The *Caenorhabditis elegans* gene *lin-17*, which is required for certain asymmetric cell divisions, encodes a putative seven-transmembrane protein similar to the *Drosophila* Frizzled protein

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Mutations in the gene *lin-17* result in the disruption of a variety of asymmetric cell divisions in *Caenorhabditis elegans*. We have found that *lin-17* encodes a protein with seven putative transmembrane domains. The LIN-17 protein is most similar to the *Drosophila* Frizzled protein and its vertebrate homologs. Studies using a *lin-17*-green fluorescent protein translational fusion indicate that *lin-17* is expressed in mother cells before asymmetric cell divisions and in both daughter cells after the divisions. Our results suggest that *lin-17* encodes a receptor that regulates the polarities of cells undergoing asymmetric cell divisions and raise the possibility that the LIN-17 protein acts as a receptor for the Wnt protein LIN-44, which also controls asymmetric cell divisions.

[Key Words: Asymmetric cell division; cell polarity; *lin-17*, *lin-44*, frizzled; Wnt genes; *C. elegans*]

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Asymmetric cell division produces daughter cells with different cell fates and is fundamental to the generation of cellular diversity during development [Horvitz and Herskowitz 1992]. The daughter cells generated by an asymmetric cell division may initially be of equivalent developmental potential but become different from each other as a consequence of distinct environmental influences after their formation. Alternatively, such daughter cells may be intrinsically different. In the latter case, the differences between the daughter cells must be caused by qualitative or quantitative differences in inheritance from their mother cells. The polarized localization of cell-fate determinants within the mother cell can provide one basis for such differences in inheritance. In *Drosophila*, asymmetric cell divisions of sense organ precursor cells and neuroblasts involve the polarized localization of the products of the *numb* and *prospero* genes in the mother cells [Rhyu et al. 1994; Hirata et al. 1995; Knoblich et al. 1995; Spana and Doe 1995]. These proteins are segregated to only one of the two daughter cells and are required for the subsequent fate of that cell. Similarly, during the development of the mammalian cerebral cortex, the polarized localization of the Notch1 protein within cortical progenitor cells precedes its subsequent segregation into future neurons upon asymmetric cell division [Chenn and McConnell 1995]. In neither of these cases is it known how the polarities of the mother cells are established and regulated.

In *lin-17* mutants of the nematode *Caenorhabditis elegans*, certain asymmetric cell divisions are abnormal [Sternberg and Horvitz 1988; Way et al. 1992; Chamberlin and Sternberg 1995]. *lin-17* mutations affect the divisions of a variety of cells, including ectodermal, gonadal, and neural cells that are not related by their lineage histories, by their positions, or by the developmental stages at which they divide. For example, in *lin-17* mutants an abnormal division of the cell P7.p in the mid-body causes the hermaphrodite to have an ectopic vulva-like protrusion (the multivulva phenotype), while defects in the divisions of the B, T, P10.p, P11.p cells in the male tail result in abnormal tail structures [Ferguson et al. 1987; Sternberg and Horvitz 1988]. In most cases, the affected cell divisions are asymmetric in wild-type animals but symmetric in *lin-17* animals, producing sister cells with similar cell fates. In addition, *lin-17* mutations cause divisions that would normally produce sister cells of unequal size to instead generate cells of equal size. For this reason, it has been suggested that *lin-17* functions prior to or during cell division to establish the polarity of mother cells and that this polarity determines the asymmetry of these divisions [Sternberg and Horvitz 1988].

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Sawa et al.

We cloned lin-17 and found that this gene encodes a protein with seven putative transmembrane domains. The LIN-17 protein is most similar to the product of the frizzled gene of Drosophila (Vinson et al. 1989). frizzled mutations disrupt the polarities of a number of cells and tissues but apparently do not affect cell divisions (Adler 1992; Gubb 1993). We propose that the LIN-17 protein acts as a receptor to regulate cell polarity.

Results

Positional cloning of lin-17

The lin-17 gene was localized previously to the left arm of chromosome I (Ferguson and Horvitz 1985) and was recently mapped 0.8 map unit (mu) left of the pop-1 gene, located on the cosmid BF8 on the physical map of the C. elegans genome [Lin et al. 1995; Fig. 1]. We mapped lin-17 0.15 mu right of restriction fragment length polymorphism (RFLP) nP58, which we localized near the left end of the cosmid C51H11 (see Materials and Methods). Our germline transformation experiments [Mello et al. 1991] indicated that the cosmids C51H11 and W09G8 could not rescue the lin-17 mutant phenotype (data not shown), suggesting that lin-17 lies between W09G8 and BF8 in a region of the physical map not covered by cosmids. We identified a genomic phage clone k6-2 that extends into the cosmid gap from W09G8. This phage clone detected RFLPs in two lin-17 mutants, rh41 and sy277 [data not shown]. Using fragments of λ-2 as probes, we identified cDNAs from an embryonic C. elegans cDNA library (Okkema and Fire 1994). We determined the sequence of a 1.9-kb cDNA and the corresponding part of the genomic sequence of λ-2 and found that λ-2 lacks 322 bp of sequence at the 3’ end of the cDNA. λ-2 did not rescue the lin-17 phenotype (Table 1). We failed to identify genomic clones that contained the entire cDNA sequence from a genomic phage library. To determine whether the 1.9-kb cDNA was derived from lin-17, we constructed an expression plasmid [pSH2] in which a 13-kb HindIII-SphI fragment of k6-2 containing the 5’ portion of the gene was ligated to the 3’ portion of the cDNA at a SphI site in exon 7 [Fig. 1]. We generated transgenic animals carrying pSH2 as a transgene in a lin-17 background and scored three aspects of the lin-17 mutant phenotype: multivulva formation in the hermaphrodite, abnormal tail structure in the male, and defective phasmids in the hermaphrodite (the phasms are a bilateral pair of sensory structures in the tail). We found that the transgene containing pSH2 could rescue all three defects (Table 1). The lin-17 phenotype was also rescued by the expression of the 1.9-kb cDNA under the control of a heat-shock promoter [Stringham et al. 1992; pSH4 in Table 1]. These results, together with the identification of mutations within the gene that encodes the 1.9-kb cDNA [see below], demonstrate that the 1.9-kb cDNA corresponds to a functional lin-17 gene.

To identify the genomic sequence corresponding to the 3’ end of the lin-17 cDNA, we amplified a genomic fragment using the polymerase chain reaction (PCR) and primers complementary to exon 8 and to the sequence near the 3’ end of the cDNA. We identified a 2 kb-intron 8 and exon 9 in the amplified fragment [S1A2]. Thus, our results indicate that lin-17 contains nine exons.

lin-17 encodes a protein similar to Frizzled

The 1.9-kb cDNA contained 10 nucleotides of an SL1 trans-spliced leader sequence [Krause and Hirsh 1987] and six nucleotides of a polyA tail, indicating that this cDNA represents a full-length lin-17 transcript [Fig. 2]. The cDNA has a 1674-bp open reading frame (ORF) beginning at the first ATG codon. Hydrophobicity analysis [Kyte and Doolittle 1982; data not shown] of the predicted protein from the ORF indicated that the protein has a potential signal sequence at its amino terminus and seven putative transmembrane domains [Figs. 2 and 3]. A search of available protein data bases revealed that the LIN-17 protein is 25-30% identical to the frizzled gene product of Drosophila (Vinson et al. 1989) and its vertebrate homologs [Chan et al. 1992; Zhao et al. 1995; Wang et al. 1996]. Similarity was found in the putative amino-terminal extracellular domain, as well as between

Figure 1. Molecular cloning of lin-17. [A] Genetic map of part of the left arm of chromosome I (above), and cosmid clones from a part of the physical map (Coulson et al. 1988) of this region (below). See Materials and Methods for mapping data. [B] Molecular map of the lin-17 locus. Restriction sites shown: E, EcoRI; H, HindIII, S, SphI. Only EcoRI sites are shown on λ-2. EcoRI sites at both ends of λ-2 are in the vector sequence. S1A2: A PCR fragment obtained using primers complementary to exons 8 and 9. The 4.8-kb and 1.2-kb EcoRI fragments of k6-2 are shown, respectively. Solid boxes indicate the open reading frame. SL1 is a trans-spliced leader sequence [Krause and Hirsh 1987]. The rescuing construct pSH2 and the expression construct pSH6 are shown below schematically. Open boxes represent exon sequences from the cDNA. The shaded box represents the coding sequence of the GFP marker gene [Chalfie et al. 1994].
The transmembrane domains [Fig. 3]. The identity is not as high as that between the Drosophila Frizzled protein and the mammalian proteins shown in Figure 3 [41–44% identity]. Strikingly, all 17 cysteine residues in the putative extracellular regions (indicated by asterisks in Fig. 3) are highly conserved among the Frizzled family proteins, including LIN-17 (some of the Frizzled family proteins not shown in Fig. 3 do not have all of the cysteine residues). The amino-terminal-most 10 of these cysteines are completely conserved and are also found in a variant of mouse type XVIII collagen in a domain not conserved among collagens (Rehn and Pihlajaniemi 1995). LIN-17 has a potential Src-homology-3 (SH3) binding consensus sequence (Yu et al. 1994) near its carboxyl terminus (Fig. 3), such a sequence is not seen in the other Frizzled family proteins. Although the seven-transmembrane topology is characteristic of G-protein-coupled receptors (Dohlman et al. 1991; Strader et al. 1994), interactions between Frizzled/LIN-17-like proteins and G proteins have not been reported.

Molecular lesions and phenotypic consequences of lin-17 mutations

To identify the molecular lesions in the 12 existing lin-17 alleles, we amplified genomic DNA from mutant animals by PCR and determined the sequences of the coding regions and the splice junctions. We found DNA alterations in all 12 alleles (Figs. 2, 3, and 4). We also quantified the penetrance of the phasmid defect caused by each allele (Table 2). This defect is likely to be caused by an absence of phasmid socket cells, as a result of the abnormal asymmetric division of the T cells (Stemberg and Horvitz 1988).

The weakest allele, n698, changes an invariant splice-donor GT sequence to AT in intron 1. Three other alleles (e1456, e2257, and n2370) have single-base changes in splice-acceptor sites (AG to AA). The second weakest allele, rh71, substitutes glycine for aspartate at codon 467. In each of these alleles, the stop codon is not in exon 1 but in intron 2, which is trans-spliced to the 5' leader sequence (Fig. 2). This alteration results in a potential new splice-junction site that gives rise to a new open reading frame, which codes for a truncated protein with a putative nitrylase-like enzymatic activity (Mladenov et al. 1993; Worrell et al. 1995). A possible explanation for the inability of these alleles to rescue the lin-17 phasmid defect is that the truncated protein is unable to interact with wild-type LIN-17.

Table 1. Rescue of the lin-17 phenotype

| DNA                              | Heat shock | Multivula (%) | Abnormal male tail (%) | Abnormal phasmsids (%) | Rescue |
|----------------------------------|------------|---------------|------------------------|------------------------|--------|
| None                             |            |               |                        |                        |        |
| x6-2                             |            |               |                        |                        |        |
| pSH2 (lin-17 genomic-cDNA)       |            |               |                        |                        |        |
| pSH4 (hs-promoter-lin-17 cDNA)   |            |               |                        |                        |        |
| pSH6 (lin-17 genomic-cDNA-GFP)   |            |               |                        |                        |        |

pSH2 contains a genomic fragment from x6-2 ligated to the 3' portion of the lin-17 cDNA. pSH4 has the lin-17 cDNA sequence downstream of a heat-shock promoter, hspl6-2 (Stringham et al. 1992). pSH6 contains the genomic fragment, a portion of the lin-17 cDNA, and the GFP reporter gene. See also Fig. 1 for pSH2 and pSH6. DNA was transformed into worms, and the GFP reporter gene was scored, respectively. Each side of animals has a phasmid. In addition to transgenic lines shown here, all other lines analyzed (10 lines with pSH2, two lines with pSH4 with heat-shock treatment, and two lines with pSH6) showed similar rescue of the lin-17 phenotype.

Figure 2. lin-17 cDNA sequence. Nucleotide sequence of the 1.9-kb lin-17 cDNA c21 and its predicted amino-acid sequence. The positions of introns and a part of the SLI trans-spliced leader sequence are indicated by arrowheads and in lowercase letters, respectively. The putative polyadenylation sequence is underlined. (*) Nucleotides altered in lin-17 mutants. The potential signal sequence is shown in bold letters and putative transmembrane domains are boxed. The potential SH3-binding site is shown in bold italic letters.
The n669 mutation is a missense mutation that converts an invariant glycine at codon 486 to arginine. One of the frizzled mutations of Drosophila (FzHCS0) contains the same glycine-to-arginine substitution at the equivalent codon (Jones et al. 1996), suggesting the importance of this residue for the function of LIN-17/Frizzled family proteins. The residues mutated in rh71 and n669 animals could define interaction sites with ligands or effectors.

Among the alleles with the greatest effect on phenotype, n3091 was found to be an ochre mutation located close to the start of the coding sequence. The predicted n3091 mutant product has only 34 amino acids in addition to the presumptive signal sequence and does not have any transmembrane domains. Therefore, it is quite likely that n3091 is a null allele. Three other similarly strong lin-17 alleles (n671, n677, and rh75) also contain nonsense mutations and might be null mutations.

The sy277 allele contains a deletion that starts 51 bp upstream of the SL1 splice site and ends in the second exon 26 bp downstream of the splice acceptor site. Because this allele is not as severe as n3091, it is likely that sy277 generates residual lin-17 activity, perhaps by initiating translation from an internal methionine, such as methionine 69. The rh41 allele appears to have a deletion or a rearrangement of at least a portion of the 9th exon (see Materials and Methods).

**lin-17 is expressed both before and after asymmetric cell divisions**

To analyze the expression of lin-17, we constructed a plasmid (pSH6) in which a reporter gene that encodes the green fluorescent protein (GFP, Chalfie et al. 1994) was fused translationally to lin-17 near the 3' end of the lin-17 coding region (Fig. 1). The fusion protein produced from pSH6 contained the complete LIN-17 amino acid sequence except for the carboxy-terminal eight amino acids. When transformed into a lin-17 (rh75) mutant, pSH6 rescued the lin-17 phenotype (Table 1). This result indicated that the LIN-17–GFP fusion protein has LIN-17 protein function and is consistent with the hypothesis that the subcellular localization of the fusion protein is similar to that of the endogenous LIN-17 protein.

Because lin-17 affects the development of the hermaphrodite vulva and the male tail (Ferguson and Horvitz 1985), we examined the expression of the LIN-17–GFP fusion protein in these tissues. In all experiments described below, the fluorescence of the LIN-17–GFP fusion protein was detected in the region of cell membranes, as expected for a protein with presumptive transmembrane domains. We detected weak lin-17 expression in the cells that form the vulva only after the vulval precursor cells [P5.p, P6.p, and P7.p] had divided twice, i.e., in all of the Pn.pxx cells [Fig. 5A]. We observed lin-17 expression in the male tail throughout development.
with expression particularly strong at the late L3 stage in most descendants of the V6 and T cells in the lateral hypodermis, which produce the ray structures (Fig. 5B). Although defects in these lineages at the L3 stage in lin-17 mutants have not been reported, rays are variably missing in most lin-17 males (data not shown).

We analyzed lin-17 expression in specific cells [P7.p, P10.p, P11.p, Z1, Z4, B, and T cells] with divisions known to be affected by lin-17 mutations [Sternberg and Horvitz 1988; see Fig. 6]. The asymmetry of the divisions of these cells is frequently disrupted in lin-17 mutants. We detected lin-17 expression in the P11.p and B cells. This expression was almost uniform around the cell surface prior to their asymmetric divisions (Fig. 5C,E). lin-17 was also expressed in both daughter cells and all the granddaughter cells of the P11.p and B cells (Fig. 5D,F, also data not shown). Expression in the daughter cells was also almost uniform around the cells. In addition, we detected weak lin-17 expression in the P10.p cell and both of its daughter cells (Fig. 5E,F). We failed to detect lin-17 expression in P7.p, Z1, Z4, and T cells, possibly because of an insufficient sensitivity of our method.

Although in these experiments lin-17 expression did not strictly correlate with known sites of lin-17 function, these results nonetheless indicate that lin-17 is expressed in mother cells before asymmetric cell division and probably in both daughter cells after division. It will be important to obtain more direct evidence concerning the localization of endogenous LIN-17 protein once antibodies against the LIN-17 protein are available.

Table 2. lin-17 phenotypes

| Genotype | Abnormal phasmids (%) |
|----------|-----------------------|
| Wild-type | 1 [n = 222] |
| n698     | 73 [n = 346] |
| rh71     | 81 [n = 400] |
| sy277    | 86 [n = 394] |
| n2370    | 88 [n = 300] |
| e2257    | 90 [n = 300] |
| n669     | 92 [n = 400] |
| n671     | 95 [n = 400] |
| n3091    | 95 [n = 516] |
| n677     | 96 [n = 400] |
| rh41     | 97 [n = 332] |
| rh75     | 97 [n = 400] |
| e1456    | 97 [n = 300] |

Wild-type and lin-17 mutant animals were grown at 25°C and the penetrance of their phasmid defects was scored as described in Materials and Methods. n: number of phasmids scored.

Discussion

lin-17 and frizzled regulate cell polarities

We have shown that lin-17 encodes a putative seven-transmembrane protein similar to the frizzled gene product of Drosophila. The frizzled gene is one of a number of tissue-polarity genes that are required for the correct polarities of many tissues, such as ommatidia, hairs, bristles, and tarsi segments [Adler 1992; Gubb 1993]. The orientation of these structures reflects the coordination of cell polarities within the plane of the underlying epidermis. Therefore, both lin-17 and frizzled seem to function in regulating the polarities of cells. The axis of cell polarities controlled by both genes is not the apical–basal axis typical of many polar cells. Rather, the direction of asymmetric cell divisions affected by lin-17 mutations (e.g., divisions of B, P10.p, and P11.p cells) and that of the hairs affected by frizzled mutations is orthogonal to the apical–basal axis.

lin-17 and frizzled are members of a gene family that includes vertebrate homologs; for example, seven such genes have been identified in the mouse [Wang et al. 1996]. Some of the vertebrate family members also may be involved in the regulation of cell polarities. However, none of the vertebrate proteins is strikingly similar to the LIN-17 protein, and it is possible that a vertebrate ortholog of lin-17 remains to be discovered.

Regulation of asymmetric cell divisions by lin-17

The Frizzled protein has been suggested to act as a receptor for an intercellular polarity signal [Vinson et al. 1989; Adler et al. 1990; Park et al. 1994]. Some frizzled alleles behave cell-autonomously, suggesting that frizzled function is required in intercellular signaling for the
reception of a signal [Vinson and Adler 1987]. The Frizzled protein is an integral membrane protein with an odd number of transmembrane domains, most likely seven [Park et al. 1994]. Based on its similarities to the Frizzled protein in amino acid sequence and hydrophyt, the LIN-17 protein is likely to have the same membrane topology. Consistent with this hypothesis, the LIN-17-GFP fusion protein was localized in or near cell membranes. This fusion protein was expressed before asymmetric cell divisions and, unlike the Numb and Prospero proteins of Drosophila, was not asymmetrically segregated into only one of the daughter cells. Thus, it is unlikely that the LIN-17 protein is an asymmetrically segregated determinant of cell fates. These considerations are consistent with the earlier hypothesis that lin-17 is required to establish the asymmetry of the mother cell, based upon the finding that lin-17 mutations can affect the relative sizes of the two daughter cells [Sternberg and Horvitz 1988; Chamberlin and Sternberg 1995]. Together, these observations suggest that the LIN-17 protein is a seven-transmembrane receptor that regulates the polarities of certain mother cells and the consequent differences between their daughter cells.

LIN-17 may be a receptor for the LIN-44 Wnt protein

Like mutations in lin-17, mutations in lin-44 affect a number of asymmetric cell divisions in C. elegans [Herman and Horvitz 1994]. In lin-44 mutants, the polarities of certain asymmetric cell divisions are reversed, i.e., the fates of anterior daughter cells are transformed to those of the corresponding posterior daughters and vice versa. In a lin-17 lin-44 double mutant, the divisions of the B and T cells are symmetric as in lin-17 single mutants, indicating that lin-17 function is required for lin-44 mutations to have their effects and thus suggesting that lin-17 acts downstream of lin-44 to regulate the polarities of the B and T cells [Herman and Horvitz 1994]. lin-44 encodes a putative signaling protein that is a member of the Wnt gene family [Herman et al. 1995]. Wnt genes encode signaling proteins involved in many aspects of development [Nusse and Varmus 1992]. Receptors of the Wnt proteins have not been identified.

Our finding that lin-17 encodes a receptor-like protein raises the possibility that the LIN-17 protein acts as a receptor for the LIN-44 protein. lin-44 is expressed in hypodermal cells at the tip of the tail and regulates the polarities of cells close to and anterior to the lin-44-expressing cells [Herman et al. 1995; Fig. 6A]. Therefore, the LIN-44 protein may activate the LIN-17 receptor at the posterior side of cells such as the B and T cells to give them a posterior-directed polarity [wild-type in Fig. 6B]. In addition to the division of the B and T cells, the divisions of cells further anterior (Z1, Z4, P7.p, P10.p, and P11.p cells) are affected by lin-17 mutations (Sternberg and Horvitz 1988; Fig. 6A). However, these cells are not affected by lin-44 mutations (Herman and Horvitz 1994).

We postulate that an unidentified signaling molecule, which we refer to as ‘X,” expressed in the mid-body region regulates the divisions of these anterior cells through the LIN-17 receptor (Fig. 6A). A Wnt gene of C. elegans other than lin-44 [Shackleford et al. 1993] might encode such a signal. In the absence of lin-44 activity, X might affect the polarities of the B and T cells in addition to those of the anterior cells. Specifically, in this case, signal X could act through the LIN-17 receptor on the anterior side of the B and T cells, conferring a reversed anterior-directed polarity, which would account for the reversed-polarity phenotype of lin-44 mutants [lin-44 in Fig. 6B]. In lin-17 mutants, because the B and T cells can not respond to either X or the LIN-44 signal, cell polarity is not established, resulting in symmetric divisions [lin-17 in Fig. 6B].

No ligand for the Drosophila Frizzled protein has been identified. However, wingless, a Drosophila Wnt gene, has been suggested to act in the regulation of tissue polarity [Theisen et al. 1994]. In addition, the gene dishevelled is known to act downstream of both frizzled and wingless [Noordermeer et al. 1994; Siegfried et al. 1994; Krasnow et al. 1995]. Therefore, the Frizzled protein and/or its Drosophila homologs (e.g., Dfz2) [Wang et al. 1996] may act as a receptor for the Wingless protein and/or its homologs [Russel et al. 1992] in Drosophila. Similarly, we propose that mammalian LIN-17/Frizzled homologs may also act as receptors of Wnt proteins. Together, such proteins might act in a signaling pathway to regulate asymmetric cell divisions in mammals, as in C. elegans.

Materials and methods

General methods

Methods for the culture and genetic manipulation of C. elegans were as described previously [Brenner 1974]. Animals were

![Figure 6](genesdev.cshlp.org)
grown at 22.5°C unless otherwise noted. N2 was used as the wild-type strain. Standard molecular biology techniques were used [Sambrook et al. 1989]. DNA sequences were determined using an ABI 373A automated sequencer.

Identification and mapping of RFLPs
We identified RFLPs by hybridizing cloned genomic DNAs to Southern blots containing DNAs from N2 and lin-17 mutants. nP58 was identified in DNA from strain MT4198, which carries the lin-17 mutation th75, by HindIII digestion using cosmid C30A7 as a probe (data not shown). A 4-kb fragment of C30A7 that could detect nP58 hybridized to a left-end fragment of C51H11 (8.2-kb HindIII fragment, which included the vector sequence), indicating that nP58 is near the left end of C51H11. To map nP58, we isolated Lin-17 non-Sup recombinants from nP58 lin-17(th75) sup-11(a403)/++ + animals and Lin-17 non-Lin-6 recombinants from lin-6(e1466) nP58 lin-17(th75)/dpy-5(e61) animals and determined the presence of nP58 in the recombinants. All 24 Lin-17 non-Sup recombinants and 38 of 41 Lin-17 non-Lin-6 recombinants carried nP58, placing lin-17 about 0.15 μm right of C30A7.

Isolation and characterization of lin-17 cDNAs
K6-2 was isolated by Kerry Kornfeld [pers. comm.] from a C. elegans genomic phage library using a YAC clone, Y171F as a probe. By Southern blot hybridization experiments, we found that K6-2 detected RFLPs in rh41 and sy277. To identify lin-17 cDNAs, we screened a cDNA library constructed from C. elegans embryonic RNA (Ottkema and Fire 1994) using 4.8-kb and 1.2-kb EcoRI fragments of K6-2. We obtained five clones (a1–a5) that hybridized to only the 4.8-kb fragment, 14 clones (b1–b14) that hybridized to only the 1.2-kb fragment, and 63 clones (c1–c63) that hybridized to both fragments. We amplified inserts of 10 of these putative cDNAs (a2, b11, c14, c21, c37, c40, c46, c50, c57, and c63) and determined the sequences of each of their ends. All but two cDNAs had polyA sequences at the same site. The a2 and c14 cDNAs did not have a polyA sequence, and their 3’ ends corresponded to nucleotides 785 and 1903, respectively, of cDNA c21, as shown in Figure 2. The 5’ ends of the 10 clones were at nucleotides 247 (a2), 601 (b11), 94 (c14), 1 (c21), 12 (c37), 26 (c40), 8 (c46), 1 (c50), 10 (c57), and 14 (c63). The lengths of these cDNAs and their terminal sequences [the sequences of 300–400 bp at the 5’ and 3’ ends of each were determined] are consistent with the idea that all derived from a single transcript. One of the longest cDNA clones (c21) was digested with BsiWI and subcloned in both directions into plasmid pSL1190 [Pharmacia] to yield plasmids pc21F and pc21R. The sequences of both strands of this cDNA clone were determined by constructing nested deletions of these plasmids with Exonuclease III. Data-base searching was done at the National Center for Biotechnology Information using the BLAST network service.

Transformation rescue
Both a 13-kb HindIII–SphI fragment of K6-2 and a 0.9-kb SphI–PvuII fragment of pc21R were cloned upstream of the unc-54 3’ end untranslated sequence [Fire et al. 1990], giving rise to the rescuing plasmid pSH2. pSH2 was constructed by cloning the entire cDNA fragment [KpnI–PvuII fragment of c21] downstream of the hsp16-2 heat-shock promoter [Stringham et al. 1992] and upstream of the unc-54 3’-end sequence. Plasmids were transformed into lin-17(th75), him-8(e1489) animals with plasmid pRF4, which contains the dominant roller mutation rol-6(su1006) [Mello et al. 1991]. him-8 was used to produce spontaneous male progenies [Hodgkin et al. 1979]. Stable roller lines were established, and the penetrances of the defects of transformants in the vulva, plasmids, and male tail structure were scored. In case of pSH4, progeny of the transformants were heat shocked twice each at 33°C for 30 min, once at an embryonic stage, and once 24 hr after the first treatment. Animals subjected to a single heat shock were not fully rescued for the lin-17 defects (data not shown). Abnormalities in the structure of the male tail and the hermaphrodite vulva were scored in transformed adult animals by use of a dissecting microscope. Defects in the plasmodia of adult hermaphrodites were scored using a dye-filling assay, as described previously [Herman and Horvitz 1994]. Briefly, animals were soaked in a 10 μg/ml solution of 3,3′-dioctadecyloxycarbamide (DiO) in M9 buffer for 2 hr and analyzed using an FITC filter set and epifluorescence microscopy.

Identification of lin-17 mutations
Genomic DNAs from lin-17 mutant animals were amplified by PCR and their sequences determined in both primers complementary to sequences upstream and downstream of each exon. A polymorphism in th41 was detected on a Southern blot when K6-2 was used as a probe after digestion with EcoRI but not after digestion with Hhal or HaeIII (data not shown). When the 1.9-kb lin-17 cDNA was used as a probe, we detected a polymorphism in all three digests (data not shown), suggesting that the polymorphism is in or very close to the ninth exon, which is not covered by K6-2. Using combinations of primers complementary to the 9th exon and upstream sequences, we failed to amplify DNA fragments containing the 9th exon from th41 animals by PCR, consistent with the hypothesis that the corresponding DNA sequences are absent or rearranged. The sequences of the primers used for these experiments are available upon request.

Expression analyses
To construct the lin-17–GFP fusion plasmid, we used a deletion clone [Ra8] of the lin-17 cDNA. Ra8 has nucleotides 1–1690 of the c21 cDNA sequence. pSH6 was constructed by cloning both the 13-kb HindIII–SphI fragment of K6-2 and a 0.65-kb SphI–NsiI fragment of Ra8 into a derivative of the vector TU#62 [Chalfie et al. 1994], which has a missense mutation in the GFP coding sequence. The mutation is equivalent to the serine to threonine mutation at position 65 described previously [Heim et al. 1995] and enhances the fluorescence of GFP in nematodes [Y. Lin, pers. comm.]. Transgenic lines containing pSH6 were established in a him-5(e1467) unc-76(e911) background using rescue of the unc phenotype conferred by the unc-76-containing plasmid pRF4 as the transgenic marker. GFP expression was analyzed using an FITC filter set and epifluorescence microscopy, as described previously [Chisholm and Horvitz 1995].

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