C. elegans HAM-1 positions the cleavage plane and regulates apoptosis in asymmetric neuroblast divisions

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Abstract

Asymmetric cell division occurs when a mother cell divides to generate two distinct daughter cells, a process that promotes the generation of cellular diversity in metazoans. During Caenorhabditis elegans development, the asymmetric divisions of neural progenitors generate neurons, neural support cells and apoptotic cells. C. elegans HAM-1 is an asymmetrically distributed cortical protein that regulates several of these asymmetric neuroblast divisions. Here, we show that HAM-1 is a novel protein and define residues important for HAM-1 function and distribution to the cell cortex. Our phenotypic analysis of ham-1 mutant embryos suggests that HAM-1 controls only neuroblast divisions that produce apoptotic cells. Moreover, ham-1 mutant embryos contain many unusually large cell-death corpses. An investigation of this corpse phenotype revealed that it results from a reversal of neuroblast polarity. A misplacement of the neuroblast cleavage plane generates daughter cells of abnormal size, with the apoptotic daughters larger than normal. Thus, HAM-1 regulates the position of the cleavage plane, apoptosis and mitotic potential in C. elegans asymmetric cell divisions.

Keywords: Caenorhabditis elegans; HAM-1; Asymmetric cell division; Neuroblast; Apoptosis

Introduction

Asymmetric cell division occurs when a mother cell generates two distinct daughter cells, a process that promotes the generation of cellular diversity in metazoans (Horvitz and Herskowitz, 1992). An important class of asymmetric cell division occurs in nervous system development when neuroblasts divide to produce daughter cells of distinct fate.

Asymmetric neuroblast divisions have been studied extensively in Drosophila melanogaster. In the Drosophila central nervous system (CNS), several asymmetrically localized molecules ensure the fidelity of neuroblast divisions. Three are found in a conserved complex: Bazooka (Baz; PDZ domain), DmPAR-6 (PDZ domain), and atypical protein kinase C (aPKC) (Petronczki and Knoblich, 2001; Wodarz et al., 2000). This complex recruits other factors, like Inscurable (Ins) and Partner of Inscurable (Pins), to the apical cortex of CNS neuroblasts. In turn, Ins and Pins direct the plane of cell division and the appropriate distribution of neuronal fate determinants, like Prospero or Numb (Kraut et al., 1996; Parmentier et al., 2000; Schaefer et al., 2000; Schober et al., 1999; Wodarz et al., 1999; Yu et al., 2000).

All Caenorhabditis elegans neurons are generated by asymmetric neuroblast divisions, but little is known about the molecular underpinnings of this process in the nem-
We previously demonstrated that *ham-1* mutations disrupt several asymmetric neuroblast divisions, including the divisions of the two HSN/PHB neuroblasts (ABpl/rappap) (Guenther and Garriga, 1996). In wild-type embryos, each HSN/PHB neuroblast divides asymmetrically to generate a smaller anterior daughter that undergoes programmed cell death and a larger posterior daughter, the HSN/PHB precursor, which subsequently divides to generate an HSN motor neuron and a PHB sensory neuron. In *ham-1* mutants, the anterior daughter can inappropriately survive, resulting in extra HSN and PHB neurons.

Unlike Numb and Prospero, HAM-1 does not behave like a cell-fate determinant. HAM-1 localizes to the posterior cortex of the HSN/PHB neuroblast and is inherited by the HSN/PHB precursor (Guenther and Garriga, 1996). Yet, it is the anterior HSN/PHB neuroblast daughter, the cell that normally does not inherit HAM-1, that is transformed in *ham-1* mutants.

In this study, we describe the role for HAM-1 in cell division. We identified six *ham-1* alleles that revealed residues of HAM-1 important for its function and localization. We observed that some apoptotic cells are abnormally large in *ham-1* mutants, a phenotype correlated with defects in asymmetric neuroblast division. We present evidence that this phenotype results from an altered cleavage plane in dividing neuroblasts. Our data suggest a mechanism by which HAM-1 could distribute developmental potential during asymmetric neuroblast divisions.

**Materials and methods**

*C. elegans strains and genetics*

Nematodes were maintained as described (Brenner, 1974). The wild-type strain N2 was used along with strains with the following genotypes: *Linkage Group (LG) I*: ynsIs45[flp-15::gfp] (Li et al., 1999), kysIs39[sva-6::gfp] (Troemel et al., 1995); *LG II*: gmls20[hlh-14::gfp] (Frank et al., 2003); *LG III*: gmls12[srb-6::gfp] (Frank et al., 2003); *LG IV*: *ham-1*(n1438), *ham-1*(n1810), *ham-1*(n1811) (Desai et al., 1988), *ham-1*(gm214), *ham-1*(gm267), *ham-1*(gm279) (this study), *dpy-20*(e1282) (Hosono et al., 1982), unc-30(e191) (Brenner, 1974), unc-31(e169) (Brenner, 1974); *LG V*: *akls7[nmr-1::gfp]* (Brockie et al., 2001); *LG X*: gmls18[ceh-23::gfp] (Wither et al., 2004); extrachromosomal array: gmeEx285[nlp-1::gfp] (Li et al., 1999).

**Isolation of *ham-1* mutants**

Initial characterizations of *ham-1*(n1438), *ham-1*(n1810), and *ham-1*(n1811) have been described (Desai et al., 1988; Guenther and Garriga, 1996). *ham-1*(gm214), *ham-1*(gm267), and *ham-1*(gm279) were isolated in a genetic screen in which gmls12 worms were mutagenized with 50 mM EMS for 4 h, and mutants with extra GFP-positive PHB neurons were identified among the progeny of individual F1 hermaphrodites.

***ham-1* cloning, gene structure, and rescue**

*ham-1* mapped genetically between RFLPs on the cosmids C08G1 and C13H6 on *LG IV*. The injection of the intervening cosmid C03F9 into *ham-1*(n1811) mutants rescued aspects of the *Ham-1* phenotype. A 12 kb *Bst EII/Mlu I* subclone of C03F9 called p3F9M2 also rescued. DNA within this clone encodes the 1.8 kb *ham-1* mRNA.

The amplification of cDNAs using a primer complementary to SL1 (5’ TCTAGAATTCCGCGGTAAAATTACC-CAAGTTTG 3’) and a second primer hm9 (5’ GCATGAAGCCCCATGTAA 3’) demonstrated that the 5’ end of the *ham-1* transcript is generated by trans-splicing to SL1.

**Identification of *ham-1* lesions**

The PCR primers Kg37 (5’ GCTTTTCATTTTGTTCGTG 3’) and hm9 (5’ GCATGAAGCCCCATGTAA 3’) yielded a 508 bp fragment from a wild-type genomic DNA template and a 270 bp fragment from a *ham-1*(n1438) template, revealing a 138 bp deletion in *ham-1*(n1438) mutants. For other lesions, the PCR primers ham-g1 (5’ TCTTGTCTTCTCTCTCCTCGC 3’) and ham-g2 (5’ AGACAACGTTTGTAGGGTG 3’) were used to amplify genomic DNA from *ham-1* mutants. The sequencing primers hm8 (5’ CGGAAGTGCATCTGTCA 3’) and hm28 (5’ CGAGATCGACCATGTGACA 3’) detected the 138 bp deletion in *ham-1*(n1810), *ham-1*(n1811), *ham-1*(gm267), and *ham-1*(gm279) lesions. The primer hm16 (5’ ATTCGTATGTTGTTGG 3’) detected the *ham-1*(gm214) lesion.

***Ham-1* antibodies and immunostaining**

*HAM-1* antisera were obtained from rats and rabbits injected with the N-terminal half of *HAM-1* as described previously (Guenther and Garriga, 1996). Antisera were affinity purified using an MBP-*HAM-1* fusion generated from the vector pMALc. Mixe stage wild-type and *ham-1* embryos were immunostained as described (Guenther and Garriga, 1996).

**Microscopy**

Embryos were mounted on 5% agar pads in 2 μL of M9 and examined on a Zeiss Axioskop compound microscope. Images were captured with a Hamamatsu ORCA-ER digital camera and formatted using Adobe Photoshop.

We used anti-serotonin staining to detect HSNs in adult hermaphrodites (Garriga et al., 1993), anti-GABA staining to detect both the PHA and PHB plasmid neurons (Troemel et al., 1995), the *nlp-1::gfp* reporter gmeEx285 to...
detect PHBs (Li et al., 1999), the flp-15::gfp reporter yns45 to
detect the PHAs and I2 neurons (Li et al., 1999), and the nmr-
1::gfp reporter aks7 to detect the AVA and RIM neurons
(Brockie et al., 2001). We followed the divisions of the HSN/
PHB neuroblasts by Nomarski optics and by GFP fluorescence
using the hlh-14::gfp reporter gmIs20 (Frank et al., 2003).
Embryonic cells in the AVA/OLQsoV and the CEPso
cell lineages were identified by Nomarski optics.

Additional cells that were not affected by ham-1 mutations
were: the GABAergic neurons AVL, DVB, DD and VD; the
FMRFamideergic neurons VC and ALA; the serotonergic
neurons ADF, NSM, RIG, RIH and I5; the dopaminergic
neuron PDE; the flp-15::gfp-expressing neuron PHA; the
sra-6::gfp-expressing neurons PVQ and ASH; the nmr-
1::gfp-expressing neurons PVQ and ASH; the
che-23::gfp-expressing neuron CAN; and the mec-3::lacZ-
expressing neurons ALM and A/PVM. Taken together, only
four of these neurons (A/PVM, NSM, PVD and PVQ) were
derived from four lineages that contained an apoptopic cell
that was either a sister or an aunt of the neuron scored. The
other 18 unaffected neurons were derived from 18 lineages
that lacked such a closely related apoptotic cell. Thus, of the
13 lineages that contained an apoptotic cell and where we
scored the fate of a resulting neuron, nine were defective. The
Ham-1 phenotype of the affected lineages can be explained
by the apoptotic cell surviving and adopting the fate of its
sister cell. By contrast, of the 18 lineages that lacked an
apoptotic cell and where we scored the fate of a neuron in the
lineage, ham-1 mutations had no obvious effect.

Results and discussion

HAM-1 is a novel protein with distinct functional regions

We cloned ham-1 by mapping it relative to polymor-
phisms and rescuing ham-1 mutants with genomic DNA
clones (see Materials and methods). In the C. elegans
genome database, ham-1 is identified as F53B2.6. HAM-1
is a 414-amino-acid protein with few homologs and no
previously characterized domains (Fig. 1). Its closest homolog is *C. briggsae* HAM-1 (76% identical). An N-terminal stretch of HAM-1 (residues 15–170) is similar to *D. melanogaster* Knockout (Fig. 1), a protein involved in the innervation of larval muscles (Hartmann et al., 1997). This conserved stretch of HAM-1 is also similar to human and mouse sequences of unknown function (Fig. 1).

ham-1 was initially defined by the alleles n1438, n1810, and n1811 based on an HSN migration phenotype (HSN abnormal migration) (Desai et al., 1988). We later demonstrated that ham-1 mutations disrupt several asymmetric neuroblast divisions, including the ABpl/rapppap (HSN/PHB neuroblast) division (Guenther and Garriga, 1996). In wild-type embryos, the HSN/PHB neuroblast divides asymmetrically, generating a smaller anterior daughter that undergoes programmed cell death and a larger posterior daughter, the HSN/PHB precursor. The precursor then divides to produce the HSN motor neuron and the PHB sensory neuron (Sulston et al., 1983). In ham-1 mutants, the anterior daughter can inappropriately survive, resulting in extra HSNs and PHBs (Guenther and Garriga, 1996). We isolated three ham-1 alleles, gm214, gm267 and gm279, in a screen for mutants with extra PHB neurons (Materials and methods; Fig. 1). n1438 and gm214 are deletion alleles that remove 5' regulatory and 3' coding sequences, respectively. n1810, n1811 and n267 are missense mutations in the conserved N-terminal domain. gm279 is a nonsense mutation in codon 62 (Fig. 1).

Each ham-1 allele reduces or eliminates gene function. All six mutations are recessive, generate similar phenotypes, and fail to complement one another (data not shown). Hemizygous ham-1/sDf22 and homozygous sDf22 animals have the same phenotype as homozygous ham-1 mutants (Guenther and Garriga, 1996). sDf22 is a deficiency that removes ham-1 and flanking genes (Clark et al., 1988). Finally, ham-1(n1438) and ham-1(gm279) embryos, respectively, have severely reduced or no detectable HAM-1 protein (see below).

We stained the ham-1 mutants with anti-HAM-1 antibodies to determine the effects of each mutation on HAM-1 protein expression and distribution. Prior immunostaining revealed that HAM-1 localizes to the cell periphery and is distributed asymmetrically in some embryonic cells, including the HSN/PHB neuroblast (Figs. 2A and B) (Guenther and Garriga, 1996). Two ham-1 mutations, gm279 and n1438, eliminate or strongly reduce HAM-1 expression. The ham-1(gm279) nonsense mutation eliminates all anti-HAM-1 staining in embryos (Fig. 2C), and no HAM-1 is detected on Western blots of ham-1(gm279) embryo extracts (Fig. 2G). ham-1(n1438) animals produce almost no HAM-

Fig. 2. HAM-1 protein expression in wild-type and ham-1 embryos. Anti-HAM-1-stained embryos (A, C, E) and corresponding DAPI images to detect DNA (B, D, F). Anterior is to the left. (A) HAM-1 expression in a wild-type embryo. HAM-1 is at the cortex of many cells, sometimes in an asymmetric crescent and sometimes in a ring (Guenther and Garriga, 1996). Scale bar, 5 μm. (B) When HAM-1 (open arrow) is asymmetric, chromosomes (closed arrow) appear condensed, indicating that cells are mitotic. (C) Anti-HAM-1 staining of a ham-1(gm279) mutant. No HAM-1 is visible. (D) DAPI image of the ham-1(gm279) mutant embryo in panel C verifies that this embryo is at a stage in development when HAM-1 is normally detected. (E) Anti-HAM-1 staining in a ham-1(n1811) mutant. In ham-1(n1810) and ham-1(n1811) mutants, HAM-1 protein is mislocalized to the cytoplasm (arrow). (F) DAPI image of the ham-1(n1811) mutant embryo in panel E. Scale bar, 5 μm. (G) Anti-HAM-1 probed Western blot. Lanes on the corresponding protein gel were run using wild-type and ham-1(gm279) embryo extracts. HAM-1 is detected in the wild-type lane (wt), but not in the ham-1(gm279) lane.
HAM-1 protein (Guenther and Garriga, 1996). Northern analysis confirms that little ham-1 mRNA is produced in ham-1(n1438) animals (data not shown). By contrast, HAM-1 is expressed in ham-1(n1810) and ham-1(n1811) mutants, but anti-HAM-1 staining showed that the mutant HAM-1(G47D) protein made in these embryos is localized inappropriately to the cytoplasm (Fig. 2E). ham-1(gm214) and ham-1(gm267) embryos have normal HAM-1 distribution (data not shown).

The cytoplasmic localization of HAM-1(G47D) in n1810 and n1811 mutant embryos suggests that the glycine at residue 47 is important for the cortical association of HAM-1. Notably, this glycine is conserved (Fig. 1C). Other HAM-1 regions are important for its function, independent of its localization, as evidenced by normal HAM-1 localization in ham-1(gm214) and ham-1(gm267) mutants.

Large apoptotic corpses in ham-1 mutants predict an altered division plane

In wild-type embryos, the HSN/PHB neuroblast generates an anterior daughter cell that undergoes programmed cell death and a larger posterior daughter, the HSN/PHB precursor (Fig. 3A) (Sulston et al., 1983). Sometimes, the anterior daughter does not die in ham-1 mutants; instead, it becomes an extra HSN/PHB neuronal precursor (Guenther and Garriga, 1996). When the cell does die in ham-1 mutants, the cell death corpse is aberrantly large (Fig. 3B).

We wondered if this corpse appeared large in ham-1 mutants because HAM-1 influences the position of the cleavage plane of some cell divisions. Perhaps, in the absence of ham-1 function, an abnormally positioned division plane position can lead to abnormally sized daughter cells, and therefore, to corpses that are larger than normal.

To test this possibility, we utilized hlh-14::gfp, a reporter that expresses GFP in several neuroblasts, including the HSN/PHB neuroblast and its two daughters (Frank et al., 2003). In wild-type hlh-14::gfp embryos, the HSN/PHB neuroblast always divided to produce two unequally sized daughters. The anterior daughter, which normally dies, was smaller than its posterior sister, the HSN/PHB precursor. This size difference was readily detectable by GFP fluorescence (Figs. 4A, B, and C). In the absence of HAM-1 function, however, the sizes of the daughter cells were reversed; the anterior daughter cell was usually larger than its posterior sister (Figs. 4D, E and F).

To quantify size differences, we measured nuclear diameters using hlh-14::gfp (Figs. 4C and F). The nucleus occupies a large majority of the cell volume at this stage of C. elegans embryonic development. Therefore, the size of the nucleus corresponds to the size of the cell. In wild-type embryos, the anterior daughter nucleus was approximately 75% the diameter of the posterior daughter nucleus (Fig. 4E). By contrast, in ham-1 embryos, this figure ranged from 97% in mutants carrying the weak loss-of-function allele ham-1(gm267) to 116% in strong loss-of-function mutants (Fig. 4G). We note that ham-1(gm267) is the weakest ham-1 allele both by this assay and by the number of extra neurons produced (Table 1).

The HSN/PHB neuroblast daughters do not switch fates in ham-1 mutants

Because the relative sizes of the anterior and posterior HSN/PHB neuroblast daughters were reversed in ham-1 mutants, we wondered whether their fates were also reversed. In particular, we wondered whether the posterior daughter, instead of the anterior daughter, died.

To test this possibility, the HSN/PHB neuroblast lineages of hlh-14::gfp; ham-1(gm279) embryos were examined. When one of the two HSN/PHB neuroblast daughters died, it was almost always the anterior daughter (7/8 cases). In these seven divisions, the anterior daughter nucleus was 114% the size of the posterior daughter nucleus, which is not different than the 115% scored for hlh-14::gfp; ham-1(gm279) when the fates of the daughter cells were not followed. Therefore, even though the anterior and posterior HSN/PHB neuroblast daughters showed size reversals in ham-1 mutants, they did not appear to swap cell death/cell survival fates with great frequency. It is possible, however,
that a smaller size may influence the posterior HSN/PHB neuroblast daughter fate, as the single exception indicates. Such posterior daughter death may account for occasional missing HSNs and PHBs in ham-1 mutants (Guenther and Garriga, 1996).

**ham-1 mutations disrupt additional neuroblast divisions that generate apoptotic cells**

We wondered whether the Ham-1 large-corpse phenotype occurred in other lineages affected by ham-1 mutations. While examining the HSN/PHB neuroblast lineage, we noticed that ham-1 mutant embryos have other large corpses, including one in the AVA/OLQsoV lineage (Fig. 3B). A neuroblast in this lineage produces a cell fated to die and a neural precursor that will divide to produce the AVA interneuron and a socket support cell for the OLQ sensory neuron (Sulston et al., 1983). If large corpses reflect defects in asymmetric cell division, ham-1 mutants might have AVA neuron defects. To examine this possibility, we utilized nmr-1::gfp, a reporter that expresses GFP in the AVA neurons (Brockie et al., 2001). Consistent with our hypothesis, nmr-1::gfp; ham-1 animals had extra AVA-like neurons (Table 1). We demonstrated previously that ham-1 mutants have extra neurons derived from the RID, ADL, ADE/ADA, PLM/ALN, and HSN/PHB lineages (Guenther and Garriga, 1996) (Fig. 5). With GFP reporter constructs and antibodies, we examined other neuronal lineages that generate cells that undergo programmed cell death (Fig. 5).
then specifically function in asymmetric cell divisions that generate apoptotic cells? Using a variety of cell markers to identify affected lineages, we have assayed 18 lineages that did not produce an apoptotic cell and found that none of these lineages produced extra or missing cells in ham-1 mutants (Fig. 5; Materials and methods). By contrast, we assayed the fates of neurons from 14 lineages that produced an apoptotic cell and found that nine or ten produced extra neurons in ham-1 mutants (Fig. 5; Materials and methods). Thus, while HAM-1 does not appear to regulate all asymmetric divisions that produce apoptotic cells, it appears to be specific for divisions that produce apoptotic cells in the lineages that we have assayed.

Could ham-1, therefore, simply act to promote the apoptosis of specific cells? Using mutations in the caspase gene ced-3, we previously showed that HAM-1 does not behave like a protein involved only in apoptosis (Guenther and Garriga, 1996). Strong ced-3 loss-of-function mutations abolish all programmed cell deaths in C. elegans (Ellis and Horvitz, 1986; Yuan et al., 1993). However, the surviving anterior daughter of the HSN/PHB neuroblast only rarely adopts an HSN- or PHB-like fate in ced-3 mutant embryos (Guenther and Garriga, 1996). Furthermore, ham-1 ced-3 double mutants showed a striking increase in the number of extra neurons over ham-1 or ced-3 mutants alone (Guenther and Garriga, 1996) (Table 1). Thus, when the anterior HSN/PHB neuroblast daughter dies in ham-1 mutants, it apparently has the potency to produce extra neurons, but this potency is masked by the fate of apoptosis. As with the HSN/PHB lineage, the extra-neuron phenotypes that we have observed in other lineages are also enhanced by the loss of ced-3 (data not shown).

**Division plane position and cell fate**

What mechanisms regulate asymmetric cell division in HAM-1-dependent neuroblast lineages? Three classes of asymmetrically localized molecules have been implicated in asymmetric divisions: cell-fate determinants, molecules that distribute these determinants, and molecules that regulate both the distribution of cell-fate determinants and the orientation of the mitotic spindle. Because HAM-1 controls both cell fate and position of the cleavage plane, its function may be similar to that of the third class of molecules.

Of the proteins known to affect both asymmetric cell division and spindle position, some of the best characterized form the conserved PAR-3/PAR-6/PKC-3 complex (Kemphues et al., 1988; Tabuse et al., 1998; Watts et al., 1996). One function of this complex in C. elegans is to establish size asymmetry between AB and P1, the daughters of the C. elegans zygote. In wild-type embryos, the anterior daughter AB is larger than the posterior daughter P1, because the spindle is positioned posteriorly in the zygote. In the absence of par-3, par-6, or pkc-3 function, AB and P1 are the same size because the spindle is not positioned asymmetrically.
In *Drosophila*, the homologous Baz(PAR-3)/DmPAR-6(PAR-6)/aPKC(PKC-3) complex is required to establish apical localization of the proteins Insc and Pins in CNS neuroblasts (Schober et al., 1999; Wodarz et al., 1999). Insc forms a crescent at the apical cortex and is needed for neuroblasts to divide in an apical/basal orientation (Kaltschmidt et al., 2000; Kraut and Campos-Ortega, 1996; Kraut et al., 1996). In the absence of Insc, neuroblasts divide in randomized orientations. Mammals also have homologs of PAR-3, PAR-6, and PKC-3, and these homologs are localized apically at tight junctions in epithelial cells. As one might expect, they influence epithelial polarity (Gao et al., 2002; Hirose et al., 2002; Suzuki et al., 2002; Suzuki et al., 2001; Yamanaka et al., 2001).

Like the PAR proteins, HAM-1 is localized asymmetrically in dividing cells and appears to position the cleavage

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**Fig. 5.** Lineages that require HAM-1 function. The markers used to detect the cells described here are discussed in the Materials and methods section. Most of the lineages shown are bilaterally symmetric, and for most lineages, we have not labeled both bilaterally symmetric neurons in the diagrams. There are both left and right HSN and PHB neurons, for example, but we have labeled the neurons simply as HSN and PHB. When left and right neurons are labeled, it is because there are additional cells of this type. For example, there are four RME neurons: RMEL/R, RMEV and RMED. Lineages affected by *ham-1* mutations: The cells that were found to be abnormal in *ham-1* mutants are indicated in a large, green font. We previously identified five lineages (top line) that were affected by *ham-1* mutations. In this study, we identified three additional lineages that were affected by scoring the presence of extra neurons (middle line). We also found extra RME neurons in *ham-1* mutants, but could not determine whether the RMEV, RMEL/R or both lineages were affected (bottom line), so both lineages are shown. EC is our abbreviation for the excretory cell. Finally, we found that the dying aunt of the CEP socket cell is unusually large, consistent with a role for *ham-1* in this lineage. Lineages unaffected by *ham-1* mutations: The cells assayed are indicated in red type. The top line shows unaffected lineages that contain an apoptotic cell. Some neurons are generated by more than one lineage. The VD neurons, for example, are generated by three distinct lineages. apV and reV are our abbreviations for the ventral arc post V and rectal epithelial V cells, respectively.

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plane and, possibly as a consequence, to control the fates of the daughter cells. However, Ham-1 and Par phenotypes are distinct. In the HSN/PHB lineage, null ham-1 mutations do not cause a symmetric division of the neuroblast as do the par mutations in the zygote. Instead, the division is still asymmetric in size, but reversed. The presence of many large cell corpses in ham-1 embryos suggests that this cleavage phenotype is shared by many of the affected neuroblast divisions.

Our data suggest a model in which HAM-1 localizes to the posterior pole of the HSN/PHB neuroblast and restricts the mitotic spindle to the anterior side of the cell (Fig. 6). This asymmetric spindle position causes an anterior bias of the cleavage plane, generating a smaller anterior daughter and a larger posterior daughter. The reversal of the HSN/PHB neuroblast cleavage plane in ham-1 mutants suggests that a second activity of unknown origin antagonizes the activity of HAM-1. In the absence of HAM-1, this second activity pushes the spindle to the posterior of the neuroblast (Fig. 6).

It is uncertain how HAM-1 regulates the spindle in neuroblasts. In the C. elegans zygote, heterotrimeric G-proteins appear to translate spatial information provided by the PARs to direct spindle position (Colombo et al., 2003; Gotta and Ahringer, 2001; Labbe et al., 2003). These G-proteins may signal through molecules that generate forces at the cortex and help position spindles, like dynein or microfilaments. As with HAM-1, a precise mechanism of how the PARs position the spindle is not yet clear.

Two general models can explain HAM-1’s role in regulating cell fate. In one model, the cleavage plane position in neuroblasts would influence the inheritance of developmental potential. HAM-1’s positioning of the cleavage plane affects the sizes of neuroblast daughters and, possibly, the inheritance of cell-fate determinants that are distributed asymmetrically by a HAM-1-independent mechanism. Posterior displacement of the cleavage plane in ham-1 mutants could result in the inheritance by the anterior daughter of some of these determinants, allowing it to bypass its normal cell death fate and generate neurons instead. Studies of asymmetric divisions in the alga Volvox carteri indicate that the size of a daughter cell has a direct bearing on its fate (Kirk et al., 1993). Yet, while cell size might contribute to the fates of the HSN/PHB neuroblast daughter cells, it is not sufficient to specify the apoptotic fate. When a cell dies in the lineage, it is usually the anterior daughter even though it is the smaller daughter in wild-type embryos and the larger daughter in ham-1 embryos. This observation favors another model in which HAM-1 distributes cell-fate determinants to daughter cells and independently regulates the position of the cleavage plane.

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