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Asymmetric distribution of the *C. elegans* HAM-1 protein in neuroblasts enables daughter cells to adopt distinct fates

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SUMMARY

One mechanism of generating cellular diversity is to distribute developmental potential asymmetrically to daughter cells at mitosis. Two observations described in this report suggest that the *C. elegans* HAM-1 protein functions in dividing neuroblasts to produce daughter cells that adopt distinct fates. First, HAM-1 is asymmetrically distributed to the periphery of certain mitotic cells, ensuring that it will be inherited by only one daughter cell. Second, *ham-1* mutations disrupt the asymmetric divisions of five neuroblasts. In one of these divisions, loss of *ham-1* function causes the daughter cell that does not inherit HAM-1 to adopt the fate of the daughter cell that normally inherits HAM-1. We propose that asymmetric distribution of HAM-1 enables daughter cells to adopt distinct fates.

Key words: *ham-1*, *Caenorhabditis elegans*, neuroblast, cell fate

INTRODUCTION

Asymmetric cell divisions produce daughter cells that adopt distinct fates, a fundamental aspect of metazoan development that generates cellular diversity (reviewed in Horvitz and Herskowitz, 1992). One way to generate asymmetry is to produce a polar mother cell that distributes cell fate determinants unequally to its daughter cells. Recent work has shown that the protein products of the *Drosophila* genes *numb* and *prospero* are distributed to one daughter cell during certain neuroblast divisions (Rhyu et al., 1994; Spana and Doe, 1995; Spana et al., 1995; Hirata et al., 1995). During embryogenesis, division of the MP2 precursor produces two neurons, dMP2 and vMP2 (Doe, 1992). numb is asymmetrically distributed in MP2 and is inherited by dMP2 (Spana et al., 1995). Similar to numb in MP2, prospero is asymmetrically distributed in CNS neuroblasts and is inherited by one daughter, the ganglion mother cell (GMC; Hirata et al., 1995; Spana and Doe, 1995).

Genetic analysis indicated that, in neuroblast divisions, *numb* and *prospero* specify the fates of the daughter cells that inherit their protein products (Rhyu et al., 1994; Hirata et al., 1995; Spana and Doe, 1995; Spana et al., 1995). For example, loss of *numb* function causes dMP2, the cell that normally inherits numb, to adopt a vMP2 fate, whereas ectopic expression of numb in both daughter cells causes vMP2 to adopt a dMP2 fate (Spana et al., 1995). Similarly, loss of prospero alters GMC fate (Doe et al., 1991; Vaessin et al., 1991).

A previous study (Desai et al., 1988) found that the *C. elegans ham-1* (HSN abnormal migration) mutations occasionally produced additional migration-defective HSN motor neurons and defective PHB sensory neurons suggesting that *ham-1* functioned in the HSN/PHB lineage. In this report, we describe two observations suggesting that the *C. elegans* HAM-1 protein functions in neuroblasts to distribute cell fate asymmetrically to daughter cells. First, HAM-1 protein is distributed asymmetrically in many dividing cells during embryogenesis. In the lineage that produces the HSN and PHB neurons, a neuroblast on each side of the animal divides to produce a cell that undergoes programmed cell death and an HSN/PHB precursor (Fig. 1). HAM-1 protein is asymmetrically distributed in this neuroblast, ensuring that it is inherited by only one daughter cell, the HSN/PHB precursor. Second, *ham-1* mutations disrupt five neuroblast divisions, including the HSN/PHB neuroblast division, that produce a cell that undergoes programmed cell death and a neuron or neuronal precursor. In the HSN/PHB lineage, loss of *ham-1* function transforms the fate of the daughter cell that does not inherit HAM-1, the cell that dies, into the daughter cell that inherits HAM-1, the HSN/PHB precursor. Thus, in contrast to numb and prospero, HAM-1 acts to determine the fate of the daughter cell that does not inherit the HAM-1 protein. We propose that HAM-1 distributes cell fate determinants asymmetrically in dividing neuroblasts, allowing their daughter cells to adopt distinct fates.

MATERIALS AND METHODS

Strains and genetics

Strains were grown at 20°C unless stated otherwise and were maintained as described by Brenner (1974). This paper uses standard *C. elegans* nomenclature (Horvitz et al., 1979).

In addition to the standard wild-type strain N2, strains with the following mutations were used in this work:

Linkage group (LG) III: ced-4(n1162)

*LG IV: ced-3(n717), dpy-20(e1282*ts*), ham-1(n1438), ham-1(n1810), ham-1(n1811), jeIn2 (mec-3::lacZ/unc-22* antisense*), nIs2(lin-11::lacZ)*, and *unc-31(e169)*

LG V: egl-1(n986)

Chromosomal aberrations: *sDf22 (IV); nT1 (IV;V)*

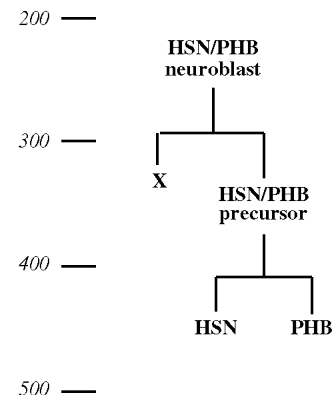

Fig. 1. Schematic representation of the wild-type HSN/PHB neuroblast lineage. At left is the time, in minutes, from first cleavage of the zygote. The HSN/PHB neuroblast divides asymmetrically at approximately 270 minutes after cleavage of the zygote to produce a smaller anterior daughter that undergoes programmed cell death (X) and a larger posterior daughter, the HSN/PHB precursor. Later during development, the HSN/PHB precursor divides to produce HSN and PHB neurons.

The isolation and initial characterization of the *ham-1* mutations were described by Desai et al. (1988). The *ced-3* and *ced-4* mutations were described by Ellis and Horvitz (1986). The *dpy-20* mutation was reported by Hosono et al. (1982). The *egl-1(n986)* mutation was described by Desai et al. (1988) and the *unc-31* mutation was described by Brenner (1974).

nIs2 lies to the left of *dpy-20* on *LG IV* (C. G. Freyd and H. R. Horvitz, personal communication), and *ham-1 nIs2* strains used in this work were generated by recombination. *dpy-20(e1282*ts*) ham-1*/+ + males were mated with *nIs2* hermaphrodites, and wild-type hermaphrodites were placed onto separate plates. From F_1 progeny segregating Dpy Egl animals (*ham-1* mutations cause a recessive Egl phenotype), Egl, non Dpy recombinant F_2 progeny were identified and placed on separate plates. Egl, non Dpy F_3 progeny were placed on separate plates and those that did not segregate Dpy progeny were scored for the presence of *nIs2*. The presence of *ham-1* was confirmed by showing that the Egl animals displayed HSN-migration and phasmid neuron dye-loading defects.

jeIn2 lies to the right of *ham-1* on *LG IV*. *jeIn2* animals twitch due to the presence of an *unc-22* antisense construct in the integrated array. *dpy-20(e1282*ts*) ham-1(n1438) jeIn2* animals were generated by mating *dpy-20 ham-1 unc-31*/+++ males to *jeIn2* hermaphrodites and placing ten heterozygous F_1 progeny on separate plates. From F_1 progeny segregating the *dpy-20 ham-1 unc-31* chromosome, we placed Dpy non Unc F_2 recombinants on separate plates. Dpy F_3 progeny that were twitching due to the presence of *jeIn2* were picked and their progeny were checked for the presence of *ham-1* as described above. All matings involving *dpy-20(e1282*ts*)* were conducted at 25°C.

sDf22 is a small deletion of *LG IV*, which removes several genes, including *ham-1* and *unc-30* but not *dpy-20* (Clark et al., 1988). To produce a large quantity of *sDf22* homozygotes, which arrest during the first larval stage of development, *dpy-20(e1282*ts*) unc-30(e191)*/ + + males were mated to *sDf22/nT1(n754*dm*)* hermaphrodites. *nT1* is a reciprocal translocation of chromosomes *IV* and *V*, and *(n754*dm) is a mutation linked to *nT1* that confers a dominant uncoordinated phenotype (Ferguson and Horvitz, 1985). 50 non Dpy Unc-30 hermaphrodites were placed on two plates and the adults were transferred daily. 2 days after the embryos were laid, when all of the F_1 progeny on each plate were young adults except the *sDf22* homozygotes, the animals were washed off the plate, fixed and stained with an antiserum that recognizes the nuclear protein EGL-43. In arrested *sDf22* homozygotes, 27% of the sides had three phasmid neurons, 61% of the sides had two phasmid neurons and 12% of the sides had one phasmid neuron that expressed EGL-43. The frequencies of extra phasmid neurons in *sDf22* homozygotes and *ham-1* mutants are similar (Table 1).

HAM-1 antiserum

To generate HAM-1 protein, we amplified a 595 bp PCR product corresponding to the amino-terminal half of the *ham-1* cDNA using the primers HAM-1A (5′-ACGCCGCTCGAGCTACTTAGCCGTT-GTGC-3′) and HAM-1B (5′-CCGGAATTCCTACGAAGCACC-CGTTTGACACTC-3′), and inserted the product into the pRSET-B vector (Invitrogen Corporation) downstream of the six histidine residues. A fusion protein of the expected size, 24.4×10^3 M_r, was generated and injected subcutaneously into mice. Polyclonal ascites produced by the mice was used in all experiments.

Analysis of additional cell types not affected by *ham-1* mutations

We included in our analysis only those terminal cell divisions that produce two daughter cells that we could distinguish by one of the methods listed below. Divisions that were symmetric or reiterated were counted as a single division. Criteria for identifying each cell type was as follows: CANL/R (Nomarski microscopy); ALML/R, AVM, FLPL/R, PVD and PVM (expression of a *mec-3::lacZ* gene fusion [Way and Chalfie, 1989]); RMGL/R and PVR (UNC-86 expression [Finney and Ruvkun, 1990]); AVL, DD1-DD2, DVB, RIS, RMED, RMEL/R, RMEV and VD1-VD13 (GABA expression [McIntire et al., 1993]); ADFL/R, NSML/R (serotonin expression [Horvitz et al.,

Table 1. Extra HSN and phasmid neurons in *ham-1* mutants

	No. of HSN neurons/side*					No. of phasmid neurons/side†			
Strain	3‡	2	1	0	N	3	2	1	N
wild type	0%	0%	100%	0%	90	0%	96%	4%	250
ham-1(n1438)	3%	23%	64%	11%	98	22%	51%	25%	208
ham-1(n1810)	0%	26%	68%	6%	122	21%	76%	3%	61
ham-1(n1811)	1%	16%	79%	4%	102	33%	59%	8%	63
ced-3(n717)§	0%	2%	98%	0%	154	7%	93%	0%	43
ham-1(n1438) ced-3(n717)¶	6%	83%	11%	0%	92	95%	5%	0%	40
ham-1(n1810) ced-3(n717)¶	7%	87%	5%	0%	82	92%	6%	2%	48
ham-1(n1811) ced-3(n717)¶	7%	83%	10%	0%	104	96%	4%	0%	48

*Average number of HSN neurons/side was deterimined by counting the number of serotonergic neurons in the tail and along the HSN migratory routes of adult hermaphrodites.

†Average number of phasmid neurons/side was determined by counting the number of EGL-43-expressing cells in the tails of first larval stage hermaphrodites.

‡When there were more than two serotonergic cells/side, one of these cells invariably was a weakly staining bipolar neuron located in the tail. These animals always had additional serotonergic cells in the tail that were not bipolar (HSN-like). Because the PHA neurons are known to take up exogenous serotonin (Horvitz et al., 1982), the extra bipolar cells could be PHA neurons that have taken up serotonin released from HSNs located in the tail.

§Similar results were obtained with *ced-4(n1162)* mutants.

¶Similar results were obtained with *ced-4(n1162); ham-1* double mutants.

1982]); PHAL/R, Z1 and Z4 (EGL-43 expression [C.G., unpublished results]). Because we only consider terminal divisions that give rise to the cell types described above, the 21 divisions that are not affected by *ham-1* mutations are an underestimate. Earlier divisions involved in producing these cells are also presumably unaffected.

Indirect immunofluorescence histochemistry

All incubations were at room temperature unless noted otherwise. Embryos were harvested by suspending gravid hermaphrodites in 5.0 mls of a 0.71 M NaOH and 4.4% NaOCl (Aldrich) solution for 10-15 minutes. The embryos were washed, fixed and permeabilized using a modified version of the procedure described by Finney and Ruvkun (1990). This procedure lacks the last two reducing and one oxidizing step. An aliquot of fixed embryos was incubated in antibody solution (1:3000 dilution of rabbit anti-LIN-26 antiserum (provided by Michel Labouesse, Universite Louis Pasteur, Strasbourg) and/or a 1:10 dilution of mouse anti-HAM-1 antiserum in PBST-A (1× PBS, 1% BSA, 0.5% Triton-X 100, 0.05% NaN_3, 1 mM EDTA)) overnight.

To collect first larval stage (L1) animals, the embryos prepared by NaOH/NaOCl treatment described above were resuspended in M9 buffer and incubated overnight at 25°C to allow the L1s to hatch. The L1s were washed, fixed and permeabilized using the method of Finney and Ruvkun (1990). The ADE and PHB neurons were visualized using an anti-EGL-43 antiserum. The ALN and PLM neurons were visualized using an anti-UNC-86 antiserum. An aliquot of fixed L1s was incubated in antibody solution (1:3000 dilution of rabbit anti-UNC-86 antiserum (Finney and Ruvkun, 1990) and 3:10 dilution of mouse anti-EGL-43 antiserum) overnight. The ADL or PLM neurons were viewed in animals carrying a *lin-11*::*lacZ* or *mec-3*::*lacZ* transgene, respectively, by incubating fixed L1s in antibody solution (0.3% rabbit anti-β-galactosidase antiserum (Cappel) and a 3:10 dilution of mouse anti-EGL-43 antiserum in PBST-A) overnight. All samples were washed with PBST-B (1× PBS, 0.1% BSA, 0.5% Triton X-100, 0.05% NaN_3, 1 mM EDTA) and incubated overnight in antibody solution (0.3% FITC-conjugated goat anti-rabbit antiserum (Cappel) and 1:300 dilution of Cy3-conjugated donkey anti-mouse antiserum (Jackson Immunoresearch) in PBST-A). The samples were washed with PBST-B and aliquots were placed on slides with a drop of 94 mM n-propyl gallate, 70% glycerol, 30 mM Tris HCl pH 9.5, 2 μg/ml DAPI (4, 6-diamidino-2-phenylindole) solution and viewed using a Zeiss fluorescence microscope.

Serotonin and FMRFamide expression were detected according to the procedure described by Garriga et al. (1993b). GABA staining was done as described in McIntire et al. (1993). The ADE neurons are dopaminergic (Sulston et al., 1975), allowing us to indirectly detect their cell bodies and processes using the activity of aromatic amino acid decarboxylase (Loer and Kenyon, 1993).

Lineage analysis

Wild-type and *ham-1* mutant embryos were allowed to develop at 20°C until approximately 260 minutes after first cleavage, at which time they were mounted in a drop of M9 buffer onto a pad of 2% agarose and viewed using Nomarski differential interference contrast microscopy. Individual embryos were followed until shortly after the division that generates the HSN and PHB neurons at 400 minutes after first cleavage. In those cases where an embryo was saved for single-worm staining, the embryo was recovered from the pad, placed on a Petri dish containing a bacterial lawn and incubated at 20°C. The adult worm was stained using the single-worm staining procedure (Garriga et al., 1993b).

RESULTS

HAM-1 protein is asymmetrically distributed during mitosis

The *C. elegans ham-1* gene encodes a novel protein of 414 amino acids (C. Guenther et al., unpublished data). To determine the times and sites of HAM-1 expression, we generated a mouse polyclonal antiserum to a His-tagged HAM-1 fusion protein expressed in *E. coli* and used the antiserum to stain whole mounts of wild-type embryos and larvae. HAM-1 is first expressed at the onset of gastrulation, approximately 100 minutes after cleavage of the zygote (Fig. 2A). At this time, HAM-1 often localizes to the periphery of cells in crescent shaped patterns that are restricted to one side of dividing cells (Fig. 2A,B). Fig. 2A shows HAM-1 expression in a cell at metaphase. Chromosomes are condensed and HAM-1 is distributed to one side of the cell. Fig. 2B shows HAM-1 expression in a cell at telophase. Two nuclear envelopes have reformed and HAM-1 is distributed to the side of the cell that will be inherited with the posterior nucleus. This division will produce a posterior daughter cell that will inherit HAM-1 and an anterior daughter cell that will not. Consistent with this hypothesis, HAM-1 staining also appears as rings that surround cells (Fig. 2C). Inheritance of asymmetrically distributed HAM-1 by one daughter cell should produce a cell with HAM-1 localized to the periphery in a ring. HAM-1 expression persists until the $1\frac{1}{2}$-fold stage of embryonic development, approximately 430 minutes after cleavage of the zygote. No HAM-1 expression can be detected later during embryonic or larval hermaphrodite development.

The anti-HAM-1 antiserum specifically recognized HAM-1 based on western blot analysis and *ham-1* mutant staining. Anti-HAM-1 antiserum recognized the bacterial HAM-1 fusion on western blots and this recognition was eliminated if HAM-1 fusion protein was included in the incubation reaction (data not shown). In addition, *ham-1* mutants showed altered HAM-1 staining. *ham-1(n1438)* embryos expressed almost no HAM-1 protein (Fig. 2D). Although a small amount of HAM-1 expression is visible in Fig. 2D, most *ham-1(n1438)* embryos produced no detectable HAM-1. The *ham-1(n1438)* allele is a small deletion that removes 5′-flanking and untranslated sequences of *ham-1*. This deletion drastically reduces the amount of *ham-1* mRNA in *ham-1(n1438)* as compared to wild type (C. Guenther et al., unpublished data).

HAM-1 protein is expressed in many mitotic and postmitotic cells. To determine whether HAM-1 is expressed in the lineage that produces the HSN and PHB neurons, we examined stained embryos at 260-300 minutes after cleavage of the zygote. At this time, the HSN/PHB neuroblast divides to produce an anterior daughter that dies and a posterior daughter, the HSN/PHB precursor (Fig. 1; the division axis is actually skewed - see Fig. 4A-C). Later, the HSN/PHB precursor divides to generate an HSN motor neuron that migrates out of the tail and a PHB sensory neuron that does not migrate (Sulston et al., 1983). We identified the HSN/PHB neuroblast nuclei by their positions relative to other nuclei in DAPI-stained embryos. Each of the two HSN/PHB neuroblast nuclei is bordered by the nuclei of a phasmid sheath cell precursor, a T blast cell, and a hyp7 cell (Sulston et al., 1983). HAM-1 is restricted to the posterior half of the HSN/PHB neuroblast, the portion of the cell that will be inherited by the HSN/PHB precursor (Fig. 2E). The identification of the HSN/PHB neuroblast as the HAM-1-expressing cell was confirmed by double staining embryos with the anti-HAM-1 antiserum and an antiserum that recognizes LIN-26, which is expressed in the nuclei of the phasmid sheath cell precursor, the T blast cell,

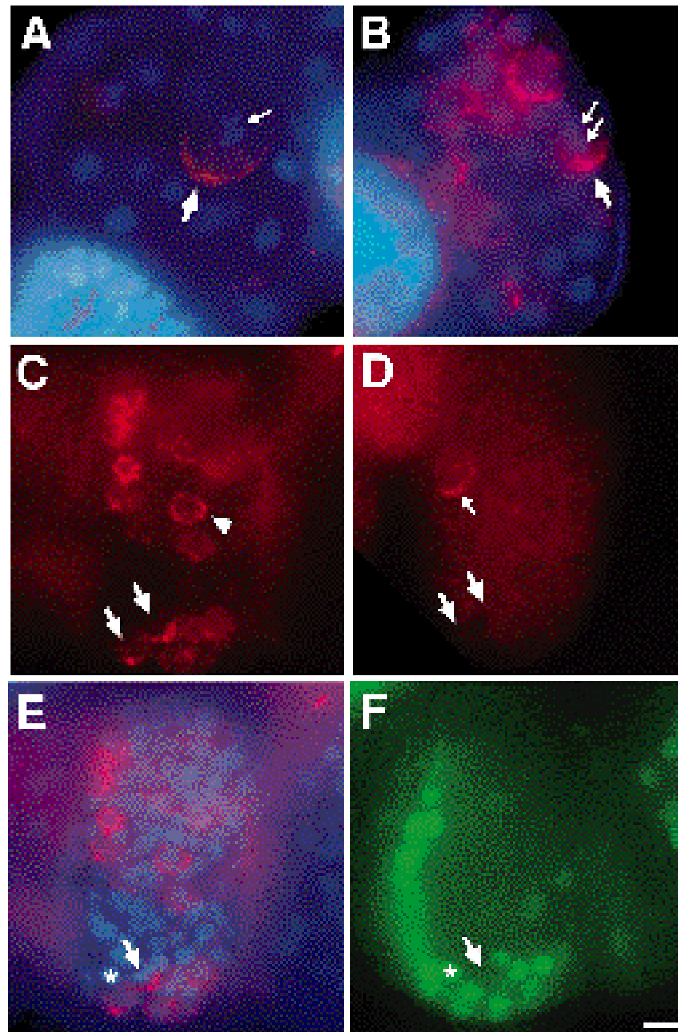

Fig. 2. HAM-1 expression in wild-type and *ham-1* mutant embryos. (A-E) Fluorescence photomicrographs of embryos stained with DAPI (blue) and a mouse anti-HAM-1 antiserum (red). (A-C, E,F) wild-type embryos, (D) *ham-1* mutant embryos. (A) Earliest expression of HAM-1 begins at the 28-cell stage of development. At this stage, HAM-1 is restricted to the cell periphery to one side of dividing cells (crescent staining). The large arrow points to the asymmetrically distributed HAM-1 protein of a dividing cell and the small arrow points to the condensed chromosomes in the same cell. (B) Fluorescence photomicrograph of an embryo later in development (mid-gastrulation) that has been stained with an anti-HAM-1 antiserum. The small arrows point to two nuclei that have just formed at the end of cell division. HAM-1 protein is associated with the plasma membrane of the posterior nucleus. (C,D) Embryos at approximately 280 minutes after the division of the zygote. (C) Ventral view of a wild-type embryo. At this stage, both rings (arrowhead) and crescents (arrows) of HAM-1 protein are visible. The arrows mark the asymmetric distribution of HAM-1 in the HSN/PHB (right arrow) and ALN/PLM (left arrow) neuroblasts prior to their divisions. (D) Ventral view of a *ham-1(n1438)* embryo. The small arrow indicates the only detectable HAM-1 expression in this embryo. This embryo is in the same orientation and at the same stage as the wild-type embryo in C. The large arrows mark the HSN/PHB (right arrow) and ALN/PLM (left arrow) neuroblasts that are not expressing HAM-1 protein. (E) Merged image showing DAPI and anti-HAM-1 staining of the same embryo as in C. The arrow points to HAM-1 localization at the posterior cell periphery of the HSN/PHB neuroblast. This portion of the neuroblast will be inherited by the HSN/PHB precursor upon division. (F) LIN-26 staining in the same embryo as in C and E. LIN-26 is not expressed in the HSN/PHB neuroblast (arrow). The asterisk in E and F marks the position of a nucleus that is expressing LIN-26 for reference. Scale bar, 5 μm.

and the hyp7 cell that border the HSN/PHB neuroblast (Fig. 2F; Labouesse et al., 1996). After division of the HSN/PHB neuroblast, HAM-1 ring staining can occasionally be seen in cells that, by position, could be the HSN/PHB precursor (data not shown). This staining, however, is usually not detectable suggesting that HAM-1 is degraded rapidly after division of the HSN/PHB neuroblast.

Additional HSN and PHB neurons in *ham-1* mutants

In agreement with a previous study (Desai et al., 1988), we found by staining with an antiserum that detects the HSN neurotransmitter serotonin that *ham-1* mutants occasionally produced additional HSN neurons. In contrast to wild-type animals, 16-23% of the sides of *ham-1* mutants produced an additional HSN neuron (Fig. 3B; Table 1). In addition, *ham-1* mutant HSNs often migrate abnormally (Fig. 3B).

ham-1 mutants also produce extra phasmid neurons, bilaterally symmetric sensory neurons located in the tail. One of the two phasmid neurons is PHB, the sister of the HSN (Fig. 1). The other is PHA, a cell lineally unrelated to the HSN. In wild-type larvae, the PHA and PHB neurons can be detected using an antiserum raised against the *egl-43* gene product, a putative transcription factor that functions in phasmid neuron development (Garriga et al., 1993a; Fig. 3C; Table 1). In contrast to wild-type animals, 22-33% of the sides of *ham-1* mutants produced an additional phasmid neuron that expressed EGL-43 (Fig. 3D; Table 1). The correlation between the frequency of extra HSN and PHB neurons suggests that the HSN/PHB lineage is defective in *ham-1* mutants.

In contrast to our results, a previous study suggested that *ham-1* mutants produced fewer phasmid neurons based on dye loading experiments (Desai et al., 1988). Using this technique, one of the phasmid neurons of *ham-1* mutants often failed to fill with dye (Fig. 3F). This observation led Desai et al. (1988) to propose that PHB was transformed into a second HSN in *ham-1* mutants. The discrepancy between the number of phasmid neurons identified using EGL-43 expression and DiI filling, however, appears to be caused by abnormal development of phasmid neuron sensilla in *ham-1* mutants (E. Hartwieg, G. G. and H. R. Horvitz, unpublished observations). Thus, while extra HSN and PHB neurons are produced in *ham-1* mutants, HSN and PHB development can be abnormal (see Discussion).

Two observations suggest that the *ham-1* mutations are strong loss-of-function mutations. First, animals homozygous (*ham-1/ham-1*) and hemizygous (*ham-1/Df*) for *ham-1(n1810)* produced similar numbers of HSNs (data not shown). Second, *ham-1* homozygotes and *sDf22* homozygotes (*sDf22* is a deficiency that removes *ham-1*) produced similar numbers of extra phasmid neurons (see Materials and Methods).

ham-1 mutations transform the sister cell of the HSN/PHB precursor

One possible explanation for the additional HSN and phasmid

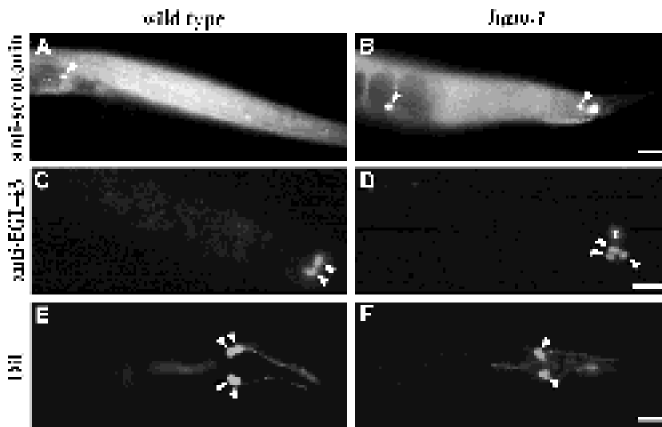

Fig. 3. HSN and PHB defects in *ham-1* mutants. Anterior is to the left. (A,B) Fluorescence photomicrographs of (A) wild-type and (B) *ham-1(n1438)* adult hermaphrodites that have been stained with an anti-serotonin antiserum to detect the HSNs. Each panel presents a left lateral view of the posterior half of an adult hermaphrodite. In wild-type embryos, each HSN migrates from the tail to a position near the center of the animal (arrowhead). The HSNs in *ham-1* mutants are migration defective and are found in the tail or along the migratory route (arrowheads). The asterisk in B indicates the position of an out-of-focus HSN on the right side that has failed to migrate out of the tail. Scale bar, 20 µm. (C,D) Fluorescence photomicrographs of (C) wild-type and (D) *ham-1(n1438)* first larval stage larvae that have been stained with an anti-EGL-43 antiserum to detect the PHA and PHB nuclei (arrowheads); (C) A left lateral view and (D) a right lateral view of the tail. In wild-type larvae, both PHA and PHB neurons express EGL-43. In this *ham-1(n1438)* mutant larva, an extra neuron expresses EGL-43. The asterisk in D indicates the position of an out-of-focus phasmid neuron nucleus on the left side of the tail. Scale bar, 3 µm. (E, F) Fluorescence photomicrographs of (E) wild-type and (F) *ham-1(n1438)* fourth larval stage hermaphrodites that have been incubated in the fluorescent dye DiI to visualize the phasmid neurons PHA and PHB. When wild-type animals are soaked in DiI, the exposed sensilla of PHA and PHB take up dye allowing visualization of both their cell bodies and processes by fluorescence microscopy (Hedgecock et al., 1985; E. Hedgecock, personal communication). Each panel presents a ventral view of the tail allowing visualization of the PHA and PHB neurons (arrowheads) on each side of the animal. In contrast to wild-type hermaphrodites, only one phasmid neuron on each side filled with DiI in this *ham-1* mutant. On average, only a single phasmid neuron filled in 78% of the sides of *ham-1(n1438)* hermaphrodites. Scale bar, 10 µm.

neurons is that *ham-1* mutations transform the sister of the HSN/PHB precursor, a cell that normally dies, into a second HSN/PHB precursor. We addressed this possibility by directly observing the HSN/PHB lineage.

The left and right HSN/PHB neuroblasts divide at about 285 minutes after cleavage of the zygote to produce a daughter cell that dies and an HSN/PHB precursor (Figs 1, 4A-C). We followed these neuroblast divisions and the death of the anterior daughter cell in five wild-type embryos using Nomarski optics (see Materials and Methods). In all ten lineages, the neuroblast divided to produce a smaller anterior cell that died and a larger posterior precursor cell.

To determine the origin of the additional HSNs and PHBs in *ham-1* mutants, we followed fourteen neuroblast divisions in *ham-1(n1810)* embryos and six neuroblast divisions in *ham-1(n1438)* embryos. Although the division of the neuroblast occurred at the normal time, two features of these divisions and the development of the daughter cells were altered in *ham-1* mutants. First, when the anterior cell died, the onset of cell death was delayed. On average, death occurred approximately 20 minutes later than in wild-type embryos. Second, three of the fourteen neuroblast divisions in *ham-1(n1810)* embryos and two of the six neuroblast divisions in *ham-1(n1438)* embryos generated two daughters that survived. Neither the daughter cells nor their descendants had died at the point that we discontinued observation (at the two-fold stage of embryogenesis, 430 minutes after first cleavage). To confirm that the sister cell of the HSN/PHB precursor produced an additional HSN when it survived, we recovered the embryos, allowed them to develop into adults and stained them with an anti-serotonin antiserum to detect the HSNs (Fig. 4D-G). All five lineages that generated two surviving neuroblast daughters produced an extra HSN; the remaining 15 lineages produced a single HSN. Thus, in *ham-1* mutants, the sister cell of the HSN/PHB precursor can be transformed into a second HSN/PHB precursor.

ham-1 does not appear to be a cell death gene

To determine whether *ham-1* mutants produce extra HSN and PHB neurons by allowing the sister of the HSN/PHB precursor to survive, we stained *ced-3* and *ced-4* mutants with anti-serotonin and anti-EGL-43 antisera. The genes *ced-3* and *ced-4* are required for the execution of all 131 programmed cell deaths that occur normally during *C. elegans* development (Ellis and Horvitz, 1986). In the absence of either of these two gene products, cells that normally die survive and in some cases differentiate into neurons that express fates similar to those of their sister cells (Ellis and Horvitz, 1986; Avery and Horvitz, 1987). Direct observations of the HSN/PHB neuroblast divisions in three *ced-3(n717)* embryos confirmed that all six anterior daughters survived. Neither *ced-3* nor *ced-4* mutants, however, produced extra HSNs or PHBs at the same frequency as *ham-1* mutants (Table 1 for *ced-3* results). Therefore, when the sister cell of the HSN/PHB precursor survives in a *ced-3* or *ced-4* mutant, it rarely divides to produce extra HSN and PHB neurons. These results are consistent with previous observations showing that cells normally destined to die do not divide when they are allowed to survive in a *ced-3* or *ced-4* mutant (Ellis and Horvitz, 1986).

Extra HSN and PHB neurons are produced, however, when the *ced-3* and *ced-4* mutations are placed in a *ham-1* background (Table 1 for *ced-3* results). In *ham-1 ced-3* or *ced-4; ham-1* double mutants, the HSN/PHB lineage almost always produced extra HSNs and PHBs. We conclude that in *ham-1* mutants, the sister cell of the HSN/PHB precursor is almost always transformed into a second HSN/PHB precursor, but the transformation is often masked by cell death. In the absence of programmed cell death, the transformation of the sister cell caused by the *ham-1* mutations is revealed. We find similar interactions between *ham-1* and *ced-3* or *ced-4* for the other lineages discussed below (data not shown).

Loss of *ham-1* function disrupts four additional divisions

Additional RID neurons in *ham-1* mutants

RID is an unpaired motor neuron in the dorsal ganglion that extends a single axon that runs along the dorsal nerve cord

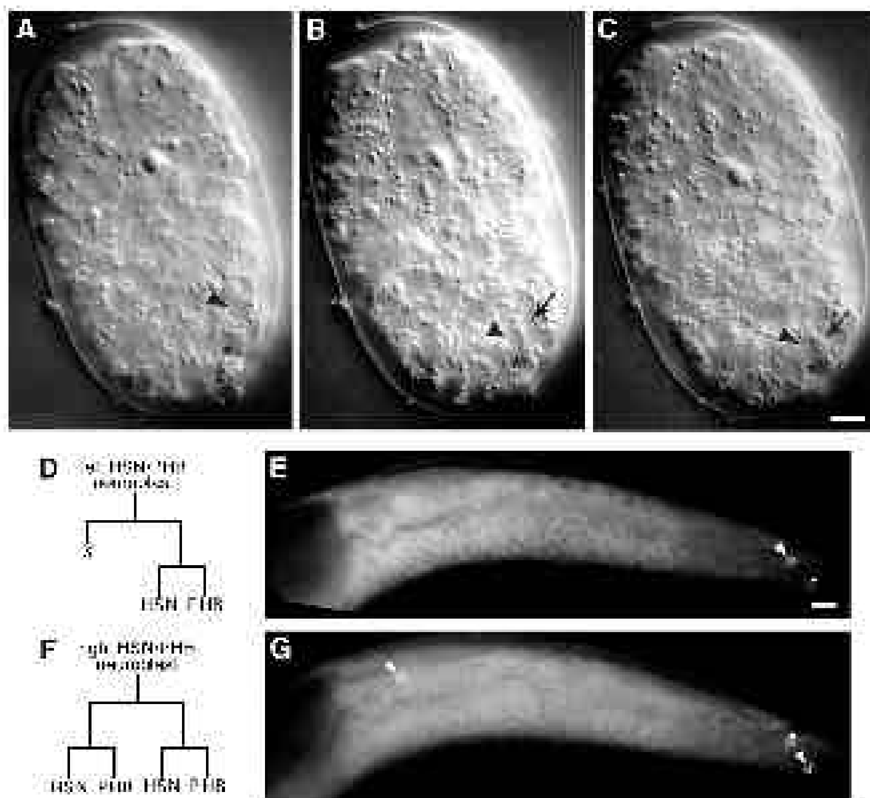

Fig. 4. The HSN/PHB neuroblast division is abnormal in *ham-1* mutant embryos. (A-C) Nomarski photomicrographs of a ventral view of a wild-type embryo following the division of the left HSN/PHB neuroblast. The asterisk marks the position of the left T blast cell, a hypodermal cell that does not divide until larval development. It is located just posterior to the left HSN/PHB neuroblast and its descendants. (A) At 270 minutes after first cleavage of the zygote the HSN/PHB neuroblasts have been produced. The arrowhead indicates the position of the left HSN/PHB neuroblast. (B) By 320 minutes, the neuroblast has divided to produce a smaller anterior cell (arrow) and a larger HSN/PHB precursor (arrowhead). (C) 10 minutes later, the anterior daughter dies (arrow) which results in a change in nuclear refractivity. Scale bar, 5 μm. (D-G) Schematic representations of observed HSN/PHB lineages (D, F) and corresponding serotonin expression patterns (E, G) of the same *ham-1(n1810)* mutant. The HSN/PHB lineage of this *ham-1* embryo was followed from 260 minutes after first cleavage to 420 minutes (two-fold stage of embryonic development) by Nomarski optics, recovered from the slide, allowed to develop to an adult and stained with an anti-serotonin antiserum. (D,E) The left HSN/PHB neuroblast division produced an anterior daughter that died and a posterior daughter that generated a single HSN neuron (arrowhead in E). Scale bar, 10 μm. (F, G) The right HSN/PHB neuroblast division produced two daughters that survived and each daughter divided to produce an HSN neuron (arrowheads in G). (E,G) The asterisk indicates the position of an out-of-focus HSN on the contralateral side of the animal.

Table 2. Extra RID, ADL and ADE neurons in *ham-1* mutants

	No. of RID neurons/animal*			No. of ADL neurons/side†,‡			No. of ADE neurons/side§,¶		
Strain	2	1	N	2	1	N	2	1	N
wild type	0%	100%	30	0%	100%	68	0%	100%	33
ham-1(n1438)	77%	23%	30	54%	43%	37	93%	7%	29
ham-1(n1810)	69%	31%	26	29%	71%	72	88%	12%	33
ham-1(n1811)	67%	33%	21	ND‖	ND	ND	83%	17%	29

*Average number of RID neurons was determined by counting the number of FMRFamide-expressing cells that were located in the dorsal ganglion and extended processes into the dorsal nerve cord. In wild-type hermaphrodites, of the 22 cells that express the neuropeptide FMRFamide in the anterior body region, only RID sends a process into the dorsal cord (Schinkmann and Li, 1992).

†Animals used in this set of experiments contained the *lin-11::lacZ* transgene, *nIs2*.

‡Average number of ADL neurons/side was determined by counting the number of cells expressing both EGL-43 and β-galactosidase from the *lin-11::lacZ* transgene, *nIs2*.

§Average number of ADE neurons/side was determined by counting the number of cells posterior to the pharynx that produced serotonin from exogenous 5-hydroxytryptophan (see Materials and Methods).

¶Similar results were obtained when L1 larvae were stained with antibodies that recognize EGL-43, which is expressed by the ADEs.

‖ND - not determined.

(DNC) (White et al., 1986) and expresses the neuropeptide FMRFamide (Fig. 5A; Schinkmann and Li, 1992). Anti-FMRFamide staining of *ham-1* mutants often revealed two RID-like cells in the dorsal ganglion (Fig. 5B; Table 2).

Additional ADL neurons in *ham-1* mutants

The bilaterally symmetric ADLs are sensory neurons located in the dorsal regions of the lateral ganglia (White et al., 1986). We identified cells that express an ADL-like fate using two criteria: expression of the *lin-11*::*lac Z* transgene, *nIs2*, (C. G. Freyd and H. R. Horvitz, personal communication) and of EGL-43 (C. G. and G. G., unpublished observations). Two sensory neurons in the lateral ganglia, ADL and ADF, express both markers. Double staining wild-type animals carrying the transgene *nIs2* with anti-β-galactosidase and anti-EGL-43 antisera revealed a single ADL neuron on either side of the dorsal midline (Fig. 5C), whereas staining *ham-1* mutant hermaphrodites carrying the *nIs2* transgene often showed an additional ADL-like neuron on either side of the dorsal midline (Fig. 5D; Table 2). Double staining of *nIs2*-containing animals with an anti-serotonin antiserum, which recognizes ADF but not ADL, and an anti-β-galactosidase antiserum confirmed that

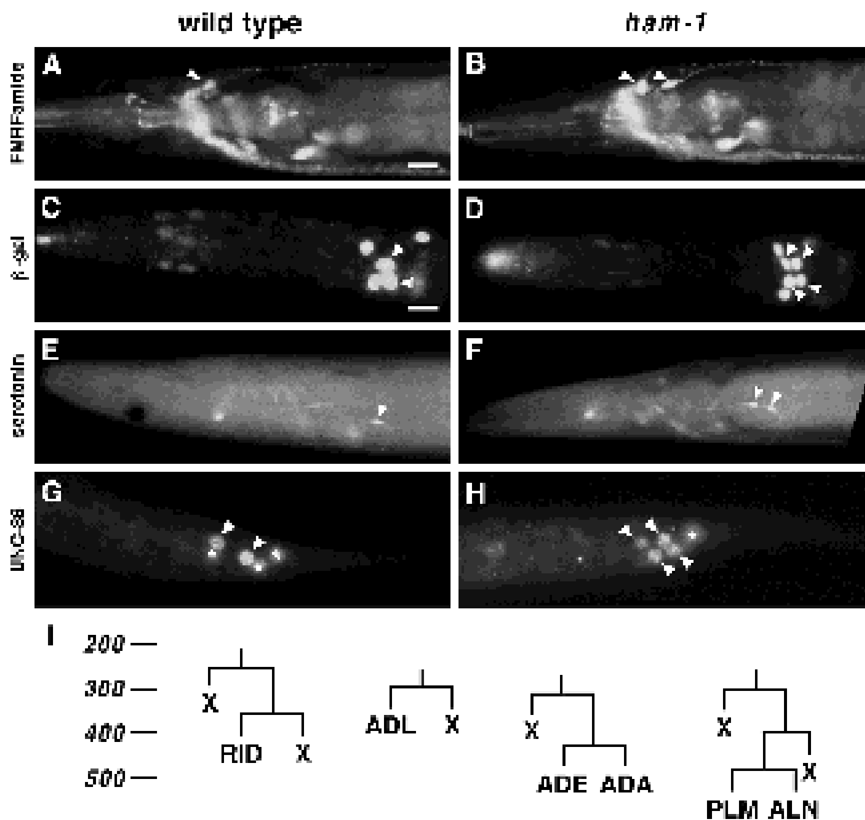

Fig. 5. Additional defects in *ham-1* mutants. (A,B) Fluorescence photomicrographs of (A) wild-type and (B) *ham-1(n1438)* adult hermaphrodites stained with an anti-FMRFamide antiserum. Each panel presents a left lateral view of the head. (A) A single RID neuron (arrowhead) in the dorsal ganglia extends a process into the dorsal nerve cord. (B) Two RID neurons (arrowheads) extend processes into the dorsal nerve cord in this *ham-1* mutant hermaphrodite. (C,D) Fluorescence photomicrographs of (C) wild-type and (D) *ham-1(n1438)* first larval stage larvae that carry a *lin-11*::*lacZ* transgene stained with an anti-β-galactosidase antiserum. Each panel presents a dorsal view of the head. (C) A single ADL neuron lies on either side of the dorsal midline in wild-type hermaphrodites (arrowheads). (D) Two ADL neurons lie on either side of the dorsal midline in this *ham-1* mutant (arrowheads). (E,F) Fluorescence photomicrographs of (E) wild-type and (F) *ham-1(n1438)* adult hermaphrodites that have been soaked in 5-hydroxytryptophan and stained with an anti-serotonin antiserum to detect the ADE neurons. Each panel presents a left lateral view of the head. (E) A single ADE neuron (arrowhead) is visible in wild-type hermaphrodites. (F) Two ADE neurons (arrowheads) are visible in this *ham-1* mutant hermaphrodite. (G,H) Fluorescence photomicrographs of (G) *egl-1(n986)* and (H) *ham-1(n1810); egl-1(n986)* first larval stage larvae stained with an anti-UNC-86 antiserum to detect the ALN and PLM neurons. Each panel presents a left lateral view of the tail. (G) Two cells on the left side of the tail, ALN and PLM, express UNC-86 in an *egl-1(n986)* larva (arrowheads). (H) Four ALN and PLM neurons express UNC-86 in this *ham-1(n1810); egl-1(n986)* mutant larva (arrowheads). (I) Schematic representations of the lineages that produce neurons affected by the *ham-1* mutations. At left is the time, in minutes, from first cleavage of the zygote. In each lineage, X denotes a cell that undergoes programmed cell death. Scale bar for adults (A,B,E,F) in panel A, 14 μm. Scale bar for L1s (C,D,G,H) in panel C, 5 μm.

the additional cells in *ham-1* mutants were ADL (data not shown).

Additional ADE and ADA neurons in *ham-1* mutants

The ADEs are a pair of ciliated sensory neurons situated laterally behind the second bulb of the pharynx (White et al., 1986). We visualized the ADE neurons using two approaches: expression of aromatic amino acid decarboxylase activity (Loer and Kenyon, 1993) and of EGL-43 (C. G., unpublished observations). Staining wild-type hermaphrodites for aromatic amino acid decarboxylase activity revealed a single ADE on either side of the animal (Fig. 5E), whereas staining *ham-1* hermaphrodites revealed extra ADE neurons (Fig. 5F; Table 2). *ham-1* mutants also produced extra neurons in the same positions that expressed EGL-43 (data not shown).

The ADA interneurons are the sisters of the ADE sensory neurons (Sulston et al., 1983). We detected the ADA neurons using an anti-UNC-86 antiserum. UNC-86 is a nuclear protein expressed in a number of neuroblasts and neurons including ADA, ALN and PLM (see below; Finney and Ruvkun, 1990). Double staining of *ham-1* mutant larvae with anti-EGL-43 and anti-UNC-86 antisera occasionally showed both extra ADE and ADA neurons, consistent with the production of an extra ADE/ADA precursor (data not shown).

Additional ALN and PLM neurons in *ham-1* mutants

The ALN and PLM sister cells are both bilaterally symmetric neurons located in the lumbar ganglia of the tail (White et al., 1986). Both the ALNs and PLMs are recognized by an anti-UNC-86 antiserum. In *ham-1* mutants, the HSNs, which also express UNC-86, often fail to migrate out of the tail. Therefore, to prevent a misplaced HSN from being scored as an additional ALN or PLM neuron in *ham-1* mutants, we analyzed the number of ALN and PLM neurons in an *egl-1* background, which causes the HSNs to die shortly after their birth (Trent et al., 1983; Desai et al., 1988). Staining *egl-1* larvae with an anti-UNC-86 antiserum revealed a single ALN and a single PLM neuron on the left side of the tail (Fig. 5G). *ham-1; egl-1* larvae often displayed additional ALN- and PLM-like neurons (Fig. 5H; Table 3). The presence of extra PLMs in *ham-1* mutants was confirmed using the *mec-3*::*lacZ* transgene, *jeIn2* (data not shown; Way and Chalfie, 1989).

We have also found that the ALN/PLM neuroblast distributes HAM-1 asymmetrically (Fig. 2C). This cell divides to produce an anterior daughter that dies and a posterior daughter that divides to produce a cell that dies and the ALN/PLM precursor. HAM-1 is restricted to the posterior half of the ALN/PLM neuroblast, ensuring that it will be inherited by the posterior daughter cell. Thus, as in the HSN/PHB lineage, the cell that is not affected by *ham-1* mutations inherits HAM-1

Table 3. Extra ALN and PLM neurons in *ham-1* mutants

	No. of ALN and PLM neurons/side*			
Strain	4	2	0	N
egl-1(n986)	0%	100%	0%	48
ham-1(n1438); egl-1(n986)	35%	54%	11%	46
ham-1(n1810); egl-1(n986)	41%	57%	2%	42
ham-1(n1811); egl-1(n986)	46%	50%	4%	46

*Average number of ALN and PLM neurons was determined by counting the number of UNC-86-expressing cells on the right and left sides of the tails of L1 larva. To prevent misplaced HSNs, which express UNC-86, from being included in the counts, the frequency of ALN and PLM cell duplications was determined in animals homozygous for the *egl-1(n986)* mutation. The HSNs die during embryogenesis in *egl-1(n986)* homozygotes. Because the right side contains a third neuron, PVR, that expresses UNC-86, the number of ALN and PLM neurons on the right was calculated as the total number of UNC-86-expressing cells minus one.

protein. This observation suggests that HAM-1 acts similarly in each identified neuroblast division.

DISCUSSION

We show that during embryonic development HAM-1 is distributed to the posterior of the HSN/PHB neuroblast, which divides to produce a smaller anterior cell that dies and a larger posterior HSN/PHB precursor that inherits the HAM-1 protein. Loss of *ham-1* function transforms the anterior daughter into an HSN/PHB precursor suggesting that HAM-1 distributes developmental potential in the dividing neuroblast. We propose that HAM-1 distributes cell fate determinants asymmetrically in dividing neuroblasts, allowing their daughter cells to adopt distinct fates.

Asymmetric cell divisions that produce a cell that dies require HAM-1

The five divisions that require *ham-1* function share two features. First, each affected division occurs between 200 and 300 minutes after cleavage of the zygote. Second, each affected division produces a daughter that undergoes programmed cell death and a neuron or neuronal precursor (Figs 1, 5). These features raise the question of whether *ham-1* functions specifically at this time in cell divisions that produce a cell that dies. 28 additional cell divisions produce a cell that dies during this time interval.

Two observations suggest that at least some additional divisions that produce cells that die at this time are affected by *ham-1* mutations. First, many cells in L1 larvae, including descendants of three of the twenty-eight divisions described above, express EGL-43 (C. G. and C. Bargmann, unpublished observations), and *ham-1* mutant larvae contain extra unidentified cells that express EGL-43 (data not shown). Second, many of the cell deaths that occur between 200 and 300 minutes after cleavage of the zygote in *ham-1* mutants, including the sister of the HSN/PHB precursor, produce cell corpses that are larger and persist longer than the corresponding corpses in wild-type embryos (data not shown). Large cell corpses and corpses that persist are features of abnormal programmed cell deaths (Hedgecock et al., 1983; Ellis et al., 1991; Hengartner et al., 1992).

We have analyzed the descendants from twenty-one additional embryonic and postembryonic cell divisions (see Materials and Methods). Eleven of the cell divisions occur between 200 and 300 minutes after cleavage of the zygote, the time of the divisions that are affected by *ham-1* mutations, but do not produce a cell that dies. Ten of the cell divisions, three of which produce a cell that dies, occur later. None of these cell divisions are defective in *ham-1* mutants suggesting that HAM-1 functions in a small subset of the asymmetric cell divisions that occur during *C. elegans* development.

HAM-1 is asymmetrically distributed in many dividing cells

We observed HAM-1 protein in many mitotic and postmitotic cells beginning at the onset of gastrulation, approximately 100 minutes after cleavage of the zygote. *ham-1* mutations, however, do not disrupt the divisions of all precursors that express HAM-1 protein asymmetrically. One possible explanation for this discrepancy between *ham-1* expression and phenotype is that other gene products can provide similar functions in the absence of *ham-1*. Redundancy may be a common feature of asymmetric cell divisions. For example, numb is asymmetrically localized in all neuroectodermal cells of *Drosophila* including the GMCs, which divide asymmetrically to produce neurons (Goodman and Doe, 1993; Rhyu et al., 1994; Spana et al., 1995). Loss of *numb* function, however, fails to perturb the fates of the GMC daughter cells (Uemura et al., 1989; Spana et al., 1995).

Creating cellular diversity through a polar mother cell

One mechanism of producing daughter cells that adopt distinct fates is for a polar mother cell to asymmetrically distribute developmental potential to its daughters. Asymmetric distribution of HAM-1 in the HSN/PHB neuroblast indicates that this precursor is polar, and *ham-1* mutant phenotypes demonstrate that HAM-1 distributes developmental potential asymmetrically to daughter cells during this division. We propose that HAM-1 functions similarly in the four other divisions affected by *ham-1* mutations.

Several other proteins have been shown to be asymmetrically distributed during mitosis. numb and prospero are asymmetrically distributed in neuroblasts and inherited by only one daughter cell. Genetic analysis has indicated that numb and prospero behave like determinants that specify the fates of the daughter cells that inherit them (Doe et al., 1991; Rhyu et al., 1994; Hirata et al., 1995; Spana et al., 1995; Spana and Doe, 1995).

The PAR-1 and PAR-3 proteins are asymmetrically distributed in the *C. elegans* zygote (Guo and Kemphues, 1995; Etemad-Moghadam et al., 1995), which divides to produce a larger anterior blast cell AB and a smaller posterior blast cell P1 (Sulston et al., 1983). PAR-3 is distributed to the anterior of the zygote and is inherited by AB, and PAR-1 is distributed to the posterior of the zygote and is inherited by P1. Like numb and prospero, PAR-3 and PAR-1 specify traits of the cells that inherit them. In wild-type embryos, AB divides transversely, while P1 divides longitudinally after a 90° rotation of its duplicated centrosomes. In *par-3* mutants, the AB centrosomes rotate 90° as they normally would in P1, whereas in *par-1* mutants the P1 centrosomes occasionally fail to rotate (Kemphues et al., 1988) suggesting that PAR-3 specifies AB

fate while PAR-1 specifies P1 fate. PAR-1 function, however, is more complicated. SKN-1, a putative transcription factor that is expressed preferentially by P1 and specifies the fates of P1 descendants, is expressed equally by AB and P1 in *par-1* mutants, causing AB to adopt certain P1 traits (Bowerman et al., 1992, 1993). Thus, PAR-1 is inherited by P1, but appears to control both AB and P1 traits.

In contrast to numb, prospero and PAR-3, HAM-1 specifies the fate of the cell that does not inherit HAM-1 protein. Thus, HAM-1 does not behave like a cell fate determinant. One model that can account for this suprising observation is that HAM-1 restricts cell fate determinants to the presumptive HSN/PHB precursor cell. By tethering the determinants to the posterior periphery of the dividing neuroblast, HAM-1 would ensure that only the posterior daughter cell adopts the specified fate. In the absence of HAM-1, the cell fate determinants would distribute equally to both daughter cells causing both cells to adopt the HSN/PHB precursor fate.

Although the most striking phenotypes of *ham-1* mutants are extra neurons, the neurons that are produced by the affected lineages can be abnormal. For example, the HSNs fail to migrate normally and occasionally fail to express serotonin, and the PHBs fail to project their sensory cilia to the environment. One prediction of the HAM-1 tether model is that loss of HAM-1 would alter in daughter cells the levels of cell fate determinants that are normally distributed to only one daughter. In this model, the HSN/PHB precursor would normally inherit all of the determinants but, in *ham-1* mutants, the determinants would be shared by both daughter cells. Perhaps reducing the dosage of the proposed determinants in the daughter cells affects HSN and PHB differentiation.

A model in which HAM-1 regulates interactions between daughter cells can also account for our observations. Asymmetric neuroblast divisions that require *Drosophila* numb also require Notch and Delta, cell-surface proteins that have been proposed to mediate interactions between daughter cells of the asymmetric divisions (Hartenstein and Posakony, 1990; Parks and Muskavitch, 1993). Spana et al. (1995) have proposed that asymmetrically distributed numb inhibits *Notch* activity, biasing the Notch/Delta interactions to generate an asymmetric division. HAM-1 could also regulate interactions between the HSN/PHB neuroblast daughter cells. If cell interactions between daughter cells of *ham-1*-dependent divisions occur, however, they would occur rapidly; the daughter cell that dies becomes refractile within 10 minutes of being produced. The interactions would also occur in the absence of *lin-12* and *glp-1*, the *C. elegans Notch* homologs (Yochem et al., 1988; Yochem and Greenwald, 1989); mutations in *lin-12* or *glp-1* or in *lag-1* or *lag-2*, genes that are required for both *lin-12* and *glp-1* activity (Lambie and Kimble, 1991), do not produce extra HSN or PHB neurons (G.G. and C.G., unpublished observations).

How are molecules like HAM-1 asymmetrically distributed? Intrinsic information from previous cell divisions or signaling from surrounding cells could distribute proteins asymmetrically during cell division. In the yeast *S. cerevisiae*, the genes *BUD1-BUD9* use intrinsic information from the previous bud site to select the new bud site (reviewed in Chant, 1994). Specifically, Bud3p localization at the bud site in one cell cycle is proposed to mark the site of axial budding in the subsequent cell cycle (Chant et al., 1995). Alternatively, cell signaling may orient asymmetric divisions during metazoan development. In *C. elegans*, secretion of LIN-44, a Wnt protein, by cells in the tail may orient the division planes of blast cells located in more anterior positions (Herman and Horvitz, 1994; Herman et al., 1995). It will be interesting to learn whether different neuroblasts that require *ham-1* use the same or distinct mechanisms to distribute HAM-1 and to determine whether those mechanisms are intrinsic or extrinsic.

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