Development 120, 2359-2368 (1994)
Printed in Great Britain © The Company of Biologists Limited 1994

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

Michel Labouesse[1,2], Satish Sookhareea[2] and H. Robert Horvitz[1]

[1]Howard Hughes Medical Institute, Department of Biology, Massachusetts Institute of Technology, Room 68-425, 77 Massachusetts Avenue, Cambridge, MA 02139, USA
[2]U. 184 INSERM - LGME CNRS, Faculté de Médecine, 11, Rue Humann, 67085 Strasbourg Cedex, France

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: *mnDf88 unc-4(e120)/mnC1[dpy-10(e128) unc-52(e444)]*, SP781: *mnDf97 unc-4(e120)/mnC1[dpy-10(e128) unc-52(e444)]*, SP803: *mnDf105 unc-4(e120)/ mnC1[dpy-10(e128) unc-52(e444)]*, SP804: *mnDf106 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 F_1 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 F_2s 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 F_1 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 *mcx* can be *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(mc1)/lin-26(mc4)* embryos, which were obtained by crossing *dpy-2(e489) lin-26(mc1) unc-4(e120)/ mnC1* hermaphrodites with *dpy-2(e489) lin-26(mc4) 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 of 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). F_1 rollers and stable F_2 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 K06E5, C18C9, F31B8 and F18A1 rescued *lin-26(n156)*, whereas the cosmids C36F4, C43A1, C18D9, C24G10, C01B9 did not rescue *lin-26(n156)*. A 17 kb *Mlu*I-*Spe*I fragment from the cosmid K06E5 was subcloned into the plasmid pIBI20 (IBI) (plasmid pMLW003). A 9 kb *Bam*HI and an 11 kb *Hin*dIII 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 *Sac*I or *Sph*I and religation. Frameshift mutations within pMLW006 were made by partial digestion with *Nde*I or *Eco*RI followed by end filling with Klenow enzyme; as a result the *Eco*RI site (G/AATTC) was transformed into an *Ase*I site (AT/TAAT). DNA manipulations were performed as decribed by Sambrook et al. (1988).

DNA sequencing, northern blots, isolation of cDNAs

The DNA sequence of both strands of the region bracketed by an *Sph*I site on the left and a *Hin*dIII site on the right (see Fig. 4) were determined using the dideoxy 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

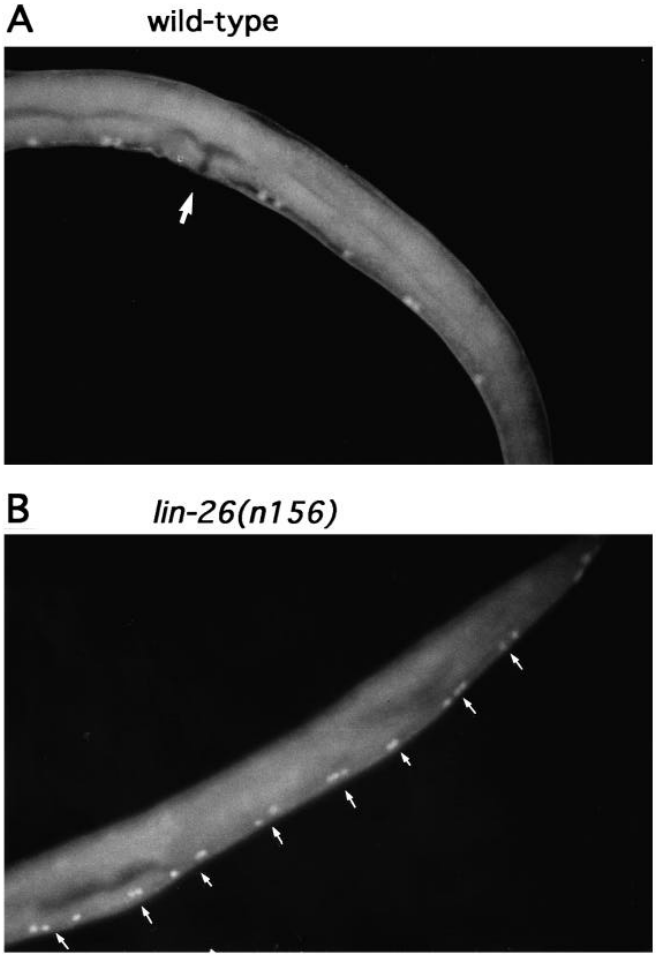


Fig. 1. *lin-26(n156)* animals generate extra GABAergic neurons. N2 and *lin-26(n156)* adults were fixed and stained with antibodies directed against the neurotransmitter GABA (see Materials and Methods). Anterior is to the left, and ventral is down. (A) Body of a wild-type (N2) animal. Note the regular pattern of one GABA-positive cell body followed by two GABA-positive cell bodies; N2 animals have on average 19±1 GABA-positive cells in their ventral nerve cords (n=54, of which 51 animals had 19 GABA-positive cells). The arrow marks the position of the vulva. (B) Body of a *lin-26(n156)* animal with eight extra GABA-positive cell bodies (arrows) in its ventral nerve cord. Note the abnormal pattern of two GABA-positive followed by three GABA-positive cell bodies. *lin-26(n156)* animals have on average 26±5 GABA-positive cells in their ventral nerve cords (n=82). *lin-26(n156)* animals lack a vulva (Ferguson and Horvitz, 1985).

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

Genotype	External epithelium			Excretory organ[d]	Anus/rectum[e]	Total[f]
	dumpy[a]	abnormal tail[b]	lack of Pn.p[c]			
+/+	0	0	2%	0	0	125
lin-26(n156)	2%	3%	100%	12%	0	109
lin-26(n156)/mnDf 88	48%	57%	100%	61%	24%	229

Animals were obtained as described in Materials and Methods.

[a]Animals with shorter size and larger cross section than usual.

[b]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 body and head of 25 other *lin-26(n156)/Df* hemizygous animals.

[c]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.

[d]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% animals of this class, the excretory pore was absent or not distinguishable.

[e]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.

[f]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 *mnC1* balancer chromosome.

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 1.5 kb RNA. Further cDNA clones for the 1.4/1.45/1.5 kb RNAs were generated using reverse transcriptase and the polymerase chain reaction (RT-PCR; see Sambrook et al., 1988) using oligonucleotides corresponding to the most 5′ and 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(mc1)* or *lin-26(mc2)* 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′TACGGATCCGACGCCGTCTCGGAAAGCTGAAG with
5′TCTAAGCTTTAATAACCTGATTAAATTTATTATTTG,
5′CGTGGATCCATGATTCTTCATGGCTTT with
5′TCTAAGCTTGTGCTGCTCCTCCAGCA,
5′TACGGATCCTCTTCATCATCAACTATCTAC with
5′TACGGATCCACAGAGTGCACACTTGTA,
5′TCTAAGCTTGAAGAGAGAGTCGATCA with
5′TACGGATCCCATCTATGAATAGCTGCTTCGAAC,
5′ATGCTTCCCAGATTATGTGC with
5′CTCGAATGCTGATGAAAGAATGC.

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-amino-butyric 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.


A
B
C
D
E
F
G
H
I
J
K
L
M
N

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(mc1)* and *lin-26(mc2)* were obtained as described in Materials and Methods. Anterior is to the left. Scale bar, 5 μm. (G,H,M) wild-type; (I,J,K,L) *lin-26(mc1)*; (N) *lin-26(mc2)*. Pictures of *lin-26(mc1)* 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(mc1)* embryo that has stopped elongation slightly before the comma stage. Some hypodermal cells have migrated around the embryo (compare to embryo in 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(mc1)* 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(mc1)* 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(mc1)* 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(mc1)* 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 collagens 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(mc1)*, *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(mc1)* 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(mc1)* 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(mc1)* comma-stage embryos (*n*=24) and 17.7±5.7 in *lin-26(mc1)* terminal-stage embryos (*n*=30), 19.5±4.5 in *lin-26(mc4)* comma-stage embryos (*n*=36) and 16±7 in *lin-26(mc4)* terminal-stage

Genotype	early arrest	lima bean	comma	two-fold	three-fold	pretzel	larva	Total
+/+		<1%	<1%				>99%	645
lin-26(mc1)	<1%	23%	71%	1%	2%	4%		249
lin-26(mc1)/mnDf88	1%	44%	54%			1%		125
lin-26(mc4)	3%	20%	77%			<1%		227
lin-26(mc4)/mnDf88	1%	39%	52%		1%	5%	1%*	146
lin-26(mc4)/lin-26(mc1)	6%	27%	63%	1%	2%	1%		96
lin-26(mc2)				3%	78%	16%	3%*	143
lin-26(mc2)/mnDf88		2%	9%	42%	31%	12%	3%*	178
lin-26(mc2)/lin-26(mc1)		3%	16%	37%	26%	15%	3%*	212
lin-26(mc2)/lin-26(mc4)	2%	7%	12%	47%	27%	4%	1%*	136
lin-26(n156)			2%	2%	1%	1%	94%	332
lin-26(n156)/mnDf88		<1%	<1%	4%	7%	6%	83%*	137
lin-26(n156)/lin-26(mc1)			2%	12%	18%	46%	32%*	134
lin-26(n156)/lin-26(mc4)	3%	2%	5%		6%	6%	80%*	341

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. *mnDf88* 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.

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 embryo elongation (Priess and Hirsh, 1986). By contrast, non-hypodermal organs appeared normal: *lin-26(mc1)* and *lin-26(mc4)* embryos formed a buccal cavity and a shortened but otherwise normal pharynx (Fig. 2H,J,L), an intestine, a gonad primordium and muscles that could twitch (data not shown).

Homozygous *lin-26(mc2)* embryos elongated normally but generally did not hatch (Fig. 3). At the end of embryogenesis, entire cells and droplets were seen floating within the egg shell, suggesting leakage of cells and cytoplasm through holes in 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; $P \leq 0.01$). 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; $P \leq 0.01$). 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.

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

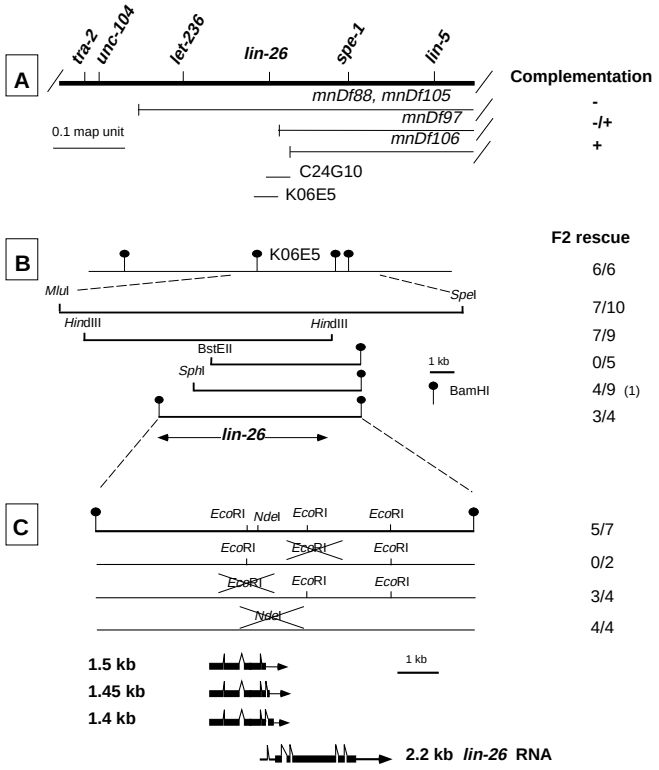


Fig. 4. Cloning of *lin-26*. For details, see Materials and Methods. **(**A) Part of the genetic map of LGII showing the extents of the four deficiencies used to map *lin-26*. The deficiency *mnDf97* complemented *lin-26(n156)* only partially, suggesting that *lin-26* is located very close to its left breakpoint. The cosmid C24G10 identified the left breakpoint of *mnDf97*. The overlapping cosmid K06E5 rescued the vulvaless phenotype of *lin-26(n156)* animals (see B). (B) *lin-26* was localized to an 8 kb region limited by a *Bam*HI site on the left and an *Hin*dIII site on the right. Plasmids carrying different fragments from cosmid K06E5 were tested for rescue of the vulvaless phenotype caused by *lin-26(n156)*. The fraction of independently derived F2 transformants rescued for the vulvaless 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 *Bam*HI 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 vulvaless 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

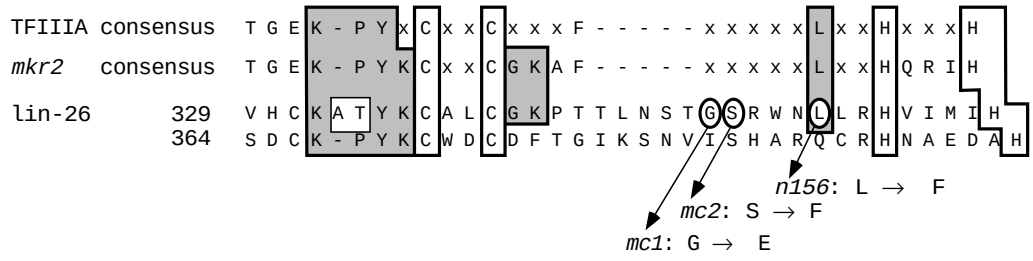
A

```
   1 GAATTCCAATGGAGTCAATTGTTAGATCAATGCTTCGGAGTCACATATCGAAAGTGGAAG
  61 CATCATAAGGAGGATACGCAGCACACCGGTGGGTTACTGTTCGATGACGACGAGTTGAGA
 121 GGCGGGTGATGAAGATTACGGTCACGAGCATCATTTTTCACCGGAGGAAGATGTAATGAC
 181 AACTTTATGATTCTTCATGGCTTTCACTTGTCGACGTCTTCCCATTGTCTTCCATTCATC
   1       M  I  L  H  G  F  H  L  S  T  S  S  H  C  L  P  F  I
 241 TTTCGTCATCTCTTCATATTTCACTCAATCACTGCCTTGTGCGATCATATGCTTTCTAAA
  19 F  R  H  L  F  I  F  H  S  I  T  A  L  C  D  H  M  L  S  K
 301 TTTGTGGTAGTCGAAGTAAGCAACTCAAATAACACAATGACACTGGTGGAATACTTGGTA
  39 F  V  V  V  V  E  V  S  N  S  N  N  T  M  T  L  V  E  Y  L  V
 361 ACGCATGGATTCGACAAGTTTGCAGTTGTAACAGATTTAGTTGATCACCCTTCTTTCAAG
  59 T  H  G  F  D  K  F  A  V  V  T  D  L  V  D  H  P  S  F  K
 421 TACAAGGATGGTTCATCAAGTCCCGAATCTCCATCAACAACTGCTTCAACTGCTGCCCAA
  79 Y  K  D  G  S  S  S  P  E  S  P  S  T  T  A  S  T  A  A  Q
                 xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx
 481 CACACACCACCACGAACAGCAGTTTCAACACCTACATCCATCAACACTCCAGTTCCACCA
  99 H  T  P  P  R  T  A  V  S  T  P  T  S  I  N  T  P  V  P  P
                                 xxxxxxxxxxxxxxxxxx
 541 CATCAAAACAAACAGAGGCATTCAATTGACAAAATCGCTGCAAATTTATCCACCAAAAAG
 119 H  Q  N  K  Q  R  H  S  I  D  K  I  A  A  N  L  S  T  K  K
 601 GTTTCACCATCATCTATTGAGAAGCAGCTCCAAAGAACATCTCATAATCCTCTTCATCAA
 139 V  S  P  S  S  I  E  K  Q  L  Q  R  T  S  H  N  P  L  H  Q
 661 CTATCTACCCCACATGCATTGAGTCAACTACAGAAATTGCTGGAGGAGCAGCACAAGATT
 159 L  S  T  P  H  A  L  S  Q  L  Q  K  L  L  E  E  Q  H  K  I
                                                      EcoRI
 721 AATCAGATGAATATTCAGAAAAAGGAACAGGAGAGGCAGCAAGCTGAGATTCAAAGAATT
 179 N  Q  M  N  I  Q  K  K  E  Q  E  R  Q  Q  A  E  I  Q  R  I
 781 CTGCTCCAACAGGCAAATGCTGCTCAGATTAATCACTCATTTGGATTAGAAAGGCTAACA
 199 L  L  Q  Q  A  N  A  A  Q  I  N  H  S  F  G  L  E  R  L  T
                                                   NheI.
 841 CCAGAATATGATGATAATCATCATAGTGAAACAATATCTAAAGCTAGCTCTGAAGATTTG
 219 P  E  Y  D  D  N  H  H  S  E  T  I  S  K  A  S  S  E  D  L
 901 AAGACTGAGCCCGATTCAACTGATTTTGGGCTTGGAACAAGCGACGATCAAGTACGTGCT
 239 K  T  E  P  D  S  T  D  F  G  L  G  T  S  D  D  Q  V  R  A
 961 TCAATGCTTCATCTGCTTCATCCAGTATTTGCTCCAGCTTTTGGGATGCTTGATGCCGAG
 259 S  M  L  H  L  L  H  P  V  F  A  P  A  F  G  M  L  D  A  E
1021 AATATCTTTGGAGCTGCATCAAAACCAACGACGCCTGCATCAAAACGACGAAACACTGAT
 279 N  I  F  G  A  A  S  K  P  T  T  P  A  S  K  R  R  N  T  D
```

```
1081 TCAAATGGTGCTCCATCGAAAAAACATCGTTGGCTCCCAGTCAATGAGCTTGAAGAGAGT
 299 S  N  G  A  P  S  K  K  H  R  W  L  P  V  N  E  L  E  E  S
1141 CGATCATCGCGAGGAAAGAACTGCGGAAGAGTTCATTGCAAGGCCACCTACAAGTGTGCA
 319 R  S  S  R  G  K  N  C  G  R  V  H  C  K  A  T  Y  K  C  A
1201 CTCTGTGGAAAGCCAACAACACTCAACTCAACAGGATCTCGTTGGAATCTTCTGCGCCAC
 339 L  C  G  K  P  T  T  L  N  S  T  G  S  R  W  N  L  L  R  H
1261  GTCATCATGATCCATTCGGATTGTAAGCCATATAAGTGTTGGGATTGTGACTTCACCGGA
 359  V  I  M  I  H  S  D  C  K  P  Y  K  C  W  D  C  D  F  T  G
1321 ATAAAATCCAACGTTATCTCCCACGCCAGGCAATGTCGTCACAACGCCGAAGATGCCCAT
 379 I  K  S  N  V  I  S  H  A  R  Q  C  R  H  N  A  E  D  A  H
1381 GACATTACTACCGACGAGATGCGTGCCGAATGGAATCTCCGCCTTCACGAATGCTTCCCA
 399 D  I  T  T  D  E  M  R  A  E  W  N  L  R  L  H  E  C  F  P
                                  ↑ mc4
1441 GATTATGTGCGTGCTAAAGAACGTGGATGGCAACCAGAGGAGGTTACTGTAAAGAAAGAA
 419 D  Y  V  R  A  K  E  R  G  W  Q  P  E  E  V  T  V  K  K  E
1501 GAAGTAGAGGAGTCTCCAACTCTTGTCAAACAAGAACTCACTCTTGTTAAACAAGAACCC
 439 E  V  E  E  S  P  T  L  V  K  Q  E  L  T  L  V  K  Q  E  P
1561 ACTTTTGCCGAGCAACTCGAGCCAATGGCTCAACCATTGGTGTAGTTCGAAGCAGCTATT
 459 T  F  A  E  Q  L  E  P  M  A  Q  P  L  V  *                 472
1621 CATAGATGAATTTTGCAATTCAATACTAGCCTTTAATTACTTTTTTCTATTGTTATGATG
1681 ATACATATCTTAGCCCTCCCCAATCAATATCTATGCACATTGATCACCTTGCCCACTTTT
1721 TAACAAACCATTCCAATCTGAATACACCTTGCCTCTCATTTGCTTTTTCATTCCTTCCGA
1801 TTATTGTTCTGTGATATTTCCTTCCAGTATTTCGTTCTGGTTGTGCCTTTTCATTCACCC
1861 GCAGGTCCGGTTAATTGAAAGTTCACTTTGCCCCCACAAATTTTGCCTTTATCTTCTCTG
1921 TTACAGTGCCTTAATTCACTTTTTCCATTGTACACCTTTTTTAACTTTACTCTTTGCCAC
1981 ACCATTCAGTTTCTCGACGGGTATTTATGTTGAATGATAGCATTCTTTCATCAGCATTCG
2041 AGATTTCTTATGTACATCTGATCATATTTTCTCAAGCATAATCTGAAATTCTTTCCATTT
2101 TCCCTGTAACGCATAATATGCAATTTAGCATAATGCGTTTTTTTGCAATTCTCTAGAAGC
2161 ATCTAATAAAAATTAAATAATACGGAATTC
```

B

C



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 allow the expression of the hypodermal cell fate or to allow the maintenance of the hypodermal cell fate once expressed. That in *lin-26* animals some presumptive hypodermal cells express a neuronal fate suggests that *lin-26* might in those cells specify the hypodermal fate as opposed to the neuronal fate. That many hypodermal cells die in *lin-26(mc1)* or *lin-26(mc4)* mutant embryos also supports the notion that *lin-26* might be required for cells to acquire the hypodermal fate. Since differentiated hypodermal cells can degenerate in *lin-26(n156)/Df*

Fig. 5. *lin-26* sequence and mutations. (A) Nucleotide sequence and predicted amino acid sequence of the longest *lin-26* cDNA. Nucleotides are numbered beginning at the first nucleotide of the cDNA, which includes an artificial *Eco*RI linker 5′GAATTCC at both ends. Amino acids are numbered beginning at the presumed ATG initiator codon (nucleotide 187) and ending at the presumed TAG stop codon (nucleotide 1602). The deduced protein is 472 amino acids long. Five potentially important functional domains have been underlined (see text): an amino-terminal region with 25% serines or threonines (dotted line); a middle region with 25% glutamines (single line); a carboxy-terminal region containing several negatively charged amino acids (each marked with an asterisk); two repeats of a cysteine-histidine motif (double line); a potential protein degradation PEST sequence (xxx), which scores over 14 by the algorithm of Rogers et al. (1987) (above 5 is considered to be a good match). The mutation *lin-26(mc4)* transforms a TGG codon (the tryptophan residue 409 marked with an arrow) into a TAG amber codon. (B) Schematic drawing showing the relative position of the five potentially important functional domains described in A. The PEST region overlaps with the SER/THR rich region (diagonals). (C) Alignment of the two LIN-26 potential zinc-finger motifs with the canonical TFIIIA zinc-finger consensus sequence (Rhodes and Klug, 1986) and positions of *lin-26* mutations. The region with the cysteine-histidine repeats is the only region of the LIN-26 protein to show similarity to other known protein sequences and is most similar to the zinc-finger motif in the mouse protein *mkr2* (Chowdhury et al., 1987). A consensus sequence for the nine *mkr2* zinc-fingers is shown (second line). By comparison with the TFIIIA and *mkr2* consensus zinc-fingers, there are five additional amino acids inserted in the LIN-26 zinc-finger motifs (marked by - - - - - within the TFIIIA and *mkr2* consensus). We have positioned the insertion in such a way as to respect the alpha-helix starting four amino acids before the first histidine; that alpha-helix is responsible for the specificity of interaction with DNA (Nardelli et al., 1991; Pavletitch and Pabo, 1991). The two LIN-26 cysteine-histidine repeats are shown below the *mkr2* consensus sequence. Three *lin-26* mutants are altered in the first LIN-26 potential zinc finger. The residue affected is circled, and the amino acid change is shown below. The mutation *lin-26(mc1)* transforms a GGA codon into a GAA codon; the mutation *lin-26(mc2)* transforms a TCT codon into a TTT codon; and the mutation *lin-26(n156)* transforms a CTT codon into a TTT codon.

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 Erik 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 ATIPE 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 Z32673.

REFERENCES

Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics* **77**, 71-94.

Chowdhury, K., Dressler, G., Breier, G., Deutsch, U. and Gruss, P. (1987). A multigene family encoding several ‘finger’ structure is present and differentially active in mammalian genomes. *Cell* **48**, 771-778.

Coulson, A., Waterston, R., Kiff, J., Sulston, J. and Kohara, Y. (1988). Genome linking with yeast artificial chromosomes. *Nature* **335**, 184-186.

Ellis, R. E., Yuan, J. and Horvitz, H. R. (1991). Mechanisms and functions of cell death. *Annu. Rev. Cell. Biol.* **7**, 663-698.

Ferguson, E. L. and Horvitz, H. R. (1985). Identification and characterization of 22 genes that affect the vulval cell lineages of the nematode *Caenorhabditis elegans. Genetics* **110**, 17-72.

Ferguson, E. L., Sternberg, P. W. and Horvitz, H. R. (1987). A genetic pathway for the specification of the vulval cell lineages of *Caenorhabditis elegans*. *Nature* **326**, 259-267.

Ghysen, A. and Dambly-Chaudière, C. (1988). From DNA to form: the *achaete-scute* complex. *Genes Dev.* **2**, 495-501.

Greenwald, I. S. and Horvitz, H. R. (1980). *unc-93(e1500)*: A behavioral mutant of *Caenorhabditis elegans* that defines a gene with a wild-type null phenotype. *Genetics* **96**, 147-64.

Hasty, P., Bradley, A., Morris, J. H., Edmondson, D. G., Venuti, J. M., Olson, E. N. and Klein, W. H. (1993). Muscle deficiency and neonatal death in mice with a targeted mutation in the myogenin gene. *Nature* **364**, 501-506.

Hengartner, M. O., Ellis, R. E. and Horvitz, H. R. (1992). *Caenorhabditis elegans* gene *ced-9* protects cells from programmed cell death. *Nature* **356**, 494-499.

Herman, R. K. (1978). Crossover suppressors and balanced recessive lethals in *Caenorhabditis elegans. Genetics* **88**, 49-65.

Hodgkin, J. A. and Brenner, S. (1977). Mutations causing transformation of sexual phenotype in the nematode *Caenorhabditis elegans*. *Genetics* **77**, 71-94.

Horvitz, H. R., Brenner, S., Hodgkin, J. and Herman, R. K. (1979). A uniform genetic nomenclature for the nematode *Caenorhabditis elegans*. *Mol. Gen. Genet.* **175**, 129-33.

Kim, S. and Horvitz, H. R. (1990). The *Caenorhabditis elegans* gene *lin-10* is broadly expressed while required specifically for the determination of vulval cell fates. *Genes Dev.* **4**, 357-371.

Kramer, J. M., Johnson, J. J., Edgar, R. S., Basch, C. and Roberts, S. (1988). The *sqt-1* gene of *C. elegans* encodes a collagen critical for organismal morphogenesis. *Cell* **55**, 555-565.

McIntire, S. L., Jorgensen, E., Kaplan, J. and Horvitz, H. R. (1993). The GABAergic nervous system of *Caenorhabditis elegans*. *Nature* **364**, 337-341.

Mello, C. C., Kramer, J. M., Stinchcomb, D. and Ambros, V. (1992). Efficient gene transfer in *C. elegans*: extrachromosomal maintenance and integration of transforming sequences. *EMBO J.* **10**, 3959-3970.

Meneely, P. M. and Herman, R. K. (1979). Lethals, steriles and deficiencies in a region of the X chromosome of *Caenorhabditis elegans*. *Genetics* **92**, 99-115.

Miller, D. M., Shen, M. M., Shamu, C. E., Bürglin, T. R., Ruvkun, G., Dubois, M. L., Ghee, M. and Wilson, L. (1992). *C. elegans unc-4* gene encodes a homeodomain protein that determines the pattern of synaptic input to specific motor neurons. *Nature* **355**, 841-845.

Nabeshima, Y., Hanaoka, K., Hasayaka, M., Esumi, S. E., Li, S., Nonaka, I. and Nabeshima, Y. (1993). Myogenin gene disruption results in perinatal lethality because of severe muscle defect. *Nature* **364**, 532-535.

Nardelli, J. Gibson, T. J., Vesque, C. and Charnay, P. (1991). Base sequence discrimination by zinc-finger DNA-binding domains. *Nature* **349**, 175-178.

Nelson, F. K. and Riddle, D. L. (1984). Functional study of the *Caenorhabditis elegans* secretory-excretory system using laser microsurgery. *J. Exp. Zool.* **231**, 45-56.

Pavletitch, N. P. and Pabo, C. O. (1991). Zinc finger-DNA recognition: crystal structure of a Zif268-DNA complex at 2.1 Å. *Science* **252**, 809-817.

Pavletich, N. P. and Pabo, C. O. (1993). Crystal structure of a five-finger GLI-DNA complex: New perspectives on zinc fingers. *Science* **261**, 1701-1707.

Priess, J. R. and Hirsh, D. I. (1986). *Caenorhabditis elegans* morphogenesis: the role of the cytoskeleton in elongation of the embryo. *Dev. Biol.* **117**, 156-73.

Ptashne, M. (1988). How eukaryotic transcriptional activators work. *Nature* **335**, 683-689.

Rhodes, D. and Klug, A. (1986). An underlying repeat in some transcriptional control sequences corresponding to half a double helical turn of DNA. *Cell* **46**, 123-132.

Rodriguez, I., Hernandez, R., Modolell, J. and Ruiz-Gomez, (1990). Competence to develop sensory organs is temporally and spatially regulated in *Drosophila* epidermal primordia. *EMBO J.* **9**, 3583-3592.

Rogers, S., Wells, R. and Rechsteiner, M. (1986). Amino acid sequences common to rapidly degraded protein: the PEST hypothesis. *Science* **234**, 364-368.

Rudnicki, M. A., Schnegelsberg, P. N. J., Stead, R. H., Braun, T., Arnold, H.-H. and Jaenisch, R. J. (1993). MyoD or Myf-5 is required for the formation of skeletal muscle. *Cell* **75**, 1351-1359.

Sambrook, J., Fristch, E. F. and Maniatis, T. (1988). *Molecular Cloning, a Laboratory Manual*. 2nd Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Sulston, J. E. and Horvitz, H. R. (1977). Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans. Dev. Biol.* **56**, 110-56.

Sulston, J. E., Schierenberg, E., White, J. G. and Thomson, J. N. (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Dev. Biol.* **100**, 64-119.

von, Mende, N., Bird, D. M., Albert, P. S. and Riddle, D. L. (1988). *dpy-13*: a nematode collagen gene that affects body shape. *Cell* **55**, 567-76.

Weintraub, H., Davis, R., Tapscott, S., Thayer, M., Krause, M., Benezra, R., Blackwell, T. K., Turner, D., Rupp, R., Hollenberg, S., Zhuang, Y. and Lassar, A. (1991). The *myoD* gene family: nodal point during specification of the muscle cell lineage. *Science* **251**, 761-766.

White, J. G. (1988). The anatomy. In *The Nematode Caenorhabditis elegans'*, (ed. W.B. Wood et al.) Monograph 17. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory, pp 81-121.

Williams, B. D., Schrank, B., Huynh, C., Shownkeen, R. and Waterston, R. H. (1992). A genetic mapping system in *Caenorhabditis elegans* based on polymorphic sequence-tagged sites. *Genetics* **131**, 609-624.

Wood,W. B. et al. (1988). *The Nematode Caenorhabditis elegans*. Monograph 17. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory, pp 81-121.

(Accepted 6 June 1994)