The *Caenorhabditis elegans* gene *lin-44* controls the polarity of asymmetric cell divisions

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SUMMARY

The generation and orientation of cellular and organismic polarity are fundamental aspects of development. Mutations in the gene *lin-44* of the nematode *Caenorhabditis elegans* reverse both the relative positions of specific sister cells and the apparent polarities of these cells. Thus, *lin-44* mutants appear to generate polar cells but to misorient these cells along the body axis of the animal. We postulate that *lin-44* acts to specify the orientation of polar cells.

Key words: *C. elegans*, cell lineage, cell polarity, polarity reversal

INTRODUCTION

The generation of polarity during metazoan development requires the formation of asymmetric cells and multicellular structures and the orientation of these cells and structures with respect to the rest of the organism. Much of what is known about the generation and orientation of polar structures comes from the study of single cells, the budding yeast *Saccharomyces cerevisiae* and the syncytial embryo of the fruit fly *Drosophila melanogaster*.

In budding yeast, genes involved in bud formation function to establish or maintain cell asymmetry (Hartwell et al., 1973, 1974; Adams et al., 1990; Chant et al., 1991). The orientation of this asymmetry depends both upon cell interactions and upon a set of genes that act to define budding pattern. Specifically, during the process of mating, yeast cells orient to the neighboring cell producing the highest level of mating pheromone and generate a projection toward that cell (Jackson and Hartwell, 1990; Jackson et al., 1991). Genes that determine where a new bud is formed include those that seem to act to localize proteins required for bud formation to a specific site within the cell (Chant et al., 1991; Chant and Herskowitz, 1991; Snyder et al., 1991; Ruggieri et al., 1992).

During the development of the single-celled embryo of *Drosophila melanogaster*, anteroposterior polarity is generated by intracellular gradients of the *bicoid* and *nanos* morphogens, while dorsoventral polarity is established by a localized signal from outside the egg cell (reviewed by St. Johnston and Nusslein-Volhard, 1992). *bicoid* determines anterior pole identity, whereas *nanos* determines posterior pole identity. The protein product of *staufen* is required for the final localization of *bicoid* mRNA (St. Johnston et al., 1991) as well as for the localization of *oskar* mRNA, which in turn determines the localization of *nanos* mRNA (Ephrussi et al., 1991). The products of two additional genes, *cappuccino* and *spire* (Manseau and Schüpbach, 1989), are required for the localization of both *oskar* mRNA (Ephrussi et al., 1991) and *staufen* protein (St. Johnston et al., 1991). How *cappuccino* and *spire* cause such localization is unknown.

Little is known about how cell polarity becomes oriented in multicellular organisms. After the generation of the single-celled zygote, metazoan development involves extensive cell proliferation that results in an adult organism with a large number of cells and a diversity of cell types. Many of the cell divisions involved in this process can be regarded as asymmetric, since they produce sister cells that have distinct fates (see review by Horvitz and Herskowitz, 1992). What are the mechanisms responsible for the asymmetry of such cell divisions, and how does the asymmetry become oriented? To address these questions, we have been studying the cellular and genetic control of the cell lineage of the nematode *Caenorhabditis elegans*. The complete cell lineage of *C. elegans* is known (Sulston and Horvitz, 1977; Kimble and Hirsh, 1979; Sulston et al., 1983). Of the 949 non-gonadal cell divisions that occur during the development of the *C. elegans* hermaphrodite, 807 are asymmetric, generating sister cells that differ in their fates (Horvitz and Herskowitz, 1992). We have discovered a mutation that does not disrupt the generation of asymmetry in this cell lineage but causes the orientation of some of these cell divisions to be reversed with respect to the body axis of the animal. This mutation defines a new gene, *lin-44* (*lin*, cell lineage abnormal).

MATERIALS AND METHODS

Strains and alleles

Nematode strains were cultured by standard techniques (Brenner,

1974; Sulston and Hodgkin, 1988). N2 is the wild-type parent of all strains used in this work (Brenner, 1974). Mutations used are described by Hodgkin et al. (1988) or are noted below. Alleles used were: *LGI: lin-6(e1466), unc-11(e47), unc-73(e936), unc-89(e1460), let-353(h46), let-504(h448), let-505(h426), let-506(h300), let-507(h439), let-503(h313)* (Howell et al., 1987; Howell and Rose, 1990), *unc-74(e883), unc-57(e406), dpy-5(e61), unc-13(e450), unc-54(e190); LGV: him-5(e1490), LGX: mec-2(e75), dpy-7 (e1364ts), sup-7(st5cs), unc-9(e101)*. Rearrangements used were: *LGI: hDf6, hDf7* (Howell and Rose, 1990), *sDp2, hDp2, hDp7* (McKim and Rose, 1990); *LGII: mnC1 dpy-10(e128) unc-52(e444); LGIII, V: eT1(III, V); LGIV, V: nT1(IV, V)*. Male characteristics were studied using strains containing the *him-5(e1490)* mutation, which increases the frequency of spontaneous male progeny (Hodgkin et al., 1979).

Isolation of *lin-44* alleles

him-5 hermaphrodites were mutagenized with ethyl methanesulfonate (EMS; Brenner, 1974), individual F1 progeny were transferred to a Petri plate, and their progeny were screened. The mutation *lin-44(n1792)* was identified on the basis of gross defects in the structure of the male tail (Fig. 1A). The second *lin-44* allele, *n2111*, was isolated in a screen for mutations that failed to complement *lin-44(n1792)* for the phasmid dye-filling defect in hermaphrodites (see below). *unc-57 I; unc-9 X* hermaphrodites were mutagenized with EMS and mated with *unc-73 lin-44(n1792)/ + +* males. About 20,000 F1 hermaphrodite progeny were screened for defects in phasmid DiO-filling. Since *lin-44(n1792)/hDf6* and *lin-44(n1792)/hDf7* strains are viable and have defective phasmids (see Table 1), we could identify and recover complete loss-of-function alleles in this way.

Dye-filling assays of the phasmids

The phasmids and amphids are tail and head sensory structures, respectively, that are exposed to the external environment (Ward et al., 1975; Ware et al., 1975; Hall and Russell, 1991). When wild-type hermaphrodites are soaked in fluorescent dyes, such as fluorescein isothiocyanate (FITC) or 3,3′-dioctadecyloxacarbocyanine (DiO), the two neurons of each phasmid as well as six neurons of each amphid fill with dye and can be visualized in living animals (Hedgecock et al., 1985 and personal communication). Briefly, adult animals were soaked in a 10 μg/ml solution of DiO in M9 salts for 2 hours, and L1 larvae were placed on agar plates containing 0.1 mg/ml FITC for 4 hours (Hedgecock et al., 1985). For the non-complementation screen and strain constructions (see below), dye-filled animals were examined on Petri plates using a Wild M420 macro-dissecting microscope equipped with epifluorescence. For quantitation and microphotography, dye-filled animals were mounted on a slide in a small drop of M9 salts (Brenner, 1974) on a 5% agar pad containing 10 mM sodium azide as an anesthetic and examined on a Zeiss Standard or Axioplan microscope equipped with epifluorescence. Generally, the amphids and phasmids of N2 animals filled with dye; by contrast, in *lin-44* animals the amphids filled but the phasmids often did not. Occasionally, both the amphids and phasmids of an N2 or *lin-44* animal, usually a young hermaphrodite, failed to fill with dye. For this reason, to score the phasmid defect of *lin-44* animals, we counted as phasmid-defective only those individuals that showed amphid filling but no phasmid filling.

Genetic mapping

lin-44(n1792) was mapped to *LGI* (data not shown) (Fig. 1B). Three-factor crosses further defined the map position of *lin-44*. From heterozygotes of genotype *unc-73 + dpy-5/ + lin-44 +*, 16/18 Dpy non-Unc and 0/13 Unc non-Dpy recombinants segregated *lin-44*. From heterozygotes of genotype *+ unc-74 dpy-5/lin-44 + +*, 11/11 Dpy non-Unc recombinants and 0/17 Unc non-Dpy recombinants segregated *lin-44*. From heterozygotes of genotype *unc-89 + dpy-5/ + lin-44 +*, 0/15 Unc non-Dpy and 13/13 Dpy non-Unc recombinants segregated *lin-44*. From heterozygotes of genotype *+ lin-44 dpy-5/unc-89*

Table 1. *lin-44* mutations block dye-filling of the hermaphrodite phasmid

Genotype	n	% Phasmids filling with DiO
wild type	472	98
lin-44(n1792)	550	1
lin-44(n2111)	436	4
lin-44(n1792)/+	416	96
lin-44(n2111)/+	230	99
lin-44(n1792)/hDf6; him-5	588	7
lin-44(n1792)/hDf7; him-5	492	6
lin-44(n2111)/hDf7	406	2
hDf6/+	202	99
hDf7/+	124	97
lin-44(n1792)/lin-44(n2111); him-5	338	5
lin-44(n1792); dpy-7	214	4
lin-44(n1792); dpy-7 sup-7/+ +	124	93
lin-44(n1792); dpy-7 sup-7	222	99
lin-44(n1792); him-5	558	3
lin-44(n1792); him-5; dpy-7	212	5
lin-44(n1792); him-5; dpy-7 sup-7	206	99
lin-44(n2111); dpy-7	208	15
lin-44(n2111); dpy-7 sup-7	267	8
dpy-7 sup-7	294	99
dpy-7 (25°C)	110	100

The hermaphrodite phasmid defect was scored as described in Materials and Methods. The *dpy-7(e1324ts)* mutation was used to mark the presence of the *sup-7* mutation. Strains containing a *lin-44* allele in combination with the *dpy-7(ts)* mutation only were scored to control for effects of the *dpy* mutation on the phasmid defect. Since *dpy-7(ts)* animals are only slightly Dpy at 20°C, we scored *dpy-7(ts)* animals at 25°C to see whether a more severe Dpy phenotype had an effect of phasmid filling. *n*, number of phasmids (sides of animals) scored for each genotype; there are two phasmids per side.

+ + 5/5 Dpy non-Lin and 0/22 Lin non-Dpy recombinants segregated *unc-89*. Thus, *lin-44* maps very close to *unc-89 I* (Fig. 1B).

The deficiencies *hDf6* and *hDf7* failed to complement *lin-44(n1792)* and *lin-44(n2111)* (see Fig. 1B, Table 1). The duplications *hDp2* and *hDp7* were tested for complementation of *lin-44* by constructing strains of general genotype *lin-44 dpy-5; hDp* and scoring hermaphrodite phasmid dye-filling. Both of the duplications *hDp2* and *hDp7* failed to complement *lin-44* (data not shown) (Fig. 1B). *hDp2* covers *let-503* and *let-507* and *hDp7* covers *let-503* (see below) but neither covers *unc-89* (McKim and Rose, 1990) Since *hDf7* uncovers *let-503* and *let-507* but not *unc-89* (Howell and Rose, 1990), *lin-44* maps to the right of *unc-89* (Fig. 1B).

Complementation tests with lethal mutations in the *hDf7* interval

lin-44(n1792) dpy-5/+ + males were mated with hermaphrodites of general genotype *let dpy-5(e61) unc-13(e450); sDp2*, and the Dpy cross progeny were scored for the phasmid dye-filling defect. Mutations in all known *let* genes in the *hDf7* interval (Howell and Rose, 1990) (Fig. 1B) complemented *lin-44(n1792)* (data not shown).

To subdivide the *hDf7* interval, we tested the *let* genes for complementation with the duplications *hDp2* and *hDp7* (data not shown). These experiments revealed that *hDp7* complemented only *let-503*, and *hDp2* complemented only *let-503* and *let-507*. Thus, *let-353, let-504, let-505, let-506,* and *lin-44* map to the interval of *hDf7* not covered by *hDp2* (Fig. 1B).

Amber suppression studies

Both *lin-44* alleles were tested for suppression by the amber suppressor *sup-7(st5cs) X* (Waterston, 1981). The *sup-7(st5cs)* mutant is inviable at 15°C, and this mutation causes stronger suppression at 20°C than at 25°C. The *dpy-7(e1364ts) X* mutation, not an amber, is heat-sensitive: at 25°C animals are dumpy (Dpy) and at 20°C only

slightly Dpy. To test for single-copy suppression of *lin-44(n1792)*, we constructed a *lin-44(n1792); + dpy-7 sup-7/mec-2 + +* strain. We tested for suppression by scoring the non-Mec animals for suppression of *lin-44* at 20°C. Each animal scored was picked to ascertain its genotype based on the progeny it segregated; those animals that segregated Mec progeny were counted as being *+ dpy-7 sup-7/mec-2 + +* (Table 1). From hermaphrodites of genotype *lin-44(n1792)/+; dpy-7/+*, 6/12 Lin progeny segregated Dpy animals and 12/12 Dpy progeny segregated Lin animals, indicating that the suppression was not caused by the presence of the *dpy-7(e1364)* allele. The penetrances of the phasmid defect for suppressed and non-suppressed strains are shown in Table 1.

The *lin-44(n2111)* allele was suppressed by neither one nor two copies of *sup-7(st5cs)*. From hermaphrodites of genotype *lin-44(n2111); dpy-7 sup-7/+ +*, 8/12 Lin progeny segregated Dpy animals and 8/10 Dpy progeny segregated Lin animals. A *lin-44(n2111); dpy-7(e1364ts) sup-7(st5cs)* strain was constructed, and animals of this genotype were also Lin (Table 1).

Cell lineage analysis and laser killing experiments

Living animals were observed using Nomarski differential interference contrast optics; cell nomenclature and cell lineage analysis were according to Sulston and Horvitz (1977). Nuclei were destroyed by a laser microbeam, as described by Avery and Horvitz (1987). For kills of AB.prappppap in the embryo, cells were identified using cell deaths and other landmarks provided in the diagrams of Sulston et al. (1983). Successful embryonic kills were verified by the absence of the descendants of the cell(s) killed in the L1 animal. Only those animals that showed no other morphological defects were studied further.

Scoring cell fates in the T cell lineage

The polarity of the T cell division can be assessed in L1 animals after the time when all four T cell granddaughters are present by the nuclear morphologies of T.aa and T.ap and by the nuclear morphologies of T.pa and T.pp or by the presence of the neural cells they generate. In wild-type animals, T.aa has the nuclear morphology of a hypodermal syncytial cell (a large oval-shaped nucleus with a flattened appearance and a large nucleolus), T.ap has the nuclear morphology of a hypodermal blast cell (a large round nucleus with large nucleolus and smooth nucleoplasm), T.pa and T.pp prior to their divisions each have the nuclear morphology of a neuroblast (a large round nucleus with a smaller nucleolus than a hypodermal cell and granular nucleoplasm), and the descendants of T.pa and T.pp all have the nuclear morphologies of neural cells (small nuclei with granular nucleoplasm and most have no visible nucleolus) (Fig. 6A). T.pppp undergoes programmed cell death and has a characteristic refractile and flattened appearance. In hermaphrodites the cell T.apap is a seam cell, a specialized hypodermal cell with a nuclear morphology similar to a hypodermal blast cell but that displays an activity at the end of each intermolt period characterized by the appearance of refractile blobs in the surrounding cytoplasm. In both males and hermaphrodites the neuron PVN (T.appp) has the nuclear morphology of a neural cell, but has a small nucleolus.

Analysis of *hDf7* homozygotes

Animals homozygous for *hDf7* hatch and arrest development as L1 larvae (Howell and Rose, 1990), suggesting that the complete loss of the zygotic functions of genes in this interval, including *lin-44*, does not cause embryonic lethality. *hDf7 dpy-5(e61) unc-13(e450)* animals were generated by mating N2 males with *hDf7 dpy-5 unc-13; sDp2* hermaphrodites, picking semi-Dpy non-Unc progeny of presumptive genotype *hDf7 dpy-5 unc-13/+ + + (dpy-5(e61)/+* animals are semi-Dpy, and animals of genotype *dpy-5/+/+*, which carry *sDp2*, are non-Dpy). From these semi-Dpy non-Unc heterozygotes, we picked Dpy Unc arrested larvae (genotype *hDf7 dpy-5 unc-13*). 57% (*n*=453) of *hDf7* homozygotes arrested as L1 larvae without any further division of T or, based upon examination of five of these arrested larvae, of

any other postembryonic blast cells. The remaining *hDf7* homozygotes arrested after varying numbers of cell divisions; based upon the number of cells and cell types produced, no animals progressed beyond the L1 stage (our unpublished observations; also Howell and Rose, 1990). The time between cell divisions in *hDf7* homozygotes was much longer than that in wild-type or *lin-44* mutant animals.

The T granddaughters are generated approximately 7.5 hours after hatching, and their differences in nuclear morphologies become apparent about 0.5 hours later. In wild-type animals, the nucleus of the cell P6 descends into the ventral cord about 8 hours after hatching, and we used the presence of P6 in the ventral cord as a marker for this time point. Thus, for wild-type and *lin-44* animals scored in Table 2, we scored the morphologies of the T granddaughter nuclei only if the P6 nucleus was in the ventral cord. However, in *hDf7* homozygotes the entry of P6 into the ventral cord was not always a reliable indicator of the developmental stage of the T cell lineage so that in some animals in which P6 had migrated the T cell granddaughters might not have been old enough for differences in nuclear morphologies to have become apparent. For this reason, the *hDf7/hDf7* animals in Table 2 were transferred to a separate plate 24 hours prior to being scored, ensuring that each *hDf7* homozygote examined was at least 24 hours old. In *hDf7/hDf7* animals in which the T cell lineage was followed, the differences in the nuclear morphologies of the T cell granddaughters, if generated, were apparent by 24 hours.

RESULTS

Isolation of *lin-44* alleles

To identify mutants abnormal in the development of the *C. elegans* male tail, we screened the F$_2$ progeny of individual *him-5* hermaphrodites mutagenized with ethyl methanesulfonate; the *him-5* mutation was used because it results in the generation of a high frequency of male XO self-progeny from XX hermaphrodites (Hodgkin et al., 1979). We isolated a mutation, *n1792*, that caused gross defects in the male tail (Fig. 1A). This mutation defined the gene *lin-44* (see Materials and Methods). As described below, *lin-44(n1792)* hermaphrodites have defective phasmids. Because this defect can be scored in living hermaphrodites and is highly penetrant (Table 1), we used it for genetic analysis and for the isolation of a second *lin-44* allele, *n2111* (see Materials and Methods). The tail defect of *lin-44* males is much more difficult to use for two reasons: first, all analyses must be done in a *him* mutant background (Hodgkin et al., 1979) to generate males; and second, *lin-44* males cannot mate, so that to recover and maintain new mutations and genetic combinations identified in males, hermaphrodite siblings must be picked and their male progeny scored. Like *lin-44(n1792)* males, *lin-44(n2111)* males have defective tails. We mapped *lin-44* to a small interval on linkage group I (see Materials and Methods) (Fig. 1B).

lin-44 mutations reverse the polarities of both the F cell and its daughters

The tail defect of *lin-44* males led us to examine the cell lineages of the male-specific blast cells in the tail (Fig. 1C). In the wild-type male the F cell undergoes a left-right symmetric division during the mid-L2 stage, generating two equivalent sister cells that subsequently undergo identical lineage patterns. During the L3 stage, the left and right F daughters F.l and F.r divide asymmetrically, and each produces four descendants (Fig. 2). We studied the F cell lineages of nine *lin-44* males. In two animals we observed division patterns that were
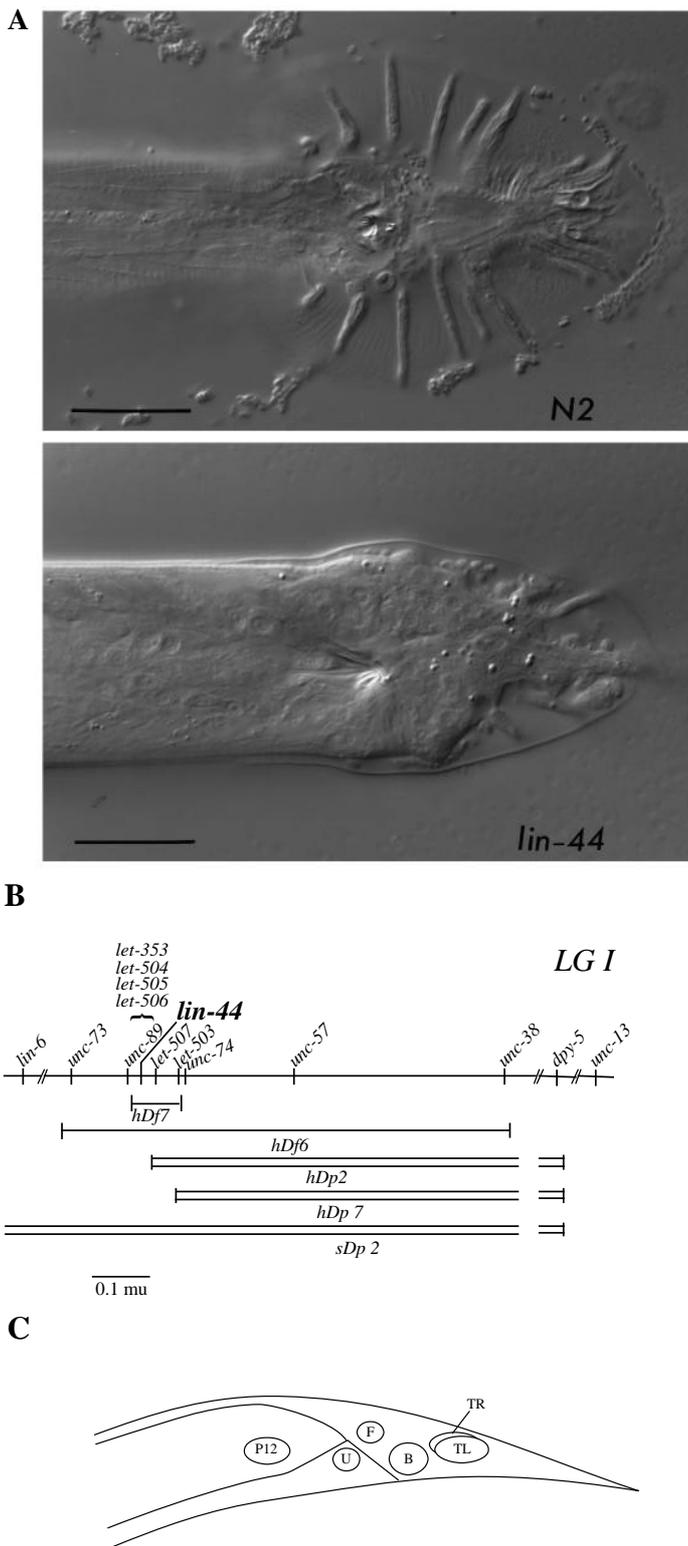
Fig. 1. (A) Nomarski photomicrographs of wild-type N2 (above) and *lin-44(n1792)* (below) adult male tails, ventral view. Bar equals 20 μm. (B) Genetic map of the *lin-44* region of *LG I*. *lin-44* maps to the region of *hDf7* not covered by *hDp2*. The genes *let-353, let-504, let-505, let-506* also map to this region and complement *lin-44*. See Materials and Methods for details. (C) Positions of cells affected by *lin-44* mutations. Left lateral view of an early L1 tail. Anterior is to the left, and ventral is down.

mirror symmetric to those of the wild type (Fig. 2). One plausible explanation is that the polarity of the first asymmetric cell division was reversed, causing the polarities of all subsequent asymmetric cell divisions also to be reversed. One other animal appeared to have a single polarity reversal of the F.l division, and two other animals had lineages with multiple apparent polarity reversals (Fig. 2). We also observed one animal with a loss of asymmetry in the F cell lineage, in that both F.l and F.r underwent two rounds of dorsal-ventral divisions generating a total of eight neuronal cells, and three animals with wild-type F cell division patterns.

lin-44 mutations appear to reverse the polarity of the B cell division

In the wild-type male, the B cell divides to generate neurons and structural cells that constitute a large portion of the tail (Sulston and Horvitz, 1977; Sulston et al., 1980). B divides asymmetrically during the late L1 stage to generate a large anterodorsal cell B.a and a smaller posteroventral cell B.p. By the end of the L2 stage B.a has undergone three rounds of division and B.p only one, generating a total of ten cells that lie in characteristic positions (Fig. 2). During the mid-L3 stage these ten cells begin to divide, eventually generating a total of 42 descendants.

We followed the B cell lineage in eleven *lin-44* males through the end of the L2 stage. In eight *lin-44* males B.a was small and underwent one round of division, and B.p was large and underwent three rounds (Fig. 2). This observation suggests that in these *lin-44* males either the fates of B.a and B.p were reciprocally transformed or the polarity of the asymmetric division of B in the L1 stage was reversed. Based on our observations of the F cell lineages of *lin-44* males, we suggest that the B cell defect also results from a reversal in the polarity of an asymmetric cell division. This reversal in polarity is evidenced by a change in cell size at the time of cell division, suggesting that *lin-44* acts prior to or at the time of cell division (also see below). In a few *lin-44* males the B cell divided symmetrically, so that B.a and B.p were of equal size and underwent similar patterns of division. In one of eleven *lin-44* males the B cell divided according to the wild-type pattern.

lin-44 mutations might also reverse polarity in the U cell lineage

U is another male-specific blast cell in the tail. In wild-type males U undergoes a left-right symmetric division during the mid-L2 stage. During the L3 stage U.l and U.r divide, and either one or both of the anterior daughters, U.la or U.ra, divide again (Sulston et al., 1980) (Fig. 2). This pattern gives the U lineage a variably asymmetric division. We observed that in three *lin-44* males U.lp or U.rp divided, indicating that a polarity reversal might have occurred (Fig. 2). In five other *lin-44* males neither U.la nor U.ra divided, indicating a possible loss of asymmetry (Fig. 2). In four *lin-44* males U divided according to the wild-type pattern.

Interpretations of polarity reversals

Our interpretations of the F cell, B cell and other cell lineage defects seen in *lin-44* animals are summarized in Fig. 3. The elliptical arrows indicate divisions with apparent polarity reversals. The horizontal arrows indicate possible reciprocal transformations. Fig. 3A shows a polarity reversal of a single
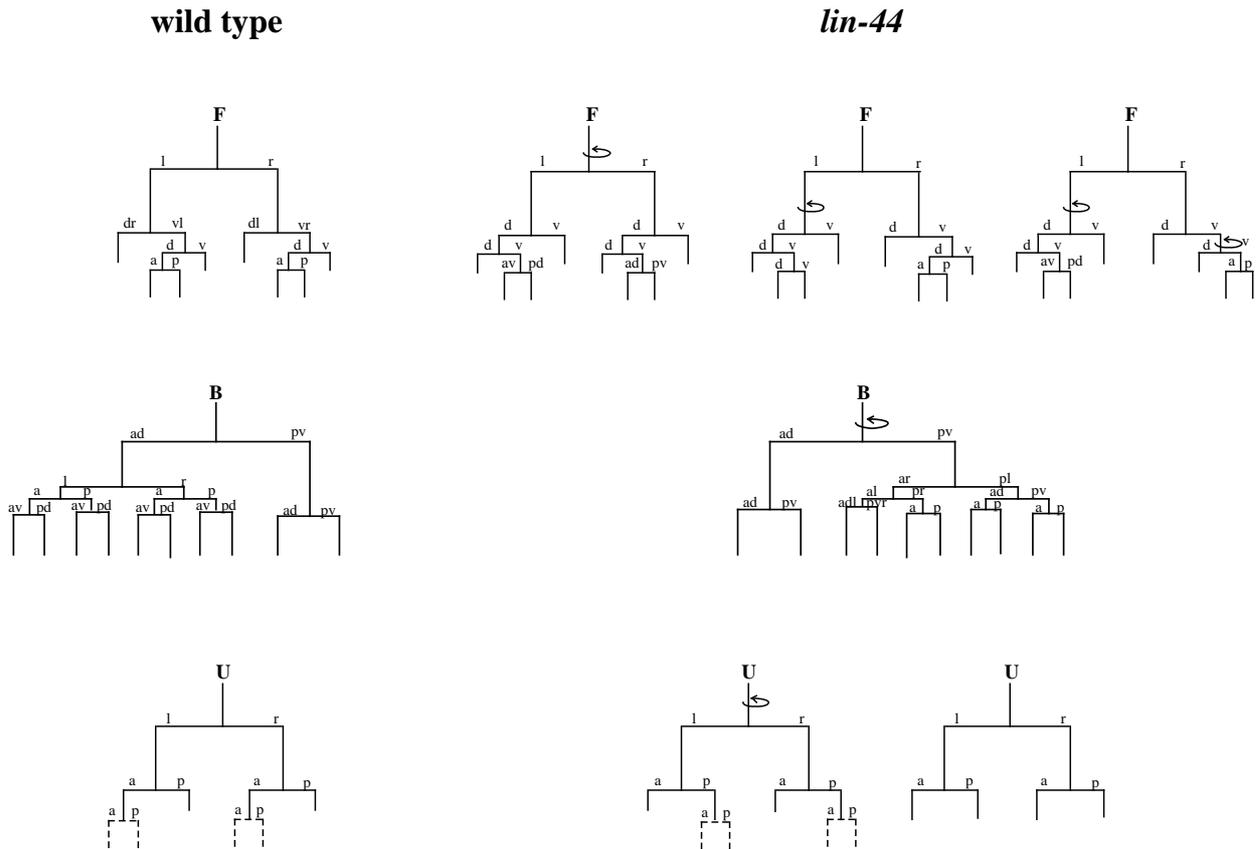
Fig. 2. Complete cell lineages of the F and U cells and cell lineages of the B cell through the L2 stage in wild-type and representative *lin-44* males. Division planes are indicated by labels denoting the relative positions of the daughter cells after division: a, anterior; p, posterior; d, dorsal; v, ventral; l, left; r, right. Oblique division planes are indicated by a combination of letters; for example, 'ad' means this cell lies anterior and dorsal to its sister. The elliptical arrows indicate divisions we interpret to be reversed in polarity in *lin-44* lineages. 2/7 *lin-44(n1792)* males had F cell lineages of the type shown second from the left, 1/7 *lin-44(n1792)* males had the F cell lineage shown third, 1/7 *lin-44(n1792)* males had the F cell lineage shown fourth, and 1/7 *lin-44(n1792)* males had another F cell lineage with multiple polarity reversals (not shown). In 1/7 *lin-44(n1792)* males, the F cell lineage showed a loss of asymmetry, in that both F.l and F.r underwent two rounds of dorsal-ventral divisions and generated a total of eight neuronal cells (not shown). 1/7 *lin-44(n1792)* and 2/2 *lin-44(n2111)* males had a wild-type F cell division pattern. 7/9 *lin-44(n1792)* males and 1/2 *lin-44(n2111)* males were observed to have the reversed polarity B cell division pattern shown. 1/9 *lin-44(n1792)* males and 1/2 *lin-44(n2111)* males had a symmetric B cell division pattern (not shown). 1/9 *lin-44(n1792)* males had a wild-type B cell division pattern. During the L3 stage in wild-type males U.l and U.r divide, and either one or both of the anterior daughters U.la and/or U.ra divide again, as indicated by the dotted lines. 3/10 *lin-44(n1792)* males had U cell polarity reversals as shown in the middle: in 2/10 of these animals, U.lp divided, and in 1/10, U.rp divided. In 4/10 *lin-44(n1792)* and 1/2 *lin-44(n2111)* males neither U.la, U.lp, U.ra nor U.rp divided, indicating a possible loss of asymmetry in the U cell lineage, as shown on the right. 3/10 *lin-44(n1792)* males and 1/2 *lin-44(n2111)* males had a wild-type U cell division pattern. The wild-type lineages are from Sulston and Horvitz (1977).

asymmetric cell division. If '2' and '3' are each symmetric in their fates, such a polarity reversal cannot be distinguished from a reciprocal transformation. If '2' and/or '3' are dividing cells, the behavior of their daughters can distinguish whether '2' and '3' are reversed in their polarities or have maintained their polarities and are reciprocally transformed in their fates (Fig. 3B). The mutant cell lineages we observe in *lin-44* males could in principle be explained either by heritable polarity reversals or by reciprocal transformations. Since far fewer polarity reversal events than reciprocal transformation events could account for the mutant lineages (e.g. one versus four for the first *lin-44* F cell lineage shown in Fig. 2), we prefer the hypothesis that in *lin-44* mutants cells undergo polarity reversals. Fig. 3C shows the effects of two sequential polarity reversals in a lineage. Examples of lineages with multiple

sequential polarity reversals can be seen in the *lin-44* T cell lineages shown below in Fig. 4.

In our interpretations of mutant cell division patterns we assume a polarity reversal to be a heritable event such that all divisions after the reversal are also reversed. Although each asymmetric cell division within an affected lineage could have been affected independently, this interpretation would require more primary events than does the interpretation that polarity reversals are heritable. Our usage of 'heritable' includes the possibility that once polarity is reversed, it still can be reversed again in subsequent divisions.

In general, we cannot directly observe whether a cell is '2' or 'reverse-2' in its polarity or even whether it is '2' or '3' in its type. In such cases, we must define both cell type and the polarity of a cell division based only upon whether the
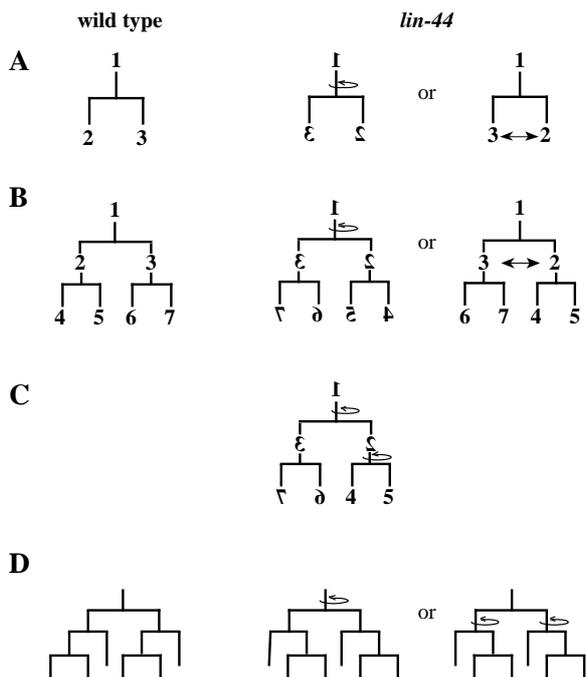
Fig. 3. Interpretations of polarity reversals seen in *lin-44* animals. The elliptical arrows indicate divisions at which we interpret there to be polarity reversals. The horizontal arrows indicate reciprocal transformations. (A) Polarity reversal (left) compared to a reciprocal transformation (right) of a single asymmetric cell division.
(B) Polarity reversal (left) compared to a reciprocal transformation (right) in a lineage with three asymmetric cell divisions. A polarity reversal can be distinguished from a reciprocal transformation by the anteroposterior order of cells, nos. 4 through 7. Normally, cells 4 through 7 are generated in the anteroposterior order 4-5-6-7. If the daughters of 1 are reciprocally transformed in their fates, these cells will be generated in the order 6-7-4-5. If the division of 1 is reversed in its polarity, these cells will be generated in the order 7-6-5-4.
(C) Two polarity reversals in a lineage with three asymmetric cell divisions. The polarity of the divisions of 1 and 2 are reversed; thus 6 and 7 are reversed in orientation, while 4 and 5 are in the wild-type orientation and the anteroposterior order is 7-6-4-5. (D) The *lin-44* mutant lineage shown can be interpreted to have either one (left) or two (right) polarity reversal events. For simplicity we interpret such lineages by assuming that the minimal possible number of polarity reversals has occurred, i.e. one rather than two in this case.

daughter cells divide and if they do divide, according to what pattern. For example, the patterns of cell division in the *lin-44* F lineages in Fig. 2 and model lineages in Fig. 3D are indicative of apparent polarity reversals. For simplicity we interpret such lineages by assuming that the minimal possible number of polarity reversals has occurred, e.g. one rather than two in the case of the example in Fig. 3D.

Many polarity reversals can occur during the T cell lineage

The T blast cells seem to be reversed in polarity in *lin-44* hermaphrodites and males. In wild-type males and hermaphrodites, the bilaterally symmetric T cells divide during the early L1 stage to produce a hypodermal cells that joins the large hyp7 hypodermal syncytium (T.aa), a hypodermal blast cell that divides later in a sex-specific manner (T.ap), and a group

of neural cells (derived from T.p) (Fig. 4). In wild-type males each T.ap blast cell divides during the L3 stage to produce the cells of three of the sensory rays of the male tail. Many of the cells produced by the T cell have distinct nuclear morphologies, so that for the T cell lineages we can assign cell fate based not only upon subsequent division pattern but also upon nuclear morphology (see Materials and Methods). We determined the complete T cell lineages of two *lin-44* males (Fig. 4). We interpret these T cell lineages to have polarity reversals at many divisions. The T cell lineage of one of the two *lin-44* males studied also had other defects: the cell T.paaaa appeared to have divided symmetrically (an extra ray cell group was formed by the cell T.paaaaa), and the cell T.papp underwent an extra division. We also followed four complete T cell lineages in *lin-44* hermaphrodites. Both *lin-44(n1792)* hermaphrodites studied showed multiple T cell lineage polarity reversals (Fig. 4). In one *lin-44(n2111)* hermaphrodite three divisions within the T cell lineage showed polarity reversals, while in the other the T cell underwent two rounds of symmetric divisions generating a syncytial hypodermal cell and three cells that appeared to have nuclear morphologies with characteristics of both a seam cell and a blast cell (see Materials and Methods) but did not divide further (data not shown).

The polarities of five of the seven L1 divisions in the T cell lineage can be determined using Nomarski optics (see Materials and Methods). We scored these five divisions by direct lineage analysis of a total of 12 *lin-44* males and five *lin-44* hermaphrodites. We observed polarity reversals for all five divisions (data not shown). The polarity of the first division of the T cell was reversed in 16 animals (the 17th had a symmetric division). The polarities of the other divisions were affected at different frequencies, e.g. the polarity of T.a was reversed in only two animals. The T cell lineages of these 17 *lin-44* animals can be explained by a total of 67 reciprocal transformations (or nonheritable polarity reversals) or by a total of 58 heritable polarity reversals. Although these numbers are not very different, based upon our observations of the F lineages in *lin-44* males we interpret these *lin-44* T cell lineages also to have heritable polarity reversals.

T lineage abnormalities cause phasmid defects

The abnormalities in the T cell lineage suggested that *lin-44* animals might have defective phasmids. The phasmids are sensory structures in the tail consisting of neurons and supporting cells (Ward et al., 1975; Ware et al., 1975; Hall and Russell, 1991). The phasmids are open to the environment, so that when animals are soaked in the fluorescent dyes FITC or DiO the two neurons of each phasmid as well as six of the eight amphidial neurons in the head fill with these dyes and can be visualized in living animals (Perkins et al., 1986; Hall and Russell, 1991). The cells T.paa and T.pap are the phasmid socket cells PHso1 and PHso2 (Fig. 4), which function to provide channels for the phasmid neurons PHA and PHB to the outside (Sulston et al., 1980; White et al., 1986).

The phasmids of *lin-44* animals failed to fill with fluorescent dyes (Fig. 5; Table 1). This defect appears to be caused by the abnormalities in the T cell lineage. During the early L1 stage, the T cells function as the phasmid socket cells (Sulston et al., 1980). We observed that 92% (*n*=100) of the phasmids in young L1 *lin-44(n1792)* animals fill with FITC, suggesting
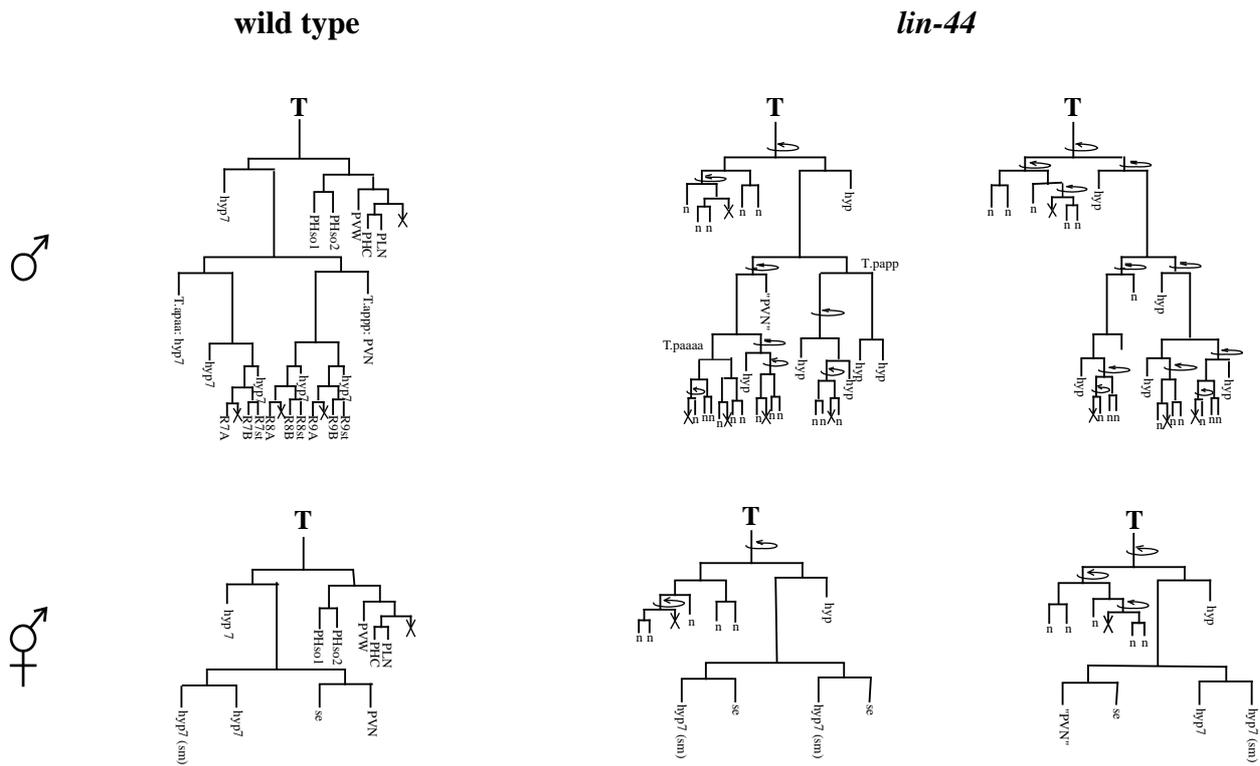
Fig. 4. Cell lineages of the T cell in wild-type and *lin-44* males and hermaphrodites. The complete cell lineage of the T cell in wild-type males (Sulston and Horvitz, 1977; Sulston et al., 1980) is shown at the upper left. In wild-type animals the fates of many different types of cells in this lineage can be distinguished by nuclear morphology using Nomarski microscopy (see Materials and Methods). The hyp7 cell T.aa joins the hypodermal syncytium. The neural cells PHso1, PHso2, PVW, PHC, and PLN have similar nuclear morphologies and cannot be distinguished from each other on that basis alone. The morphology of the cell that undergoes programmed cell death (X) is quite distinct. The neuronal cell PVN has a distinctive nuclear morphology. The sensory ray cells, RnA, RnB and Rnst all have the nuclear morphologies of neural cells. The complete cell lineages of T cells in two *lin-44(n1792)* males are shown in the upper right. The apparent polarity reversals in these lineages are indicated by the elliptical arrows. In the *lin-44* lineages, cells with the nuclear morphologies of neural cells are designated n, and those with the nuclear morphologies of hypodermal cells are designated hyp. Both *lin-44* males had multiple polarity reversals throughout the lineage. The T.paap cell in the *lin-44* lineage on the left had a nuclear morphology like that of PVN and is designated 'PVN.' The nuclear morphology of the T.paap cell in the *lin-44* lineage on the right was neuronal but could not be identified as PVN-like and is designated n. The complete cell lineage of the T cell in wild-type hermaphrodites (Sulston and Horvitz, 1977) is shown at the lower left. The fate of each cell is assigned on the basis of nuclear morphology (see Materials and Methods). Both T.apaa and T.apap are syncytial hyp7 nuclei; however, T.apaa has a smaller nucleus and is designated hyp7(sm). The seam cell (se) is a specialized hypodermal cell. The complete T cell lineages of two *lin-44(n1792)* hermaphrodites are shown on the lower right. In the *lin-44* lineage on the left, the division of hypodermal blast cell in the L2 was symmetrical and somewhat abnormal, generating two cells, each of which gave rise to a hyp7(sm) cell and a seam cell (se). 1/2 *lin-44(n2111)* hermaphrodites also displayed multiple polarity reversals within the T cell lineage. In 1/2 *lin-44(n2111)* hermaphrodites the T cell underwent two rounds of symmetric divisions, generating a syncytial hypodermal cell and three cells that appeared to have both seam cell and blast cell characteristics but did not divide further.

the phasmid neurons are not affected by *lin-44* mutations and that the phasmid defect of *lin-44* animals arises after the T cells normally divide to generate the phasmid socket cells of the older animal. In wild-type larvae and adults, the four phasmid socket cells label with antisera generated against the Lin-26 protein (M. Labouesse, personal communication). In *lin-44* mutant late-L1 and L2 larvae, Lin-26 antisera do not label cells in the normal positions of the four phasmid socket cells but do label four cells near the anus, suggesting that the phasmid socket cells are anteriorly displaced (M. Labouesse, personal communication). We have directly observed the anterior displacement of the presumptive phasmid socket cells in *lin-44* animals in our studies of T cell lineages (our unpublished observations). Thus, phasmid socket cells seem to be produced in *lin-44* animals and are normal at least with respect

to Lin-26 expression, but are displaced and do not function normally.

lin-44 mutations transform P12 to express the P11 fate

While characterizing the Lin-44 mutant phenotype, we observed one *lin-44(n1792)* male in which P12 appeared to be transformed to express the fate of P11. In wild-type animals the posterior daughter of P12, P12.p, divides to generate a small hypodermal cell, P12.pa, and a cell that undergoes programmed cell death, P12.pp. In this *lin-44(n1792)* male P12.p failed to divide during the L1 stage and instead assumed a hypodermal blast cell appearance like that of P11.p. During the L3 stage P12.p in this animal divided with a lineage pattern normally produced by P11.p, and P12.aap divided (P12.aap
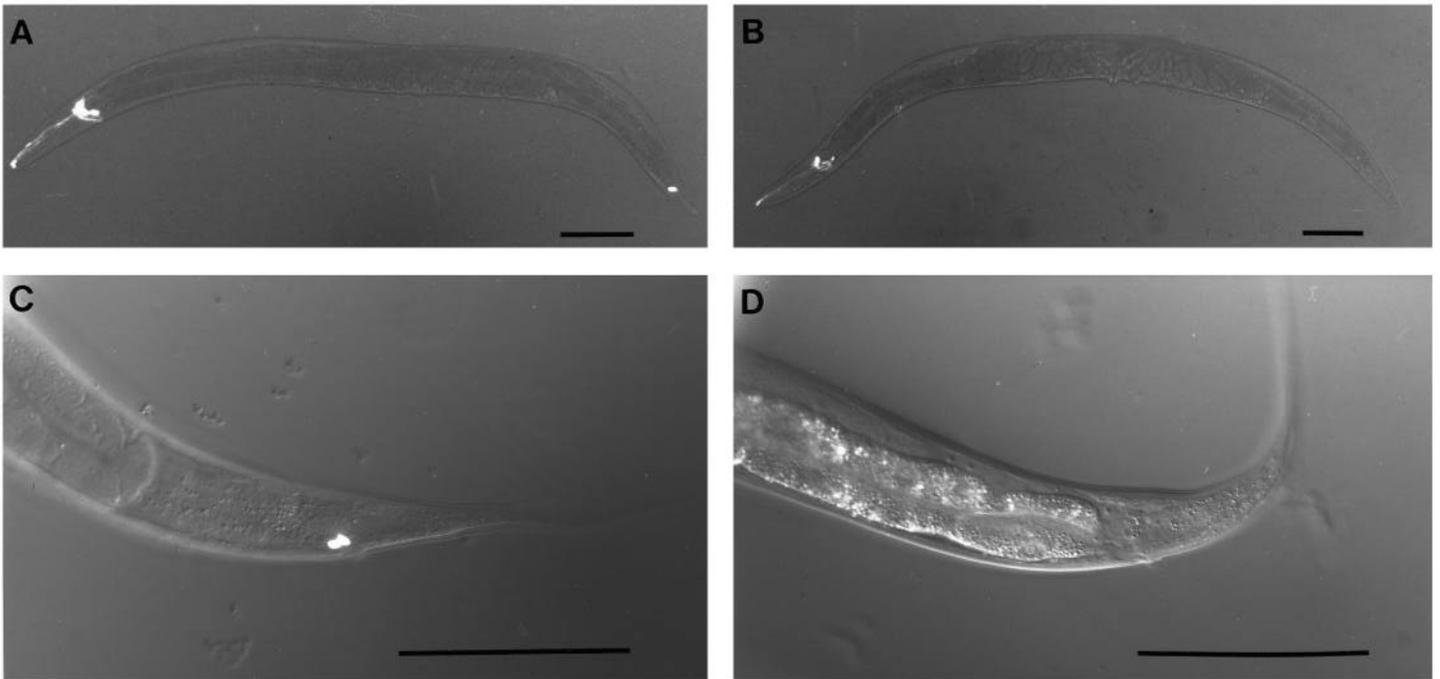
Fig. 5. Dye filling of wild-type and *lin-44* hermaphrodites. Bars, 100 μm. Anterior is to the left, and ventral is down. (A,B) Double exposure of bright-field and fluorescence images of DiO filling of (A) a wild-type hermaphrodite and (B) a *lin-44* hermaphrodite. The fluorescence in the anterior shows dye-filling of the amphids. The fluorescence in the posterior of the wild-type hermaphrodite shows dye-filling of the phasmids. The phasmids of *lin-44* hermaphrodites do not fill. (C,D) Double exposure of Nomarski and fluorescence images of DiO filling of (C) a wild-type hermaphrodite and (D) a *lin-44* hermaphrodite phasmids as viewed at higher magnification.

does not divide in wild-type males) like P11.aap normally does. Since in this *lin-44(n1792)* male both branches of the P12 lineages were like that of P11, we suspect that P12 was transformed to express the fate of P11. Subsequently, we identified another *lin-44(n1792)* male in which P12.p had failed to divide during the L1 stage and then observed the rest of the P12 lineage. In this male also P12 assumed the fate of P11. We examined the nuclear morphology of the cell in the position of P12.pa in 35 other *lin-44(n1792)* L3 males and found that in six of these animals a cell that looked like P11.p, presumably P12.p, was present instead. These observations indicate that in *lin-44* animals the cell that normally expresses the P12 fate can instead express the P11 fate.

The fates of P11 and P12 are known to be specified by cell interactions. If P12 is killed using a laser microbeam, P11 will express the fate normally expressed by P12, indicating that P12 or its descendants normally act to prevent P11 from becoming P12-like (Sulston and White, 1980) In addition, like *lin-44* mutations, mutations in the gene *let-23* can cause P12 to express the P11 fate (Fixsen et al., 1985; Aroian and Sternberg, 1991). *let-23* encodes a receptor tyrosine kinase that is probably the receptor for the inductive signal that functions during vulval induction (Aroian et al., 1990; reviewed by Horvitz and Sternberg, 1991). It seems likely that *let-23* functions in controlling P11 and P12 fates as it does in vulval induction. These considerations suggest that the transformation of P12 into a P11-like cell in *lin-44* mutant animals might result either from a primary defect in intercellular signaling or more indirectly, for example from a defect in the development of a cell that interacts with the presumptive P12 cell to specify the P12 fate.

lin-44 mutations coordinately affect the B, F and U lineages but not the B, P12 and T lineages

The cells affected by *lin-44* mutations are related by position (Fig. 1C) and not by lineage history. This observation raises the possibility that cell signaling may control the polarities of some or all of the affected cells. If so, the polarities of affected cells might be coordinately altered within given animals. The B, F, and U cell divisions were examined together in seven animals. In four animals the B, F and U lineages all showed defects, while in the other three animals the B, F, and U lineages were wild-type in polarity (data not shown). This observation suggests that the abnormalities in these three cell lineages are not caused independently.

The abnormalities of B and P12 were examined together in 26 animals: the fate of P12 was normal in 16/18 animals in which B was abnormal and in 5/8 animals in which B was wild-type in polarity. The abnormalities in B, P12 and T were examined together in 20 animals: T cell polarity was abnormal in all 20 animals, P12 was normal in 17/20 animals and B was wild-type in polarity in 6/20 animals. These results suggest that the abnormalities in the B, P12 and T lineages occur independently.

Polarity reversals in the B lineage may cause the F and U polarity reversals

F and U polarity reversals have been observed in wild-type males in which the B cell was killed with a laser microbeam (Chisholm and Hodgkin, 1989). We have confirmed the finding of Chisholm and Hodgkin (1989) by killing the B cell in three wild-type L1 males (data not shown). In addition, we

killed the mother of the B cell (AB.prppppap) in a single *him-5* male embryo. (We used the *him-5* mutation to generate males in part because we wanted to compare effects of these kills to kills in *lin-44; him-5* mutant males; see below.) Again, polarity reversals were seen in the F and U lineages (data not shown). Thus, in the absence of the B cell, polarity reversals occur in the F and U lineages, indicating that B or its descendants normally act to control polarity within these lineages. This observation is consistent with our finding described above that the B, F, and U lineages are affected coordinately in *lin-44* animals. Since *lin-44* mutants display F and U polarity reversals like those seen in the absence of a B cell, it seems likely that in *lin-44* animals these reversals are a secondary consequence of the abnormalities in the B cell lineage.

The B cell does not control polarity in the T cell lineage

By contrast, the B cell does not control polarity in the T cell lineage. We killed the mother of the B cell (AB.prppppap) in two *him-5* male embryos and observed that polarity in the T lineages was normal, based on observations of the complete T cell lineages of one animal and on the morphologies of the T cell granddaughters in the other.

Could an abnormal B cell be responsible for the polarity reversals seen in the T cell lineages of *lin-44* males? To test this possibility, we killed the mother of the B cell in four *lin-44; him-5* male embryos and one hermaphrodite embryo. The morphologies of the T cell granddaughters of both TL and TR cells were examined in each animal. Both T cells in each male were reversed, as was TR in the hermaphrodite; TL in the hermaphrodite generated four hypodermal nuclei that did not divide. Thus, T cell polarity in the absence of the B cell was indistinguishable from that seen in unoperated *lin-44* animals.

These studies establish that the B cell does not control polarity in the T cell lineages of either wild-type or *lin-44* animals.

lin-44 mutants are defective in egg laying

We have observed several other defects in *lin-44* animals: 59% (*n*=231) of *lin-44(n1792)* and 67% (*n*=245) of *lin-44(n2111)* hermaphrodites are egg-laying defective (Egl), and 10% (*n*=596) of *lin-44(n1792)* hermaphrodites and 15% (*n*=323) of *lin-44(n2111)* hermaphrodites have a protruding vulva. We determined the vulval cell lineages in 14 *lin-44(n1792)* hermaphrodites. All were normal, even in four animals that became Egl and developed a protruding vulva. The hermaphrodite sex muscles, which open the vulva and squeeze the uterus during egg laying (Horvitz et al., 1982; Trent et al., 1983; White et al., 1986), appear normal in *lin-44* animals as visualized by polarized light microscopy (M. Stern, personal communication). The HSN neurons, which innervate the sex muscles and drive egg-laying (Desai et al., 1988), are normal in staining and morphology as visualized with fluorescent antibodies to serotonin (G. Garriga, personal communication). Thus, the basis of the *lin-44* Egl defect is unclear.

We have determined the lineages of many other cells throughout the body in *lin-44(n1792)* animals and have not observed any additional defects. The cells examined include: H1 (*n*=2), H2 (*n*=2) and V1-V6 (*n*=2) (lateral hypodermal syncytial and seam cells from the head to the posterior body region); V5 (*n*=3) in the male (postdeirid sensillum, one male sensory ray and lateral hypodermal syncytial and seam cells); QL (*n*=2) and QR (*n*=2) (neuroblasts that generate cells that migrate anteriorly and posteriorly); G2 (*n*=2) (neurons and the excretory pore cell in the head); K (*n*=2) (DVB neuron and a rectal epithelial cell in the tail); P3.p-P8.p in hermaphrodites (*n*=14) (vulva equivalence group), P9.p-P11.p in the male (*n*=10) (preanal equivalence group); all other Pn.p cells (14 hermaphrodites and 10 males) (hypodermal cells in the ventral cord); and the male Y cell (*n*=2) (cloacal sensillum near the tail).

Polarity reversals are caused by the loss of *lin-44* function

The existing alleles of *lin-44* appear to be null alleles based on the phenotype they cause when heterozygous to deficiencies. Table 1 presents data for the hermaphrodite phasmid defect: both *lin-44* alleles result in recessive phenotypes, and neither is enhanced when placed in *trans* to a deficiency for *lin-44*; moreover, both *lin-44* homozygotes look similar to the *lin-44(n1792)/lin-44(n2111) trans*-heterozygote. Similarly, males from a variety of *him-5*-containing strains - *lin-44(n1792), lin-44(n1792)/hDf6, lin-44(n1792)/hDf7, lin-44(n2111), lin-44(n2111)/hDf7* and *lin-44(n1792)/lin-44(n2111)* - were examined using the dissecting microscope, and the range of male tail defects appeared similar (unpublished observations). Thus, the *n1792* and *n2111* alleles behave in these experiments like deficiencies that eliminate *lin-44* function. Furthermore, *n1792* is an amber mutation and presumably results in a truncated *lin-44* protein (see Materials and Methods) (Table 1). These results indicate that *n1792* and *n2111* reduce *lin-44* function and could be null alleles.

hDf7 fails to complement *lin-44* and is presumably null for *lin-44* function. Molecular analysis has shown that a 7.5 kb genomic DNA fragment that rescues the *lin-44* phenotype in microinjection experiments lies approximately 40 kb to the right of the left *hDf7* endpoint and approximately 160 kb to the left of the right *hDf7* endpoint (M.A.H., unpublished results), suggesting the *lin-44* locus is completely removed by *hDf7*. Because 43% of *hDf7* homozygotes hatch and initiate development (see Materials and Methods), we could determine whether polarity reversals occur in these animals. Analysis of these deficiency homozygotes indicated that complete loss of *lin-44* function most often resulted in T cell polarity reversal.

We scored the polarities of the T cell divisions by examining the nuclear morphologies of the descendants of the T cell present at late the L1 stage in wild-type, *lin-44*, and homozygous *hDf7* animals (see Materials and Methods) (Table 2). The polarity of the T cell division was normal in wild-type animals (Fig. 6A). In most *lin-44* animals and in most homozygous *hDf7* animals the polarity of the T cell division was reversed (Fig. 6). Thus, a complete absence of *lin-44* gene activity can reverse the polarity of the asymmetric T cell division. Despite the complete loss of *lin-44* gene activity the polarity reversal phenotype was not completely penetrant: *hDf7* homozygotes with T cell divisions of normal polarity or a loss of asymmetry (symmetric divisions) were observed. Furthermore, the frequencies of T cell divisions with reversed and normal polarities were similar in the *lin-44* mutants and the *hDf7* homozygotes (Table 2). The observation of divisions with normal polarity and loss of asymmetry in the *hDf7* homozygotes
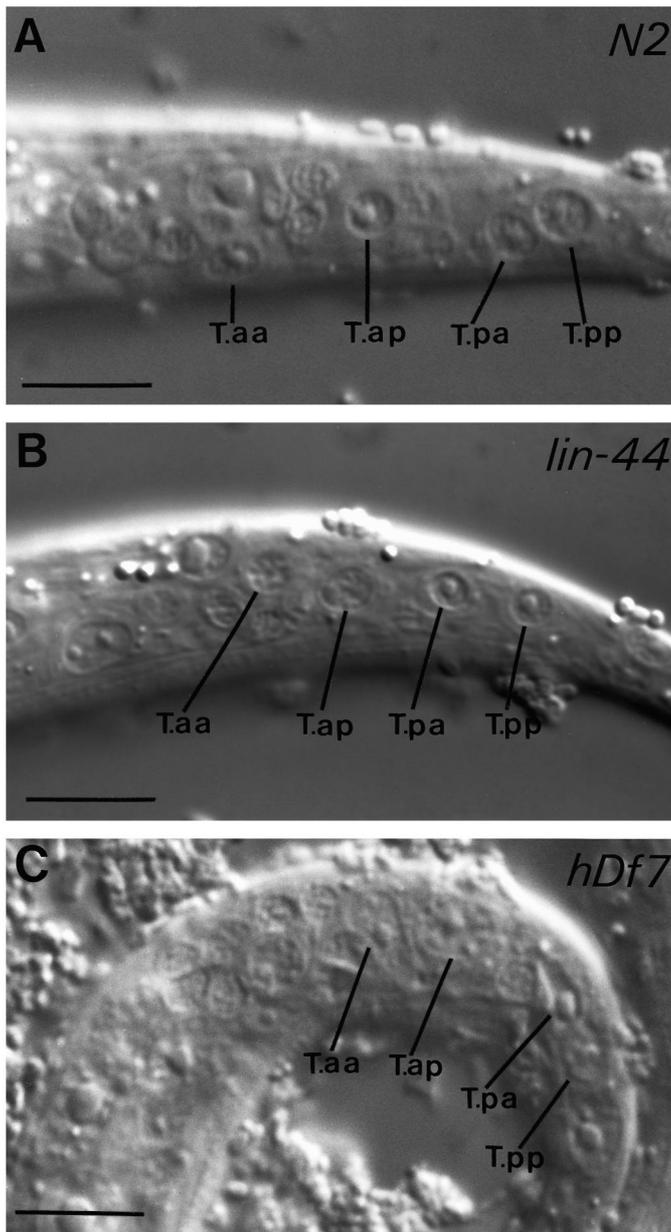
Fig. 6. (A-C) Nuclear morphologies of the T cell granddaughters. Nomarski photomicrographs of (A) wild-type, (B) *lin-44(n1792)*, and (C) *hDf7 dpy-5(e61) unc-13(e450)* L1 animals. In each panel the T cell nuclei are labeled. In wild-type animals T.aa has the nuclear morphology of a hypodermal syncytial cell, T.ap has the nuclear morphology of a hypodermal blast cell, and prior to division, T.pa and T.pp each has the nuclear morphology of a neuroblast (see Materials and Methods). Bars, 10 μm. Anterior is to the left, and ventral is down. (D) T cell lineages of wild-type animals and an *hDf7* homozygote. The lineages of the T cell in wild-type hermaphrodites (Sulston and Horvitz, 1977) and an *hDf7/hDf7* hermaphrodite are shown. We began following the T cell lineage of this *hDf7/hDf7* hermaphrodite after the T cell had divided; the dashed line indicates the inferred portion of the lineage. The lengths of the solid vertical lines of each lineage represent time and are drawn to the same scale. Nuclear morphologies are indicated: hyp, hypodermal; nb, neuroblast; n, neuronal. We followed the T cell lineages of seven other *hDf7* homozygotes (see text), observing each for at least 30 hours. We were unable to determine whether the hypodermal cells in the *hDf7* homozygotes were syncytial or blast cells because the nuclei in these animals appeared sickly and abnormal.

demonstrates that these defects are not caused by residual *lin-44* gene activity.

To confirm that these reversals were defects in cell lineage, we directly followed the T cell lineages of eight *hDf7* homozygotes. Based on the morphologies of the granddaughters of the T cells, the polarity of the T cell division was reversed in five *hDf7* homozygotes. One of these T cell lineages is shown in Fig. 6D. In one other *hDf7* animal the T cell division was of normal polarity, and in two *hDf7* animals the polarity of the T cell division could not be assessed (data not shown). Thus, direct lineage analysis of these eight *hDf7* homozygotes confirmed that a complete absence of *lin-44* gene activity can reverse the polarity of the asymmetric T cell division.

lin-44 acts prior to or at the time of division

Three of the cell divisions that can be reversed in polarity in *lin-44* animals generate daughter cells with nuclei of different sizes, which allowed us to determine if the effects of *lin-44* on the polarities of the divisions of B, T.pp and T.ppp could be observed at the time of cell division. In wild-type males, the nucleus of B.a is larger than that of B.p (Fig. 7A, Table 3). In *lin-44* males, as noted above, B.p was larger than B.a (Fig. 7B, Table 3). In wild-type animals, T.pp divides to generate a smaller anterior nucleus, T.ppa, and a larger posterior nucleus, T.ppp. In 8/15 *lin-44* animals, the polarity of the cell express-
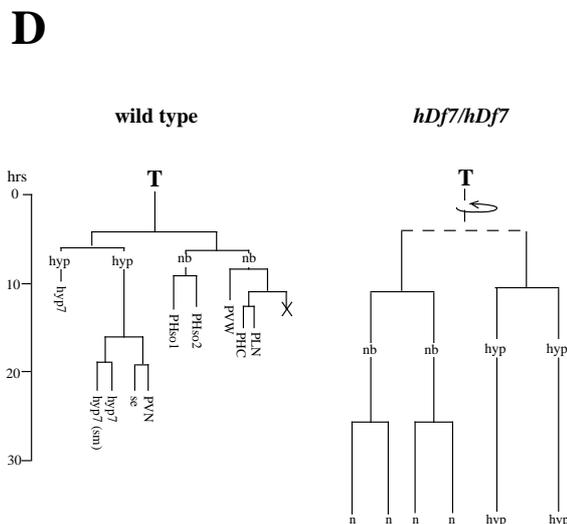
Table 2. The polarity of the T cell division is reversed in *lin-44* mutants and *hDf7* homozygotes

| Genotype | *n* | Polarity of T cell division: | | |
		% Reversed	% Symmetric	% Normal
wild type	108	0	0	100
lin-44(n1792)	103	79	10	12
lin-44(n2111)	108	68	19	14
hDf7/hDf7	124	72	19	10

The polarity of the T cell division was determined by examining the granddaughters of the T cell in L1 animals. Based on the nuclear morphologies of these cells (see Materials and Methods), the polarity of the T cell division was scored as reversed, symmetric, or normal. *n*, number of T cell divisions scored for each genotype.

Table 3. Asymmetry of male B cell division

| Genotype | *n* | Relative sizes of B.a and B.p: | | |
		%B.a > B.p	%B.a = B.p	%B.a < B.p
wild type	20+	100	0	0
lin-44; him-5	49	20	8	71
lin-17; him-5	46	11	85	4
lin-17 lin-44; him-5	48	17	77	6

The relative sizes of the daughter nuclei of the B cell division, B.a and B.p, were scored in late-L1 or early-L2 stage males using Nomarski microscopy. *n*, number of males scored for each genotype. In the case of the wild type, 20+ means that we scored 20 N2 males in this assay and that many more have been scored in the course of other work by us and others. B.a > B.p, the B.a nucleus was larger than the B.p nucleus.; B.a = B.p, the B.a nucleus was the same size as the B.p nucleus; B.a < B.p, the B.a nucleus was smaller than the B.p nucleus.

ing the T.pp fate (identification based upon its subsequent divisions pattern) was reversed, and in all eight of these animals the reversal could be seen by the reversal in the nuclear sizes of the daughters immediately after division (data not shown). Similarly, in 11/15 *lin-44* animals, the polarity of the cell expressing the T.ppp fate was reversed, and in all 11 of these animals the reversal could be seen by the reversal in the nuclear sizes of the daughters immediately after division (data not shown). Since the polarity reversals caused by *lin-44* mutations can be seen at the time of cell divisions, *lin-44* must act prior to or at the time of cell division.

lin-17 generates the B cell asymmetry oriented by *lin-44*

lin-17 mutations cause the B and T cells to lose polarity and generate daughters of the same type (Sternberg and Horvitz, 1988). In *lin-17* males, the nucleus of B.a is the same size as the nucleus of B.p (Fig. 7C, Table 3), which led Sternberg and Horvitz (1988) to propose that *lin-17* might act at or before the time of the B cell division. We constructed a *lin-17 lin-44* double mutant and observed that in this mutant the nuclei of B.a and B.p were similar in size (Fig. 7D, Table 3). Furthermore, we observed in 8/8 *lin-17 lin-44* double mutant animals the same effect on the T cell lineage as Sternberg and Horvitz (1988) saw in the *lin-17* single mutant: the T cell usually underwent two rounds of symmetric divisions generating four hypodermal cells. These observations suggest that *lin-17* is needed to generate asymmetry, i.e. to make two daughter cells different, whereas *lin-44* is needed to define the spatial orientation of that asymmetry.

DISCUSSION

### *lin-44* is needed to orient the polarities of specific cells and cell divisions

Mutations in the gene *lin-44* cause apparent polarity reversals in the B, F, U and T cell

lineages. In each case of polarity reversal, a cell divides to produce sister cells that appear to be reversed with respect to both their normal positions and their own polarities. The polarity reversals of different cells occur along different axes. The affected cell divisions in the T and U cell lineages are anterior-posterior, that of the B cell is skewed and anterior/dorsal-posterior/ventral, and those of the F cell lineage are dorsal-ventral. Thus, *lin-44* seems to act in a general process involved in orienting the polarities of asymmetric cell divisions rather than in one involved in specifying polarities along a single axis.

Two considerations indicate that the reversals in cell polarity caused by *lin-44* mutations probably occur at the stage of the mother cell rather than after the two sisters have been generated (Fig. 8). First, a reversal in the polarity of the mother cell would explain the coordinate reversals of both the positions and the polarities of the sister cells. Second, certain cell divisions that can be reversed in polarity generate sister cells of different sizes, and relative cell size - which is determined prior to the time of cell division - is also reversed when a polarity reversal occurs during such a cell division. Since polarity reversals appear to be caused by a complete loss of *lin-44* function, the *lin-44* gene normally is needed to orient the polarities of the B, F, U and T cells and their descendants.
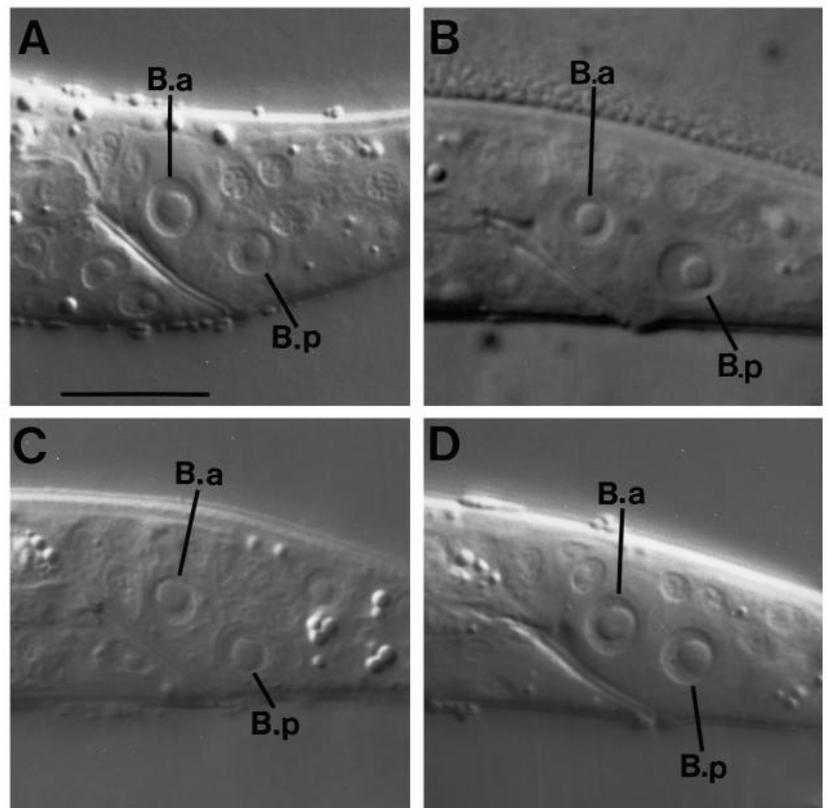
Fig. 7. Asymmetry of the B cell division. Nomarski photomicrographs of the daughters of the B cell division, B.a and B.p, in late-L1 stage males. Anterior is left, and ventral is down. (A) Wild-type. The B.a nucleus is larger than the B.p nucleus. Bar equals 10 μm. (B) *lin-44(n1792)*. The B.a nucleus is smaller than the B.p nucleus. (C) *lin-17(n671)*. The B.a nucleus and the B.p nucleus are of equal size. (D) *lin-17(n671) lin-44(n1792)*. As in the *lin-17(n671)* male, the B.a nucleus and the B.p nucleus are equal in size.
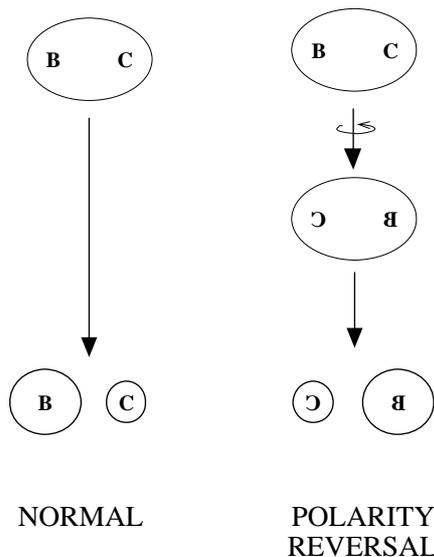
Fig. 8. Model for a polarity reversal of an asymmetric cell division. Since the sizes of the daughter cells are reversed, the polarity reversal seems likely to occur at the stage of the mother cell.

lin-44 might function in an evolutionarily recent process

In the absence of *lin-44* function, certain cells have polarities opposite to those expressed in the wild-type animal. Why should cells be generated with polarities that must be reversed by *lin-44*? One possibility is that the cell polarities seen in *lin-44* mutants reflect polarities that were expressed at an earlier stage of nematode evolution. In other words, perhaps the evolution of nematode cell lineage has involved the reversal of certain cell polarities. The suggestion that polarity reversals might function in the evolution of nematode cell lineage was made previously, based upon comparisons of homologous cell lineages both within *C. elegans* and between *C. elegans* and another free-living nematode, *Panagrellus redivivus* (Sternberg and Horvitz, 1981, 1982). In particular, it is interesting that a descendant of B, the cell B.ppa, appears to display opposite polarities in these two species.

lin-44 might act either indirectly or directly to orient cell polarity

How does *lin-44* affect the B, F, U, T, and P12 cell lineages? These cells are all located in the tail and far posterior body region (Fig. 1C). One possibility is that the defects in these cell lineages are secondary consequences of defects in another cell or cells. For example, the polarity reversals that occurred in the F and U lineages of *lin-44* animals were identical to those previously seen by Chisholm and Hodgkin (1989) in wild-type animals in which the B cell was killed with a laser microbeam. Since defective F and U lineages were seen only in *lin-44* animals with defective B lineages, it seems likely that the F and U polarity reversals were consequences of the abnormalities in the B cell lineage. By analogy, the polarity reversals in the B and T lineages of *lin-44* animals might also have been secondary consequences of abnormalities in cells that specify the polarities of these B and T cells. The effects of *lin-44* on P12 could be similarly explained: since, as discussed above,

the fate of P12 seems to be specified by cell interactions, *lin-44* could be altered in P12 cell fate as a secondary consequence of its effects on a cell that specifies this fate.

Alternatively, *lin-44* might act more directly to specify cell polarities. Polarity may be determined by a polarizing signal from a neighboring cell. *lin-44* mutations might disrupt this signal, for example if *lin-44* encodes a protein that acts in a signal transduction pathway, such as a ligand, a receptor, or a Ras protein (Beitel et al., 1990; Han and Sternberg, 1990; Aroian and Sternberg, 1991; Hill and Sternberg, 1992). Another possibility is that *lin-44* encodes a protein that responds to such a signal transduction pathway such as a transcription factor needed to specify both the fate of P12 and the polarities of cells in the B and T lineages.

A third possibility is that *lin-44* encodes a protein that acts within polar cells to specify their orientations. An analogy is provided by the two distinct sets of genes involved in polarity in the budding yeast *Saccharomyces cerevisiae*: one set is need for the generation of polarity, while the second set is needed for its orientation (Hartwell et al., 1973; Hartwell et al., 1974; Adams et al., 1990; Chant et al., 1991; Chant and Herskowitz, 1991). The *C. elegans* gene *lin-17* is needed for the generation of the polarity of the B and T cells (Sternberg and Horvitz, 1988), while *lin-44* is needed for the normal orientation of these polar cells along the body axis of the animal. It is possible that these nematode genes act analogously to the yeast genes noted above to establish and orient cellular polarity in the developing animal.

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