The *Caenorhabditis elegans* gene *lin-26* is required to specify the fates of hypodermal cells and encodes a presumptive zinc-finger transcription factor

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**SUMMARY**

The mutation *lin-26(n156)* prevents vulva formation in *C. elegans* by transforming the vulval precursor cells into neurons or neuroblasts. We have isolated and characterized three new *lin-26* alleles, which result in embryonic lethality. These mutations cause a few other hypodermal cells to express a neural fate and most hypodermal cells to degenerate. *lin-26* encodes a presumptive zinc-finger transcription factor. Our data indicate that *lin-26* is required for cells to acquire the hypodermal fate.

Key words: *Caenorhabditis elegans*, cell fate, zinc-finger, transcription factor, *lin-26*, hypodermal cell

**INTRODUCTION**

How do general categories of cell types, such as epidermal cells, neurons and muscle cells acquire their identities? In vertebrates, a family of cell-type-specific basic helix-loop-helix (b-HLH) transcription factors including MyoD, Myf-5, herculin and myogenin, controls the muscle cell fate in vitro (Weintraub et al., 1991) and in vivo (Hasty et al., 1993; Nabeshima et al., 1993; Rudnicki et al., 1993). In *Drosophila*, a family of four b-HLH transcription factors, the proneural genes of the *achaete-scute* complex, specifies the neural cell fate (Ghysen and Dambly-Chaudière, 1988; Rodriguez et al., 1990).

We have chosen to study the problem of how cell fates are specified in the nematode *Caenorhabditis elegans*. We have focused on the gene *lin-26*, which was defined by the mutation *n156*; this mutation causes neural cells to be generated instead of certain hypodermal cells (Ferguson et al., 1987). (In *C. elegans*, epidermal-like cells are termed hypodermal cells.) Specifically, in wild-type *C. elegans* first stage (L1) larvae, each of the 12 postembryonic P ectoblasts divides to generate an anterior daughter, the Pn.a cell, and a posterior daughter, the Pn.p cell (Sulston and Horvitz, 1977). The Pn.a cells are neuroblasts that undergo three rounds of cell divisions and generate up to five motor neurons. The Pn.p cells are hypodermoblasts that generate the vulva, which is used for egg laying, and other cells of the ventral hypodermis. In *lin-26(n156)* animals, the Pn.p cells generate neuronal descendants (Ferguson et al., 1987), causing these animals to lack a vulva. Indeed, the mutation *lin-26(n156)* was originally identified in a screen for vulvaless mutants (Ferguson and Horvitz, 1985).

In the present study, we characterize the role of *lin-26* during *C. elegans* hypodermal development. We have isolated three new *lin-26* alleles that we show to cause embryonic lethality and defects in all categories of hypodermal cells. The *lin-26* transcript encodes a presumptive transcription factor with two zinc-finger motifs. Based on these observations, we postulate that *lin-26* acts as a transcriptional activator to specify the fates of hypodermal cells.

**MATERIALS AND METHODS**

**Strains and general methods**

Worms were maintained as described by Brenner (1974). Animals were raised at 20°C unless otherwise stated. This paper conforms to the standard *C. elegans* genetic nomenclature described by Horvitz et al. (1979). *C. elegans* strain N2 (Brenner, 1974) was the wild-type strain and the parent of all *C. elegans* strains that we used. The following mutant chromosomes and mutant strains were used: *mnC1[dpy-10(e128) unc-52(e444)]* (Herman, 1978); *dpy-2(e489) and unc-4(e120)* (Brenner, 1974); *him-5(e1490)* (Hodgkin and Brenner, 1977); *lin-26(n156)* (Ferguson and Horvitz, 1985); *MT5823: lin-26(n156) unc-4(e120)\*/mnC1[dpy-10(e128) unc-52(e444)]; *him-5(e1490)*; SP754: *mnD198 unc-4(e120)\*/mnC1[dpy-10(e128) unc-52(e444)]; SP781: *mnD97 unc-4(e120)\*/mnC1[dpy-10(e128) unc-52(e444)]; SP803: *mnD105 unc-4(e120)\*/mnC1[dpy-10(e128) unc-52(e444)]; SP804: *mnD106 unc-4(e120)\*/mnC1[dpy-10(e128) unc-52(e444)]* (Herman, 1978).

**Isolation and characterization of *lin-26* alleles**

*lin-26(n156)\*/Df* animals are inviable (Ferguson and Horvitz, 1985). We found that the mutation *lin-26(n156)* causes larval lethality in trans to deficiencies that delete *lin-26*. Hemizygous *lin-26(n156)/Df* larvae were recognized as homozygous Unc-4 larvae in crosses between *MT5823* males and *SP754* hermaphrodites (although *lin-26(n156)/Df* larvae die, most reach a stage at which the Unc-4 phenotype can be observed, which is the late L1 stage; see Miller et al. 1992). Mothers were allowed to lay eggs for half a day and progeny were examined after 40 hours.

Because hemizygosity for lin-26(n156) causes larval lethality, we designed a screen that would allow us to recover lethal alleles of lin-26. The progeny of 4,400 F1 progeny of dpy-2(e489) unc-4(e120)/mnC1 animals mutagenized with ethyl methane sulfonate (Brenner, 1974) were examined for the presence of Dpy Unc animals. The F2s from individuals that did not segregate Dpy Unc offspring were tested for complementation with the deficiency mnDf88, which deletes lin-26 (see below), and for complementation with lin-26(n156). Two mutations, named lin-26(mc1) and lin-26(mc2), failed to complement lin-26(n156). The allele lin-26(mc4) was isolated by a similar strategy: the F1 progeny of 3700 dpy-2(e489) unc-4(e120)/mnC1 animals mutagenized with ethyl methanesulfonate raised at 22.5°C was directly examined for complementation with lin-26(mc1). They were outcrossed twice with N2 and balanced by mnC1 to maintain stocks. To examine the terminal phenotypes of lin-26(mc1), lin-26(mc2) or lin-26(mc4) embryos, heterozygous dpy-2(e489) lin-26(mcx) unc-4(e120)/mnC1 mothers carrying the corresponding lin-26(mcx) mutation (where mc1, mc2 or mc4 were allowed to lay eggs for 3 hours; the progeny were examined 14 hours later. Hemizygous lin-26(mcx) embryos were obtained by crossing heterozygous mnDf88 unc-4(e120)/+ males with dpy-2(e489) lin-26(mcx) unc-4(e120)/mnC1 hermaphrodites. Heteroallelic lin-26(mcx)/lin-26(mcy) embryos were obtained by crossing dpy-2(e489) lin-26(mcx) unc-4(e120)/mnC1 hermaphrodites with dpy-2(e489) lin-26(mcy) unc-4(e120)/mnC1; him-5(e1490) males, except lin-26(mcx)/lin-26(mcy) embryos, which were obtained by crossing dpy-2(e489) lin-26(mcy) unc-4(e120)/ lin-26(mc1) unc-4(e120)/ mnC1 hermaphrodites with dpy-2(e489) lin-26(mcy) unc-4(e120)/+ males. For each of the crosses described above, we checked that the proportion of males was as expected in a successful cross.

Mapping lin-26

The map position of lin-26 on LGII (Ferguson and Horvitz, 1985) was refined by mapping lin-26(n156) relative to deficiencies. MT5823 males were crossed with SP754, SP781, SP803 and SP804 hermaphrodites. mnDf106 complemented lin-26(n156) (36 Unc males and 129 wild-type males), mnDf97 partially complemented lin-26(n156) (two Unc males and 66 wild-type males), mnDf88 and mnDf105 failed to complement lin-26(n156) (no Unc males and over 100 wild-type males). To identify the left breakpoints of these four deficiencies, DNA from strains SP754, SP781, SP803, SP804 and N2 were analyzed by Southern blotting. For probes, we used cosmids from the region of the previously cloned LGII genes unc-104 and zyg-11 (Coulson et al., 1988). The cosmid C05B9 identified the left breakpoint in the deficiencies mnDf88 and mnDf105, the cosmid C24G10 identified the left breakpoint of the deficiency mnDf97 and the cosmid C01D8 identified the left breakpoint of the deficiency mnDf106 (data not shown).

Transformation rescue of lin-26(n156)

Cosmid DNA was microinjected into the gonad of lin-26(n156) animals at 10 ng/µl together with the plasmid pRF4, which carries the dominant mutation rol-6(su1006), at 80 ng/µl (Mello et al., 1992). F1 rollers and stable F2 roller lines were isolated, and animals were examined for their abilities to form a vulva and lay eggs. (lin-26(n156) animals never lay eggs.) lin-26 was located by testing cosmids surrounding C24G10 for their abilities to rescue the egg-laying defect of lin-26(n156) animals. The cosmids K06D5, C18C9, F31B8 and F18A1 rescued lin-26(n156), whereas the cosmids C36G4, C43A1, C18D9, C24G10, C01B9 did not rescue lin-26(n156). A 17 kb MluI-SpeI fragment from the cosmid K06D5 was subcloned into the plasmid plBI20 (IB1) (plasmid pMLW003). A 9 kb BamHI and an 11 kb HindIII fragment from pMLW003 were subcloned into the plasmid pBSKII (Stratagene) (plasmids pMLW006 and pMLW009, respectively). Plasmids pMLW003 and pMLW006 rescued the phenotypes caused by the mutations lin-26(n156), lin-26(mc1) and lin-26(mc2). Two deletions were made from pMLW006 by digestion with SacI or SphI and religation. Frameshift mutations within pMLW006 were made by partial digestion with NdeI or EcoRI followed by end filling with Klenow enzyme; as a result the EcoRI site (G/AATTC) was transformed into an Asel site (AT/TAAT). DNA manipulations were performed as described by Sambrook et al. (1988).

DNA sequencing, northern blots, isolation of cDNAs

The DNA sequence of both strands of the region bracketed by an Spht site on the left and a HindIII site on the right (see Fig. 4) were determined using the dye deoxy chain termination method and Sequenase enzyme (US Biochemical). Single-stranded templates were prepared using the phagemid pBSKII from deletion derivatives generated by limited ExoIII digestion (Sambrook et al., 1988). Northern blots were
performed using N2 or lin-26(n156) mixed-stage polyadenylated RNA, or N2 embryonic polyadenylated RNA. 500,000 plaques from a mixed-stage population cDNA library (Kim and Horvitz, 1990) were screened using the plasmid pMLW006 as a probe. Fifteen cDNAs were analyzed. Fourteen of them, ranging in size between 1.0 kb and 2.2 kb, corresponded to nearly full length or partial cDNAs for the 2.2 kb RNA; the sequences of the longest two (1.8 kb and 2.2 kb) were determined and proved to encode identical proteins (data not shown). The fifteenth corresponded to a partial 0.7 kb cDNA for the 0.7 kb RNAs differ by alternative exons in their 3′ ends and so generate sequences of the 0.7 kb cDNA or 3′ sequences of the 0.7 kb cDNA or from sequences that we tentatively predicted to be coding after inspection of putative splice donor and acceptor sites in the genomic sequence (P. Dufourcq, M. Labouesse and H. R. Horvitz, unpublished observations; a detailed account of our strategy will be presented elsewhere). These cDNAs revealed that the 1.4 kb, 1.45 kb and 1.5 kb RNAs differ by alternative exons in their 3′ ends and so generate proteins that differ in the last coding exon (data not shown).

**Sequences of lin-26 mutations**

Purified DNA from N2 or lin-26(n156) animals, or DNA from lin-26(mcl1) or lin-26(mcl2) single dead eggs treated as described by Williams et al. (1992) was amplified by the polymerase chain reaction using Taq Polymerase (Cetus). Following amplification, DNA was run on a low melting point agarose gel, the desired band was excised, reamplified with the same oligonucleotide pair, again gel purified, extracted from agarose with a GeneClean kit (Bio 101) and directly used for DNA sequence determination. The following oligonucleotide pairs were used:

5′ TACGGATCCGAGCGGTGCTCCGGAAGCTGAGGA with 5′ TCTAAGCTTTAATAACCTGATTAAATTTATATTTG.
5′ CGTTGATCATGATCTTCTTACGCTTTT with 5′ TCTAAGCTTTGCTGCTCTTCCAGCA.
5′ TACGGATCTCCCTTCTCACTAACATATCTAC with 5′ TACGGATCAGAGTGACACTTGT.
5′ TCTAAGCTTTGAGAGGAGACGTCGATA with 5′ TACGGATCTCCATCTGATAGTCTGTTCCAG.
5′ ATGCTTCCCAAGATATGTC with 5′ CTCGAAGTCTGATTAGAAGATGC.

**Table 1. Hemizygous lin-26(n156)/Df animals displayed various hypodermal defects**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>External epithelium</th>
<th>Excretory organ</th>
<th>Anus/rectum</th>
<th>Total</th>
</tr>
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<tr>
<td>+/+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>125</td>
</tr>
<tr>
<td>lin-26(n156)</td>
<td>2%</td>
<td>3%</td>
<td>100%</td>
<td>12%</td>
</tr>
<tr>
<td>lin-26(n156)/nnmDf 88</td>
<td>48%</td>
<td>57%</td>
<td>100%</td>
<td>61%</td>
</tr>
</tbody>
</table>

Animals were obtained as described in Materials and Methods.

3 Animals with shorter size and larger cross section than usual.

4 A variably truncated or vacuolated tail (the tip of the tail is made exclusively of hypodermal cells); vacuoles were also observed in hypodermal cells of the head and body and head of 25 other lin-26(n156)/Df/hemizygous animals.

4 Animals older than late L1 larvae with fewer than 12 hypodermal cells in their ventral nerve cords (Pn.p cells); in wild-type animals fewer than 2% of the animals lacked one Pn.p cell, whereas homozygous and hemizygous lin-26(n156) animals had on average one Pn.p cell, that is lacked 11 Pn.p cells in their ventral cord.

4 Animals with an abnormal excretory organ: a vacuole of variable size was found adjacent to the excretory cell nucleus and/or the excretory duct nucleus, the excretory canal was very thick with a variable length in most animals; in 20% of animals of this class, the excretory pore was absent or not distinguishable.

4 Animals with a poorly formed rectum, with a lumen that was barely visible. In six lin-26(n156)/Df/hemizygous animals displaying an abnormal rectum, the rectal cells B, F, Y, U, K, K’ were either not distinguishable or transformed into cells with neuronal-like nuclei.

A small percentage of lin-26(n156)/Df/hemizygous embryos did not hatch (see Fig. 3); 78 of the 229 larvae were L1 larvae, and all others were L2 larvae (most should have been L3 or L4 larvae). Hemizygous lin-26(n156)/Df animals almost never reached adulthood. In similar crosses in which all presumptive non-Unc animals (i.e. non-lin-26(n156)/Df) were removed from the plate, we observed four adult Unc animals among 337 Unc larvae (i.e. presumptive lin-26(n156)/Df); one was very sick and sterile, one was a male with all Pn.p cells present, the other two were egg-laying competent hermaphrodites with a normal vulva but that laid over 50% dead eggs; these last three animals might have arisen from a rare breakdown of the ncl/C balancer chromosome.

**Immunofluorescence**

Antisera against GABA were used as described by McIntire et al. (1993). Primary antibodies were revealed with FITC-coupled anti-rabbit antibodies (Cappel) incubated overnight at room temperature. DAPI at 2 µg/ml was included during this second incubation to visualize nuclei (Wood et al., 1988). Animals were mounted on a slide with phenylenediamine in 50% glycerol/50% PBS and observed with a Zeiss Axioplan microscope equipped with fluorescence capabilities.

**RESULTS**

**lin-26(n156) causes 12 hypodermoblasts to be neural**

Ferguson et al. (1987) reported that, in homozygous lin-26(n156) animals, the normally hypodermal Pn.p cells have a neuronal morphology or divide to produce neuronal-like cells, as do their sisters, the neuroblasts Pn.a. We now report that, in lin-26(n156) animals, the Pn.p cells or cells generated by the Pn.p cells can express a neuronal marker normally expressed by descendants of the Pn.a cells, the neurotransmitter GABA (γ-aminobutyric acid). Normally, there are 19 GABAergic neurons in the ventral cord of a wild-type adult animal, 12 of which are descendants of the 12 Pn.a cells (McIntire et al., 1993). By contrast, we found an average of 26±5 GABAergic neurons (n=82) present in the ventral nerve cords of lin-26(n156) animals (Fig. 1). We counted all neurons present between the ventral nerve cord neurons VD2 and VD12 (this region includes neurons derived from the postembryonic ectoblasts P2-P11 and the interspersed DA, DB, DD neurons; Sulston and Horvitz, 1977). Wild-type animals had 57.5±2.5 neurons of which 13±1 were GABAergic (n=54), whereas lin-26(n156) animals had 65.5±11.5 neurons of which 19.5±4.5 were GABAergic (n=82). These observations suggest that most Pn.p cells differentiate as GABAergic neurons and do so without dividing.

Pn.p cells are probably not the only cells that can be transformed to adopt a neural fate in lin-26(n156) animals.
Fig. 2. lin-26 mutations affect all hypodermal cells. (A-F) Hemizygous lin-26(n156) animals were obtained and observed as described in Table 1. Anterior is to the left, and ventral is down. Scale bar, 10 µm. (A,C,E) Wild-type animals, (B,D,F) lin-26(n156)/Df hemizygous animals. (A) Young wild-type L1 larva; the arrowhead points at the excretory cell nucleus. (B) Dumpy animal, presumably a lin-26(n156)/Df hemizygous larva, which has essentially no tail and numerous vacuoles. (C) Lateral surface of a wild-type L2 larva showing the excretory canal, which is a thin process (small arrows) that runs under the lateral hypodermis from the excretory cell body toward the mouth and the rectum. (D) Lateral surface of a lin-26(n156)/Df hemizygous L2 larva showing a vacuolated excretory canal (small arrows) and a large vacuole at the position of the excretory cell body (arrowhead, compare to A). (E) Wild-type L1 larva with a normal rectum (arrow). (F) Presumptive lin-26(n156)/Df hemizygous L1 larva, which has no visible rectum. (G-N) lin-26(mcl) and lin-26(mc2) were obtained as described in Materials and Methods. Anterior is to the left. Scale bar, 5 µm. (G,H,L) wild-type; (J,K,L) lin-26(mcl); (N) lin-26(mc2). Pictures of lin-26(mcl) and lin-26(mc2) embryos correspond to terminal phenotypes reached by these embryos. (G) Lateral view of a wild-type embryo at the comma-stage (beginning of elongation). Hypodermal cells have migrated around the embryo (arrows point at six hypodermal cells, the left P cells). (H) Median view of the embryo in G. Note the pharynx (arrowheads) and the rectum (large open arrow). (I) Lateral view of a lin-26(mcl) embryo that has stopped elongation slightly before the comma stage. Some hypodermal cells have migrated around the embryo (compare to G), but several are missing (only three P cells could be counted on this side, arrows) or dying (half open triangles). (J) Median view of the embryo in I. Non-ectodermal organs are normal. For instance, there is a buccal cavity (long arrow). The pharynx of lin-26(mcl) embryos (arrowheads) looks like a shortened version of a normal L1 larval pharynx (compare with A) rather than the pharynx primordium of a wild-type embryo that has reached the level of elongation at which lin-26(mcl) embryos arrest (compare with H). By contrast, the rectum primordium (large open arrow) has not developed properly and is typical of a wild-type comma-stage embryo (compare with H). (K) Lateral view of another lin-26(mcl) embryo with an abnormal large process that is probably a malformed excretory canal (small arrows). In this embryo, as in 26% of the lin-26(mcl) embryos, we observed (n=249), internal cells extruded from a region near where the excretory pore is located in wild-type embryos (marked by the three white dots in J), starting at the comma-stage; we observed this process of extrusion directly using Nomarski optics for a few embryos. In such embryos, hypodermal cells (large turning arrow) are located internally instead of superficially, leaving only neurons (arrowheads) visible at the surface of the embryo. (L) Median view of the embryo in K. The pharynx (arrowheads) has developed essentially normally, but, as a consequence of the massive leakage of cells and organs from this embryo, the pharynx became separated from the rest of the embryo. This embryo has a normal buccal cavity (arrow). (M) Lateral view of a wild-type embryo prior to hatching (pretzel stage). Arrowheads point at the smooth surface. (N) Lateral view of a lin-26(mc2) embryo that has elongated normally but will never hatch. Its surface is rough (arrowheads) and droplets (arrows) are floating in the egg.

Normally, a single GABA-positive neuron is found posterior to the rectum, the neuron DVB ( McIntire et al., 1993). We observed two GABA-positive neurons at that position in two lin-26(n156) animals (n=82), in contrast to the single GABA-positive neuron as expected in all wild-type animals examined (n=54). The additional GABAergic neuron in lin-26(n156) animals could be the cell K.a, as K.a is the sister of DVB and is normally a rectal cell.

lin-26 mutants display many hypodermal defects

The mutation lin-26(n156) is lethal in trans to a deficiency that deletes lin-26 (Ferguson and Horvitz, 1985). We examined such hemizygous lin-26(n156)/Df larvae and found that they had many hypodermal defects (Table 1). C. elegans hypodermal cells can be classified into three broad classes: cells that compose the external epithelium (White, 1988), the excretory system (Nelson and Riddle, 1984) and the rectum (White, 1988). In hemizygous lin-26(n156)/Df animals, all three classes of hypodermal cells appeared to be defective. First, about half of the lin-26(n156)/Df hemizygous animals were dumpy (Table 1; Fig. 2A, B). A dumpy body shape can be a consequence of hypodermal defects, as microfilaments present in the external epithelium are essential for embryo elongation (Priess and Hirsh, 1986) and as some dumpy mutations affect genes encoding cuticle collagen synthesized by the hypodermis (see von Mende et al., 1988; Kramer et al., 1990). Second, degeneration of the hypodermis was observed, particularly in the tail (Table 1; Fig. 2A, B). Third, the hypodermal excretory cell, canal and duct were vacuolated in 60% of hemizygous lin-26(n156)/Df animals (Table 1; Fig. 2C, D). Fourth, the hypodermal rectum appeared by Nomarski microscopy to be closed in 24% of hemizygous lin-26(n156)/Df animals (Table 1; Fig. 2E, F). In general, hypodermal cell fates were abnormal but did not appear to be transformed to an alternative fate, as they are in the case of the Pn.p cells. These hypodermal defects seem likely to be the cause of inviability of lin-26(n156)/Df animals. Most of the defects of lin-26(n156)/Df animals were also observed in homozygous lin-26(n156) animals, but at a much lower penetrance (Table 1). This observation suggests that the mutation lin-26(n156) is a weak loss-of-function mutation in a gene that affects all hypodermal cells.

lin-26 mutations cause hypodermal cells to die in embryos

We isolated three new lin-26 alleles, lin-26(mcl), lin-26(mc2) and lin-26(mc4), by a clonal non-complementation screen (see Materials and Methods). All three alleles resulted in embryonic lethality and failed to complement each other and lin-26(n156) for viability. All three new lin-26 alleles appeared to affect hypodermal cells more strongly and probably at an earlier stage of hypodermogenesis than did lin-26(n156). The defects displayed by lin-26(mcl) and lin-26(mc4) embryos are extremely similar to each other and more severe than those displayed by lin-26(mc2) embryos.

In homozygous lin-26(mcl) or lin-26(mc4) embryos, hypodermal cells became vacuolated, were difficult to see and reduced in number (Fig. 2G.I), suggesting that these cells had not been generated or that they were dying. For instance, in a wild-type comma-stage embryo there are 21 hypodermal nuclei that correspond to the nuclei of the body hypodermis (Sulston et al., 1983). We observed 21.1±1 such nuclei among 40 control embryos. By contrast, we observed 19.1±7.1 body hypodermal nuclei in lin-26(mcl) comma-stage embryos (n=24) and 17.7±5.7 in lin-26(mc1) terminal-stage embryos (n=30), 15.4±4.5 in lin-26(mc4) comma-stage embryos (n=36) and 16±7 in lin-26(mc4) terminal-stage
embryos (n=36). We presume that this reduction in hypodermal cells involved a process distinct from programmed cell death (Ellis et al., 1991), as the dominant mutation ced-9(n1950) did not prevent them. (n1950 blocks programmed cell deaths; Hengartner et al., 1992.) In addition, in lin-26(mc1) or lin-26(mc4) embryos, a few rare cells that occupied normal positions of hypodermal cells looked like neurons, suggesting that hypodermal cells might occasionally have adopted a neural fate (data not shown). Hypodermal cells that normally migrate circumferentially around the embryo starting from a dorsal position (Sulston et al., 1983) were not always found at their normal positions but rather were grouped in a dorsolateral position (data not shown). In 26% of lin-26(mc1) embryos (n=249) and 21% of lin-26(mc4) embryos (n=227), internal cells and organs burst from the ventral surface (Fig. 2K,L), presumably because hypodermal cells failed to join together in a ventral position as they should at the end of gastrulation (see Sulston et al., 1983). Finally, most lin-26(mc1) embryos and most lin-26(mc4) embryos did not elongate beyond the comma stage of embryogenesis (Fig. 3), consistent with the observation that microfilaments within the hypodermis are essential for embryogenesis (Fig. 3), consistent with the observation that microfilaments within the hypodermis (Fig. 2M,N). In addition, the hypodermis and the cuticle, which is synthesized by the hypodermis, appeared rough (Fig. 2M,N). The number and positions of hypodermal nuclei seemed normal.

We suspect that none of these three new lin-26 mutations is a null mutation. Hemizygous lin-26(mc1)/Df embryos failed to elongate and arrested at the lima-bean stage of embryogenesis more frequently than did homozygous lin-26(mc1) embryos (44% versus 23%; see Fig. 3, P≤0.01). Similarly, hemizygous lin-26(mc4)/Df embryos failed to elongate and arrested at the lima-bean stage of embryogenesis more frequently than did homozygous lin-26(mc4) embryos (39% versus 20%; see Fig. 3, P≤0.01). However, these lin-26(mc1)/Df and lin-26(mc4)/Df hemizygotes did not display a phenotype more severe than the most severe phenotype displayed by lin-26(mc1) or lin-26(mc4) homozygotes, arrest at the lima-bean stage. lin-26(mc1) and lin-26(mc4) behaved like a deficiency when heterozygous with lin-26(mc2), a partial loss-of-function mutation (see Fig. 3, lin-26(mc4) behaved also like a deficiency when heterozygous with lin-26(n156); however, lin-26(mc1) did not behave like a deficiency in this test, as fewer lin-26(n156)/lin-26(mc1) heterozygous embryos hatched than lin-26(n156)/Df hemizygous embryos hatched (Fig. 3). Together, these data suggest that the mutations lin-26(mc1) and lin-26(mc4) eliminate most but not all lin-26 gene function and raise the possibility that the null phenotype of lin-26 could be arrest at the lima bean stage of embryogenesis.

### Fig. 3. Terminal phenotypes of lin-26 mutants. The percentage of wild-type and lin-26 embryos and larvae terminating development with the terminal phenotypes is indicated. Standard developmental stages (see Wood et al., 1988) are listed and depicted schematically (in the third column, comma stage and one and a half-fold stage embryos were counted together). Embryos with fewer than 200 nuclei are considered as early arrest embryos. Embryos were obtained as described in Materials and Methods. mmDf88 is a deficiency that deletes lin-26. All lin-26 larvae with an asterisk (*), except those of genotype lin-26(n156), were scrawny and uncoordinated; these larvae died during the L1 or L2 stages with defects as described in Table 1.

**Table 1.**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>early arrest</th>
<th>lima bean</th>
<th>comma</th>
<th>two-fold</th>
<th>three-fold</th>
<th>pretzel</th>
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<td>645</td>
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<tr>
<td>lin-26(mc1)</td>
<td>&lt;1%</td>
<td>23%</td>
<td>71%</td>
<td>1%</td>
<td>2%</td>
<td>4%</td>
<td>1%</td>
<td>249</td>
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*lin-26 encodes a presumptive zinc-finger transcription factor*

We cloned the gene lin-26 by transformation rescue and localized this gene to an 8 kb restriction fragment (Fig. 4). We identified four abundant RNAs (2.2 kb, 1.5 kb, 1.45 kb and 1.4 kb) and two rare RNAs (3.2 kb and 4.4 kb; these RNAs were not further characterized) transcribed from this region. We identified the 2.2 kb transcript as the lin-26 RNA as described in Materials and Methods. In brief, we introduced frameshift mutations within the coding sequences of each of the four abundant RNAs. Only the mutation in the 2.2 kb RNA abolished...
C. elegans hypodermal cell fates

Fig. 4. Cloning of lin-26. For details, see Materials and Methods. (A) Part of the genetic map of GLI showing the extents of the four deficiencies used to map lin-26. The deficiency mnDf97 complemented lin-26(n156) only partially, suggesting that lin-26 is located very close to its left breakpoint. The cosmid C24G10 identified the left breakpoint of mnDf97. The overlapping cosmid K06E5 rescued the vulval phenotype of lin-26(n156) animals (see B). (B) lin-26 was localized to an 8 kb region limited by a BamHI site on the left and an HindIII site on the right. Plasmids carrying different fragments from cosmid K06E5 were tested for rescue of the vulval phenotype caused by lin-26(n156). The fraction of independently derived F2 transformants rescued for the vulval phenotype is shown on the right. (1) Rescue with this plasmid appeared to be incomplete, as animals had a strongly protruding vulva, suggesting an incomplete morphogenesis of the vulva. (C) Identification and structure of the lin-26 transcript. Four major RNAs were detected on northern blots using the 9 kb BamHI fragment shown in B as a probe. The structures of these RNAs were determined by comparing the nucleotide sequence of genomic DNA with the nucleotide sequences of cDNAs. The lin-26 mRNA was identified as the 2.2 kb RNA (see text for details). The 1.4/1.45/1.5 kb RNAs result from alternative splicing within their 3′ regions. The arrangement of the four major RNAs transcribed from this region is unusual. The 5′ untranslated region of the 2.2 kb RNA overlaps with the last two coding exons of the 1.4/1.45/1.5 kb RNAs, with the presumptive AUG initiator codon of the 2.2 kb RNA located immediately 3′ to the stop codons of the 1.4/1.45/1.5 kb RNAs. Thus, the coding sequence of the 2.2 kb RNA does not overlap with the coding sequences of the other three RNAs. A detailed description of the genomic features of the lin-26 region will be published elsewhere. We do not know the function(s) of the 1.4/1.45/1.5 kb RNAs.

the ability of the 8 kb restriction fragment to complement the vulval phenotype of lin-26(n156) mutants (Fig. 4C).

The deduced amino acid sequence of the protein encoded by lin-26 contains domains that strongly suggest that this protein is a transcription factor. Specifically, the LIN-26 protein has a serine/threonine-rich amino-terminal region, a glutamine-rich region, two copies of a cysteine/histidine motif (Cys/His motif), and a weakly acid-rich carboxy-terminal region (Fig. 5A, B); all of these types of domains have been associated with transcription factors (Ptashne, 1988). In addition, there is a proline/glutamic acid-serine/threonine-rich region (PEST motif), which is found in proteins with a short half-life (Rogers et al., 1986). The LIN-26 Cys/His motifs might form zinc-fingers, as they are similar to, although distinct from, the TFIIIA zinc-fingers that allow the Xenopus TFIIIA transcription factor to bind nucleic acids (Rhodes and Klug, 1986). In particular, the Cys/His motifs of the LIN-26 protein contain a normally invariant leucine and some residues of the TGEKPY stretch that links two consecutive TFIIIA zinc-fingers (Fig. 5C). Two features of the LIN-26 Cys/His motifs differ from those of canonical TFIIIA-type zinc fingers: the LIN-26 motifs lack a conserved phenylalanine and are somewhat longer than typical, with 17 residues between the second cysteine and the first histidine as opposed to the usual 12 residues. However, some zinc fingers, including two zinc-fingers of TFIIIA itself (Rhodes and Klug, 1986) lack this phenylalanine, and other zinc fingers – such as the second zinc-finger of the mammalian GLI1 protein (Pavletich and Pabo, 1993) – are nearly as long as the LIN-26 Cys/His motifs. Thus, we believe that these motifs are likely to encode TFIIIA-like zinc fingers.

Three lin-26 mutations affect the first putative zinc finger of the LIN-26 protein. The strong embryonic lethal allele lin-26(mc1) changes a glycine to glutamic acid, the weaker embryonic lethal allele lin-26(mc2) changes the adjacent serine to phenylalanine, and the viable allele lin-26(n156) changes the conserved leucine residue to phenylalanine (Fig. 5C). The fourth allele, lin-26(mc4), another strong embryonic lethal allele, is an amber mutation that affects a tryptophan residue located 11 amino acids carboxy terminal to the end of the second zinc finger (Fig. 5A).

DISCUSSION

The lin-26 gene appears to affect all C. elegans hypodermal cells. The strongest alleles, lin-26(mc1) and lin-26(mc4), caused many hypodermal cells to die during embryogenesis. As a consequence, lin-26(mc1) and lin-26(mc4) homozygous embryos did not elongate and subsequently died. Another embryonic lethal mutation, lin-26(mc2), caused hypodermal cells to fail to enclose the embryo properly. The weakest mutation, lin-26(n156), caused the hypodermal Pn.p cells to adopt a neural fate; in hemizygous lin-26(n156)/Df animals, other hypodermal cells degenerated, although their fates were generally unchanged.

The simplest interpretation of our results is that a complete loss of lin-26 function causes arrest at the lima-bean stage. First, the four lin-26 alleles we have studied result in a recessive phenotype and arose at a frequency typical for loss-of-function mutations (Brenner, 1977; Meneely and Herman, 1979; Greenwald and Horvitz, 1980). Second, two of the mutations we have characterized, lin-26(mc1) and lin-26(mc4), behave as strong loss-of-function alleles. Although a higher percentage of lin-26(mc1)/Df or lin-26(mc4)/Df hemizygous
embryos arrested at the lima bean-stage of embryogenesis than did lin-26(mc1) or lin-26(mc4) homozygous embryos, suggesting that these mutations are not null alleles, the phenotype of these hemizygous embryos was not more severe than that of the most severely affected homozygous embryos. Based upon our observations, lin-26 might function in any of three distinct ways: to specify the hypodermal cell fate, to express a neuronal fate suggests that this is required for cells to acquire the hypodermal fate. Since differential expression of three distinct ways: to specify the hypodermal cell fate, to specify the hypodermal cell fate as opposed to the neuronal fate. That many hypodermal cells die in the maintenance of the hypodermal cell fate once expressed.

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The presence of the most severely affected homozygous embryos supports the notion that the maintenance of the hypodermal cell fate once expressed.
hemizygous animals, lin-26 might also be required to maintain the differentiated state of hypodermal cells.

lin-26 encodes a protein that we presume to be a zinc-finger transcription factor. Two not mutually exclusive models could explain how lin-26 controls hypodermal differentiation. At a biochemical level, lin-26 could encode either a transcriptional activator or a transcriptional repressor. If lin-26 encodes a repressor that acts within the ectoderm, likely targets are genes that specify the neural fate. One argument in favor of a model in which lin-26 acts by preventing neural fates derives from the observation that the 12 Pn.p hypodermoblasts adopt a neural fate in lin-26(n156) mutants. An alternative hypothesis is that lin-26 acts not by preventing neural fates but rather by promoting non-neural fates. If lin-26 encodes an activator, likely targets are genes that specify the fate of each category of hypodermal cell. In this case, why do the Pn.p cells become neurons in lin-26(n156) mutants? First, lin-26 might act as a repressor of neural-specific genes in the Pn.p cells but not in other hypodermal cells. Alternatively, the lin-26(n156) mutation might confer a novel activity to the LIN-26 protein, causing it to activate the transcription of genes that are not activated by the wild-type LIN-26 protein.

We thank very much Steve McIntire for sharing his observation that lin-26(n156) animals have extra GABAergic neurons. We thank Daniella Rhodes and Grant Jacobs for useful comments about the structure of LIN-26 zinc fingers. We thank Stuart Kim for a sample of his cDNA library, and members of the laboratory of Barbara Meyer for good protocols. Some strains were provided by the Caenorhabditis Genetics Center, which is funded by the NIH National Center for Research Resources (NCRR) from the NIH. We thank Erika Jorgensen and other members of the Horvitz laboratory for numerous discussions. We are very grateful to Cori Bargmann, Michael Basson, Andrew Chisholm, Elisabeth Georges, Angela Giangrande, Erik Jorgensen, Josh Kaplan and Pat Simpson for critical reading of the manuscript. M. L. thanks Bernard Boulay for help with art work. Research at MIT was supported by US Public Health Service grants GM24663 and GM24943 to H. R. H. Research in Strasbourg was supported by a CNRS ATIP grant to M. L. and funds from the CNRS, INSERM, Centre Hospitalier Universitaire Régional, the Association pour la Recherche sur le Cancer and the Fondation pour la Recherche Médicale. M. L. was supported by Fellowships from the NIH/John Fogarty Center and the Human Frontier Science Program Organization. H. R. H. is an Investigator of the Howard Hughes Medical Institute.

The accession number within the EMBL databank for the sequence presented in Fig. 5 is X23673.

REFERENCES


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