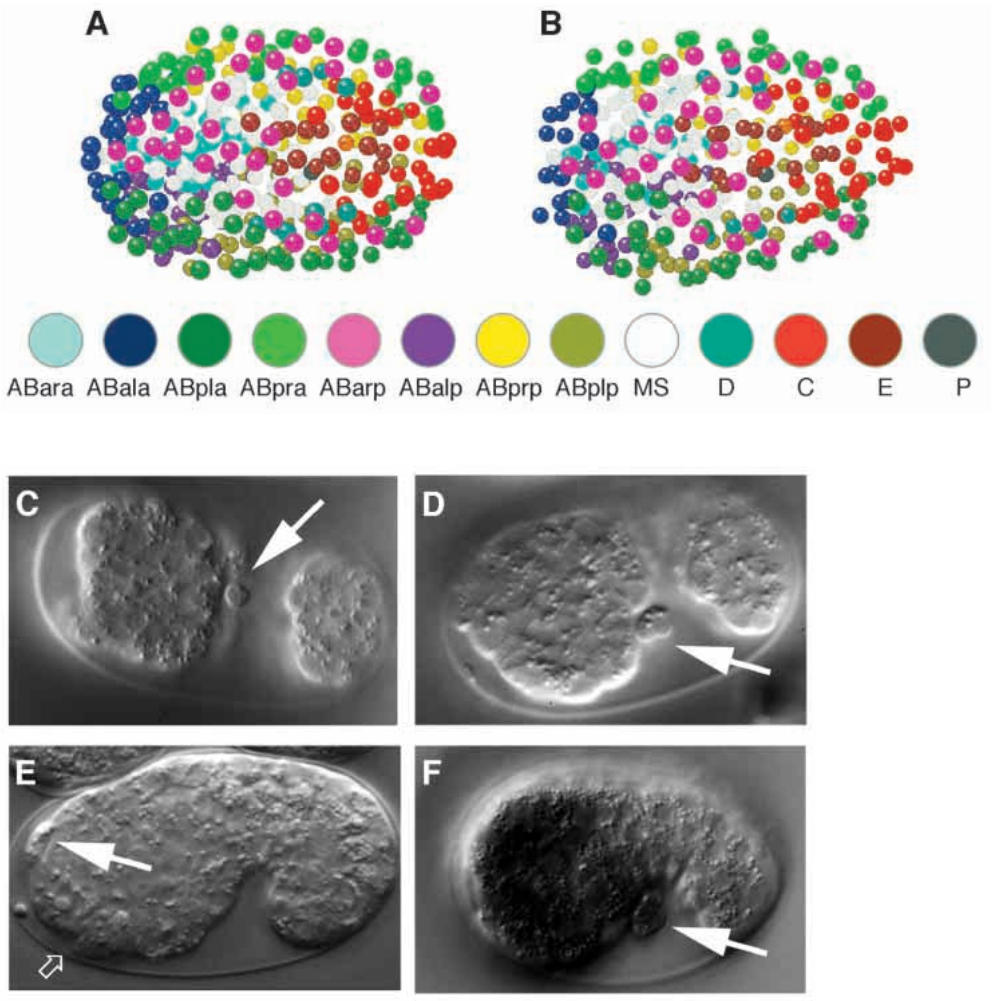

Fig. 4. Lineage analyses of *ceh-13* mutants. (A, B) Color-coded reconstructions of 240-minute embryos. The embryos were rotated to present their dorsal side in the uppermost position. Subtle variations in the positions of the nuclei can be observed between (A) a wild-type and (B) a *ceh-13* mutant embryo. (C) Ventral view of a *ceh-13*(*sw1*) mutant embryo with the neuronal precursor cell ABplppapaap (arrow) detaching. (D) Ventrolateral view of a *dpy-17*(*e164*) *ceh-13*(*sw1*) mutant embryo showing two detached neuronal precursor cells, ABprppapapa and ABpappapapp (arrow). (E) Lateral view of a *ceh-13*(*sw1*) *unc-32*(*e189*) mutant embryo. The arrow points to a hypodermal cell (hyp6) that is loosing contact with the embryo. Another detached hyp6 cell is in a lower focal plane just beneath the visible one. Moreover, there is a group of unidentified cells loosing contact with the embryo at the anterior ventral side (open arrow). The genetic markers *dpy-17*(*e164*) and *unc-32*(*e189*) have no effect on the lineage of the embryos. (F) Lateral view of a *ceh-13*(*sw1*); *unc-119::gfp* embryo showing a group of detached neuronal precursor cells (arrow). The Nomarski picture overlays with an inverted picture of GFP, thus the fluorescent GFP staining is represented by the black color.

cell V1 (data not shown). However, unlike the anterior seam cells, which were reproducibly mislocalized, the identity of the mispositioned body wall muscle cells differed in the various lineaged embryos.

DISCUSSION

In *C. elegans*, genetic and molecular approaches as well as single-cell resolution have allowed detailed analyses of the roles played by three *Hox* genes, namely *lin-39*, *mab-5* and *egl-5*, during postembryonic development (reviewed by Kenyon et al., 1997). In this paper, we present the expression pattern and the phenotypic analysis of the *C. elegans labial*-like *Hox* gene, *ceh-13*. We show that CEH-13 is present in many cells from different lineages and that the rostral boundary of its expression domain is anterior to that of *lin-39*. We also show that *ceh-13* is required for anterior epidermal and mesodermal organization in the elongating embryo, but not for the specification of epidermal and mesodermal fates. Furthermore, *ceh-13* activity is required for the fusion of anterior dorsal hypodermal cells to their neighboring cells and for the proper cell-cell adhesions of some epidermal and ventral neuronal cells.

labial class genes occupy a conserved position at the 3′ margin of the *Hox* clusters of arthropods (Akam et al., 1994), *Amphioxus* (Garcia-Fernandez and Holland, 1994), zebrafish (Prince et al., 1998), mice and human (Duboule, 1994). These genes are the first to be activated and are expressed in anterior domains of the embryonic body (Duboule and Morata, 1994). Although the *ceh-13* locus is positioned between *lin-39* (an ortholog of *pb*, *Dfd* and *Scr*) and *mab-5* (an ortholog of *Antp*, *Ubx* and *abd-A*) (Sulston et al., 1992), it is first expressed at the beginning of gastrulation, before any of the other members of the *Hox* cluster (Clark et al., 1993; Cowing and Kenyon, 1992; Wang et al., 1993; Wittmann et al., 1997). Moreover, the rostral boundary of the *ceh-13* expression domain is anterior to that of *lin-39* and consequently to those of *mab-5* and *egl-5*. Thus, the *ceh-13* expression pattern shows the same relative temporal and spatial distribution as do *labial*-like genes from other species and this does not depend on its position at the 3′ end of the *C. elegans Hox* cluster.

Approximately 97% of the *ceh-13*(*sw1*) animals arrest either during embryogenesis or at early larval stages and show severe defects in the elongation process. The remaining 3%, although also showing morphogenetic defects, are able to reach adulthood and are fertile. This suggests that the *ceh-13* phenotype is not fully penetrant. Because *sw1* behaves as a null allele and *ceh-13* is the only known sequence exhibiting

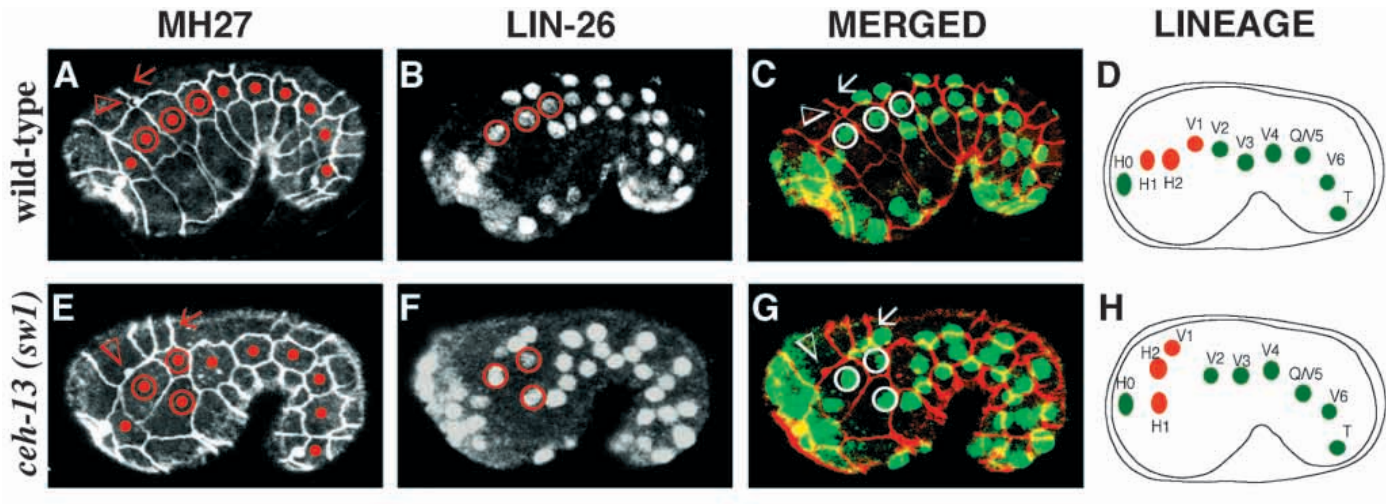

Fig. 5. Anterior hypodermal patterning defects in *ceh-13* mutant embryos. Lateral views of 360-minute wild-type (A-D) and *ceh-13*(*sw1*) mutant (E-H) embryos doubly stained with MH27 (A,E), which identifies adherens junctions between hypodermal cells, and anti-LIN-26 antibody (B,F), which specifically recognizes non-neuronal ectodermal nuclei. (C,G) Merged pictures. The ten lateral hypodermal cells are marked by dots and, specifically, the positions of H1, H2 and V1 lateral hypodermal cells and/or nuclei are indicated by circles. The arrowhead points to the deirid sensilla, which forms a circular junction. In the Ceh-13 embryo, an anterior dorsal hypodermal cell remains unfused (arrow). Both wild-type and Ceh-13 embryos contained the same numbers of anti-LIN-26-positive cells. (D,H) Schematic diagrams of embryos on which lineage analyses were performed. The positions of ten seam nuclei are indicated.

homology to the *labial*-like genes in the *C. elegans* genome, the variable expressivity of the *ceh-13* phenotype could result from the variability of the cellular defects or from the activity of partially redundant genes.

At the cellular level, the first observed defects in *ceh-13*(*sw1*) mutant embryos are subtle deviations in the positions of nuclei at the premorphogenetic stage. Since, at this stage, wild-type embryos also show some variability (Schnabel et al., 1997), it is not clear which, if any, of these misplacements are significant. At the comma stage, however, when the cells occupy precise positions in wild-type animals, mislocalizations of anterior seam and body-wall muscle cells and/or cell-shape changes become obvious in *ceh-13*(*sw1*) mutant embryos. Nevertheless, seam and mesodermal fates appear to be normal in the mutants as far as we can judge with the given methods. Interestingly, the disorganization of the seam lines is reproducibly observed in all *ceh-13*(*sw1*) embryos. The correlation between mispatterning of the hypodermal seam cells H2 and V1 and the strong expression of *ceh-13* in both of these cell types suggests that *ceh-13* may play a role in specifying some important features of their seam cell fate. In *ceh-13*(*sw1*) mutants, H2 and V1 may acquire abnormal cell affinities resulting in the formation of a disorganized seam line. Alternatively, they may be transformed into anterior or posterior homologs. However, since the different hypodermal seam cells do not execute different lineages during embryogenesis (Sulston et al., 1983), and since no specific markers exist to differentiate them from one another, we are currently unable to test this hypothesis. It remains possible that the defects observed in seam cells of Ceh-13 animals reflect a regulatory role of *ceh-13* in the expression of cytoskeletal proteins. Unlike H2 and V1, we conclude that the abnormal shape and position of the seam cell H1 does not directly depend on *ceh-13* activity since *ceh-13* expression cannot be detected in H1 cells. We favor the hypothesis that the defects in shape and positioning of H1 cells may be due to the ability of these cells to compensate for the morphological defects of their neighboring cells, H2 and V1. Plasticity of cell shape has previously been observed in mesodermal cells of *C. elegans* embryos lacking one quarter of the body wall muscle cells (Moerman et al., 1996). In addition to hypodermal mispatterning, some of the *ceh-13*(*sw1*) homozygous embryos

Table 1. Phenotypic analysis of *ceh-13(sw1)* mutants

Genotype	*n*	eggs laid/ hermaphrodite	% dead eggs	% larval arrest	% viables
N2	5	257±9	0.7±0.3	0	99.3±0.3
ceh-13/qC1	12	258±20	11.4±0.9[a]	13.4±1.0[a]	75.2±1.4
ceh-13/ceh-13 [b]	9	–[c]	42.4±4.4	54.9±4.5	2.8±0.7[d]

Data are means ± s.e.m. Hermaphrodites were picked as L4 animals and transferred to fresh plates every 24 hours. Eggs which failed to hatch after 24 hours were scored as dead eggs. Larvae that failed to develop into adults after 48 to 96 hours were scored as larval arrest.

[a]The 24.8±1.9% arrested animals resulting from the *ceh-13*/*qC1* hermaphrodites are assumed to be *ceh-13*/*ceh-13* homozygotes.

[b]*ceh-13*(*sw1*) homozygous escapers (see text).

[c]Not applicable. The mean number of eggs laid by *ceh-13*(*sw1*) homozygous escapers varied greatly between each individual. The numbers ranged from 7 to 268 eggs laid per hermaphrodite.

[d]The viable *ceh-13*(*sw1*) homozygous animals also exhibit a Vab phenotype.

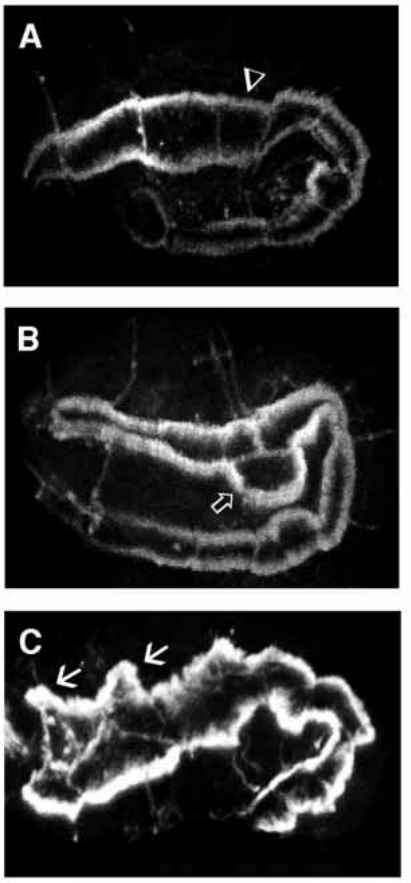

Fig. 6. Positions and shapes of seam cells in wild-type and *ceh-13* embryos. (A-C) Double staining with mAb NE2-1B4, which recognizes a seam cell specific filamentous antigen, and mAb MH27, which stains hypodermal adherens junctions. (A) Lateral view of a two-fold-stage wild-type embryo. The arrowhead indicates the position of the seam cell V1. (B) Two-fold *ceh-13*(*sw1*) mutant embryo. The open arrow points to a seam cell, which is located outside of the row. (C) Terminal stage *ceh-13*(*sw1*) mutant embryo. The thin arrows point to two seam cells, which are no longer inserted into the row. Both wild-type and *ceh-13* mutant embryos contain 20 seam cells.

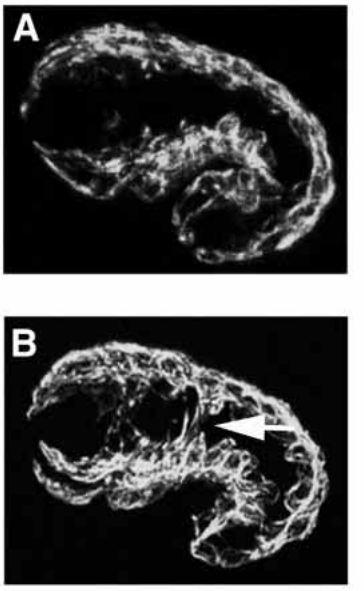

Fig. 7. Mislocalization of body-wall muscles in *ceh-13* mutant embryos. (A,B) Fluorescence micrographs showing lateral views of 1.5-fold-stage embryos labelled with the mAb5-6, which stains myosin heavy-chain A of body wall muscles. (A) Wild-type and (B) *ceh-13*(*sw1*) mutant embryos. The arrow points to strings of misplaced muscle material, which connect dorsal and ventral quadrants.

show a misplacement of the anterior body wall muscle cells where CEH-13 is also expressed. Since we also did not detect any lineage transformation in the mesodermal cells, we think that *ceh-13* plays a role in establishing or maintaining the affinities of these cells rather than specifying their proper cell fate. However, at present, we do not know whether the function of *ceh-13* is cell autonomous in the epidermal and mesodermal tissues. Nonetheless, it is interesting to note that with respect to these epidermal and mesodermal mislocalizations, the *ceh-13* phenotype is similar to that observed for mutation in the *vab-7* gene (*even-skipped* homolog), which has been demonstrated to act as a patterning gene in the posterior part of the embryo (Ahringer, 1996).

Other indications that *ceh-13* is involved in a genetic pathway determining cell affinities are given by the detachment of epidermal and neuronal precursor cells from morphogenetic stage *ceh-13*(*sw1*) mutant embryos, as well as by the lack of fusion of at least one dorsal hypodermal cell with the dorsal hypodermal syncytium. We do not know whether all these affected cells express CEH-13 but they are located in the *ceh-13* expression domain. Other *Hox* genes have been proposed to be involved in the acquisition of different cell affinities. For example, *Drosophila Ubx* mutant clones generated by genetic mosaicism in the haltere territory have a tendency to invaginate inside or less frequently to evaginate outside the surrounding wild-type tissue (Morata and Garcia-Bellido, 1976).

In contrast to the *labial*-like genes in other species, *ceh-13* mutants do not appear to alter pattern formation or the fate of restricted sets of cells (Goddard et al., 1996; Hoppler and Bienz, 1994). Nonetheless, similarly to *ceh-13*, some genes of the *labial* class have also been suggested to control cell-cell interactions. The analysis of hindbrain in *Hoxa-1*$^{-/-}$ mutant mice reveals that the remnants of hindbrain constrictions, termed rhombomeres 4 and 5, appear to be fused caudally with rhombomere 6 to form a single fourth rhombomeric structure, suggesting abnormal cellular adhesion at the rhombomere boundaries (Mark et al., 1993). Finally, in *Drosophila labial* mutants, the incomplete head involution appears to be due to a failure of mandibular and maxillary lobes to fuse with the procephalic lobe, as well as of cells to assimilate into the dorsal pouch (Merrill et al., 1989).

In *C. elegans*, as gastrulation proceeds, a variability in cell positioning and cell-cell contacts results in an essentially invariant premorphogenetic stage embryo (Schnabel et al., 1997). How positional information is generated and maintained during morphogenesis remains to be solved. Further analysis of the *ceh-13* gene and identification of upstream and downstream genes will bring insights into the pathways which link patterning specification and cell-cell interactions.

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