Summary

Achaete-Scute basic helix-loop-helix (bHLH) proteins promote neurogenesis during metazoan development. In this study, we characterize a C. elegans Achaete-Scute homolog, HLH-14. We find that a number of neuroblasts express HLH-14 in the C. elegans embryo, including the PVQ/HSN/PHB neuroblast, a cell that generates the PVQ neuron. HLH-14 promotes all three classes of neuron. The fact that HLH-14 promotes all three classes of neuron indicates that C. elegans proneural bHLH factors may act less specifically than their fly and mammalian homologs. Furthermore, neural loss in hlh-14 mutants results from a defect in an asymmetric cell division: the PVQ/HSN/PHB neuroblast inappropriately assumes characteristics of its sister cell, the hyp7/T blast cell. We argue that bHLH proteins, which control various aspects of metazoan development, can control cell fate choices in C. elegans by regulating asymmetric cell divisions. Finally, a reduction in the function of hlh-2, which encodes the C. elegans E/Daughterless bHLH homolog, results in similar neuron loss as hlh-14 mutants and enhances the effects of partially reducing hlh-14 function. We propose that HLH-14 and HLH-2 act together to specify neuroblast lineages and promote neuronal fate.

Key words: Caenorhabditis elegans, HLH-14, HLH-2, bHLH, Proneural, Neuroblast

Introduction

Basic helix-loop-helix (bHLH) transcription factors play integral roles in a number of developmental processes, including neurogenesis. Neural bHLH proteins have been characterized in organisms as diverse as nematodes, flies and vertebrates. These factors can assume a variety of tasks, depending upon when and where they are expressed. For example, proneural bHLH factors are expressed early in neurogenesis and are both necessary and sufficient to promote neuroblast lineages. By contrast, neuronal differentiation bHLH factors are expressed later, in neuronal precursors or postmitotic neurons, and help to fine tune neuronal fates (reviewed by Bertrand et al., 2002).

The first proneural genes identified were the Achaete-Scute (A-S) Complex genes in Drosophila. Genes in this family includeachaete (ac), scute (sc), lethal of scute (lsc) and asense (ase), and are required for external sense organ development (Garcia-Bellido, 1979; Gonzalez et al., 1989; Villares and Cabrera, 1987). Later work in Drosophila identified atonal (ato), the founding member of another proneural bHLH gene family, as crucial for the development of internal sense organs, the chordotonal organs (Jarman et al., 1993). Other fly Atonal family members are also involved in sense organ development and include absent of MD neurons and olfactory sensilla (amos) and cousin of atonal (cato) (Goulding et al., 2000a; Goulding et al., 2000b; Huang et al., 2000).

Proneural genes can act as developmental switches that control neural fate. In general, proneural gene expression promotes the generation of neuroblasts at the expense of adjacent cell types, like epidermal cells. Flies lacking ac and sc function, for example, are missing most of their mechanosensory and chemosensory organs (Bertrand et al., 2002; Garcia-Bellido, 1979) because of a failure to select sensory organ progenitors from the ectoderm. Ectopic expression of A-S genes in the ectoderm can induce the development of ectopic sensory organs at the expense of dermal cells (Domínguez and Campuzano, 1993; Rodríguez et al., 1990). Similarly, flies lacking ato gene function are missing their chordotonal sensory organs, and ectopic expression of Atonal family members promotes the formation of extra chordotonal organs (Chien et al., 1996; Goulding et al., 2000b; Huang et al., 2000; Jarman et al., 1993).

In vertebrates, members of the A-S, Atonal and Neurogenin (Ngn) bHLH families all exhibit proneural characteristics. Again, specification of neuroblast cell fate is a crucial function of these factors. For example, the A-S protein Ash1 is present in most, if not all, vertebrates (Allende and Weinberg, 1994; Ball et al., 1993; Ferreiro et al., 1993; Frowein et al., 2002; Verma-Kurvari et al., 1996). Mice lacking functional Mash1 (mouse Ash1) have neurogenesis defects in the ventral telencephalon and olfactory sensory epithelium (Casarosa et al., 1999; Guillemot et al., 1993; Horton et al., 1999). These defects are correlated with an absence of progenitor cells, consistent with Mash1 promoting neurogenesis.

C. elegans, like Drosophila and vertebrates, has a number
of neural bHLH proteins. To date, the most extensively characterized is LIN-32, the sole C. elegans Atonal family member (Ledent et al., 2002; Zhao and Emmons, 1995). Reminiscent of Drosophila atonal mutants, lin-32 mutants lack some sensory organs (Portman and Emmons, 2000; Zhao and Emmons, 1995). In particular, lin-32 mutant males lack rays, peripheral sensory organs of the male tail that are important for sensing hermaphrodites during mating (Zhao and Emmons, 1995). Ectopic expression of LIN-32 under a ubiquitous heat-shock promoter is sufficient to generate ectopic ray papillae structures (Zhao and Emmons, 1995).

bHLH proteins usually activate transcription of target genes as heterodimers with members of the E/Daughterless (DA) bHLH family (Cabrera and Alonso, 1991; Johnson et al., 1992; Massari and Murre, 2000). Heterodimer formation is mediated by the helices of the bHLH proteins, while the basic regions are important for binding to DNA sequences with an E-box motif, CANNTG. In C. elegans, both LIN-32 and the A-S protein HLH-3 can bind to the C. elegans E/DA homolog, HLH-2, in the presence of E-box motifs (Krause et al., 1997; Portman and Emmons, 2000; Thellmann et al., 2003). Both LIN-32 and HLH-3 are expressed in many of the same neuronal lineages as HLH-2 and probably require heterodimerization for the proper execution of some of these lineages (Krause et al., 1997; Portman and Emmons, 2000; Thellmann et al., 2003).

In this study, we characterize a new C. elegans A-S family member, HLH-14. We find that hlh-14 function is required for the production of specific neurons, notably three lineally related neurons: the PVQ interneuron, the HSN motoneuron, and the PHB sensory neuron. Like other A-S factors, HLH-14 is expressed in neuronal precursors and has proneural characteristics. Yet HLH-14 does not have a strictly proneural role; it appears to act in neuronal differentiation as well. Additionally, genetic data suggest that hlh-14 and hlh-2 act together in neurogenesis.

Surprisingly, we find that loss of hlh-14 function causes an asymmetric cell division defect. Specifically, in hlh-14 mutants, the PVQ/HSN/PHB neuroblast appears to assume characteristics of its sister cell, the hyp7/T blast cell. Taken together with previous studies in nematodes, we propose that HLH-14 functions together with previous studies in nematodes, we propose that HLH-14 functions together in neurogenesis. Additionally, genetic data suggest that hlh-14 and hlh-2 act together in neurogenesis.

Materials and methods

C. elegans strains

Nematodes were maintained as previously described (Brenner, 1974). Strains were kept at 20°C unless otherwise noted. This study uses standard C. elegans nomenclature (Horvitz et al., 1979). The wild-type strain N2 was used unless otherwise noted.

Strains with the following mutant alleles, chromosomal aberrations or transgenic arrays were used in this work. Unreferenced strains were generated in the course of this study.

Linkage Group (LG) I

unc-13(e51) (Brenner, 1974), hlh-2(bx115) (Portman and Emmons, 2000), yns45[fp-15::gfp] (Li et al., 1999), na4s11[osm-10::gfp] (Hart et al., 1999) and kyls39[sla-6::gfp] (Troemel et al., 1995).

LG II


LG III

gmls12[stb-6::gfp] (N. Hawkins, personal communication) (Troemel et al., 1995), gmls21[nlp-1::gfp] (Li et al., 1999).

LG IV

kyls179[unc-86::gfp] (Gitai et al., 2003), ham-1(n1811) (Desai et al., 1998; Guenther and Garriga, 1996), ced-3(n717) (Ellis and Horvitz, 1986).

LG V

gmls22[nlp-1::gfp] (Li et al., 1999).

Extrachromosomal arrays

gmxs281[hlh-14::gfp], le8x87[C50B6.8::gfp] (Mounsey et al., 2002).

Isolation of hlh-14 mutants and cloning of hlh-14

hlh-14(gm34) was isolated in a genetic screen for mutants with missing or misplaced HSN motoneurons (G.G., unpublished), and hlh-14(ju243) was isolated in a screen for mutants with morphological defects (W.-M. Woo and A. Chisholm, personal communication). As the two alleles displayed similar defects and mapped to the same region of LG II, a complementation test was performed, confirming that they are allelic.

hlh-14(gm34) was genetically mapped between bli-2 and lin-4 on LG II. From worms of the parental genotype hlh-14 (gm34) bli-2(st1016) lin-4(e912), 4/11 Bli nonLin recombinant progeny segregated the hlh-14(gm34) mutation. Corroborating this map position, the deficiency madf4 fails to complement hlh-14(gm34), but the deficiency ccDf5 complements hlh-14(gm34). Few C. elegans cosmide clones are in the region corresponding to these mapping data. Rescue of Hlh-14 phenotypes was achieved with injection of two of the cosmids in this region, F22C7 and C18A3, both of which contain the hlh-14-coding region, C18A3.8.

Detection of hlh-14 mutant lesions

To detect lesions in hlh-14 mutants, we PCR amplified the genomic region of hlh-14 from mutant genomic DNA and sequenced the amplicons. To obtain mutant genomic DNA, we picked about five mutant worms into the cap of a PCR tube containing 10 μl of lysis buffer (10 mM Tris pH 8.2, 50 mM KCl, 2.5 mM MgCl2, 0.45% Tween 20, 0.05% gelatin). The tubes were briefly centrifuged to bring down the lysis buffer and worms and then placed on dry ice for 10 minutes. The tubes were thawed at room temperature and then placed in the PCR machine for a lysis reaction (60 minutes at 60°C, followed by 15 minutes at 95°C). Lysate (3 μl) was used as a genomic DNA template for PCR reactions.

To amplify the hlh-14-coding region for sequencing, the primers C18A3.8-L1 (5’ AGACAATTCGAAAATTGGGAGG 3’) and C18A3.8-R1 (5’ GCTAATTGACTTCTCGCGC 3’) were used. The resulting 5.9 kb product was used as a template to reamplify the region with the primers C18A3.8-L2 (5’ TACATCCGCCTCGAAGTTGG 3’) and C18A3.8-R2 (5’ TTGTATGGGGAGGAGATGTC 3’), yielding a 5.2 kb product.

To sequence the hlh-14-coding region, the primers bg1-s1 (5’ ATACCTCCACCATTTGG 3’), bg1-s2 (5’ CACCACGCTCTT-
In this way, the integrated array individual plates and allowed to produce F2 progeny; two or three F2 extrachromosomal array line gmEx281 were found among the transgenic progeny, establishing the reporter, different GFP reporters. The in adult worms using the serotonin staining procedure as described at a concentration of 50 ng/m. The worms were irradiated in a UV Stratalinker at a strength of 250 gmEx281 animals were backcrossed to wild-type animals, and the promoter and coding regions of hlh-14 (5¢ GCTAATGTTGAGGAA- AGGCGGG 3¢) were used to amplify the promoter and coding regions of hlh-14, using the cosmid F2C27 as a template. The resulting 8.4 kb genomic product and the GFP vector pPD95.77 (A. Fire, S. Xu, J. Ahmn and G. Seydoux, unpublished) were double digested with Sall and BamHI and ligated together. The resulting construct was co-injected into hlh-14::gfp animals with the pRF4 plasmid (Mello et al., 1991) at a concentration of 50 ng/µl with the pRF4 plasmid (Mello et al., 1991) at a concentration of 50 ng/µl. Rescued hlh-14::gfp transgenes were found among the transgenic progeny, establishing the extrachromosomal array line gmEx281. Rescued animals were identified by the absence of the mnl1 GFP balancer, the presence of the array (pRF4-bearing Rol progeny), and the absence of hlh-14 morphological defects. Prior to array integration, hlh-14::gfp; gmEx281 animals were backcrossed to wild-type animals, and gmEx281 was recovered in a wild-type background.

The extrachromosomal array gmEx281 was integrated into the genome by UV irradiation. L4 stage gmEx281 worms were washed four times with M9 and placed on a NGM agar plate without bacteria. The worms were irradiated in a UV Stratalinker at a strenght of 250 µJx100 and allowed to recover on bacteria at 15°C overnight and lay eggs the next day. Approximately 150 F1 progeny were cloned to individual plates and allowed to produce F2 progeny; two or three F2 animals per F1 plate were cloned to new individual plates. F3 progeny were then scored for 100% transgenic animals (pRF4 Rol phenotype). In this way, the integrated array gmIs20 was generated.

Detection and analysis of specific neurons

The HSN neurons were detected in larvae using the unc-86::gfp reporter, kyIs179 (Giti et al., 2003). The HSN neurons were detected in adult worms using the serotonin staining procedure as described (Garriga et al., 1993). The PHB neurons were detected with two different GFP reporters. The srb-6::gfp reporter (Troemel et al., 1995), which was integrated onto LG III to form the strain gmIs12 (N. Hawkins, personal communication), detected both the PHA and PHB phasmid neurons. The nlp-1::gfp reporter (Li et al., 1999) gmEx285 was integrated onto LG III and LG V to form the arrays gmIs21 and gmIs22, respectively; these arrays specifically detected the PHB neurons. The PHA neurons were detected using the flyp-15::gfp reporter ynlIs45 (Li et al., 1999) and the osm-10::gfp reporter nldIs11 (Hart et al., 1999) (PHA-specific in adults). The PQV neurons were detected using the sra-6::gfp reporter kyIs39 (Troemel et al., 1995).

All neurons were visualized using a Zeiss Axioskop compound microscope. Some images were captured using Elite Chrome 100 color film (Kodak) and developed into slides. Other images were captured with a Hamamatsu ORCA-ER digital camera and saved as OpenLab files. Images were formatted using Adobe Photoshop.

Lineage analysis of living embryos

Lineage analysis was performed for two experiments: determining that hlh-14::gfp is expressed in the PQV/PHB neuroblast lineage; and determining the cell lineage defect of hlh-14 (gm34) embryos. For both experiments, embryos were placed on 5% agar pads in 2 µl of M9 buffer and examined on a Zeiss Axioskop compound microscope by Nomarski optics. Specific cells were identified relative to nearby landmark cell deaths (Sulston et al., 1983). In the analysis of hlh-14 (gm34) embryos, lineaging began at the four-cell stage and was followed for only one of the two bilaterally symmetric lineages. Lineages were observed until the comma stage of development.

RNA interference and analyses

RNA interference experiments were performed on two genes, hlh-14 and hlh-2. hlh-14 sequences were amplified from C. elegans cDNA library template (see 5’ and 3’ RACE Methods) using the primers B1-LHIS1 (5’ GAATCTGCAGCAAATGGGTGCTGTCG 3’) and B1-GSPR3 (5’ GCTAATGTTGAGGAAAGCGGG 3’). The resulting amplion was used as a template to detect the 5’ end of the common hlh-14 transcripts. The primers for this second, nested reaction were Clontech’s NUP primer and BG1-LHIS1 (5’ CCTAGAAGTACACA TGA TTG 3’). To control for general effects of RNAi, we injected dsRNA molecules, we obtained cDNA phage clones from Yuji Kohara: yk394g5 (egf-1), yk73c3 (cht-3) and yk726d (cht-4). The cDNAs were excised from phage following protocols provided by Yuji Kohara. After excision, the cDNAs were in the pBluescript plasmid, which is equipped with T7 and T3 transcription sites flanking the sites of cDNA insertion. Using both the T7 and T3 sites, sense and antisense transcripts were made for each clone, according to the protocol described in Promega’s RiboMax in vitro transcription kit. Amplification and RNA integrity analyses were performed as described above.

For RNA interference, dsRNA was always injected into young adult worms at the maximum possible concentration; it was never diluted above. Annealing and RNA integrity analyses were performed as described above.

To control for general effects of RNAi, we injected dsRNA molecules of genes used in a separate study. Control RNAi injections did not phenocopy hlh-14 and hlh-2 dsRNA injections. To make control dsRNA molecules, we obtained cDNA phage clones from Yuji Kohara: yk394g5 (egf-1), yk73c3 (cht-3) and yk726d (cht-4). The cDNAs were excised from phage following protocols provided by Yuji Kohara. After excision, the cDNAs were in the pBluescript plasmid, which is equipped with T7 and T3 transcription sites flanking the sites of cDNA insertion. Using both the T7 and T3 sites, sense and antisense transcripts were made for each clone, according to the protocol described in Promega’s RiboMax in vitro transcription kit. Amplification and RNA integrity analyses were performed as described above.

For RNA interference, dsRNA was always injected into young adult worms at the maximum possible concentration; it was never diluted after the annealing reactions. Injected animals were picked to plates seeded with bacteria, allowed to lay eggs for 24 hours, and then transferred to fresh plates. Progeny laid after the first 24 hours were likely to display RNAi defects.
Results

hlh-14 cloning and characterization of mutations

hlh-14 was initially defined by two mutations, hlh-14(gm34) and hlh-14(ju243). hlh-14(gm34) was isolated in a genetic screen for mutants with missing or misplaced HSN motoneurons (G.G., unpublished), and hlh-14(ju243) was isolated in a screen for mutants with morphological defects (W.-M. Woo and A. Chisholm, personal communication). On a gross level, hlh-14 mutants have morphologically disorganized posteriors (Fig. 1B), an Unc (Uncoordinated) phenotype, and an Egl (Egg-laying defective) phenotype. Both hlh-14 alleles can be maintained in homozygous strains, but homozygotes have a high degree of larval lethality and are slow to develop. Homozygotes that escape lethality and reach adulthood have low fertility (data not shown).

We cloned hlh-14 by conventional mapping and rescue experiments (see Materials and methods). In the C. elegans genome database, hlh-14 is identified as the open reading frame C18A3.8 and encodes a basic helix-loop-helix (bHLH) protein similar to members of the A-S family (Fig. 2). We detected three different forms of hlh-14 mRNA expressed in C. elegans by performing 5’ and 3’ RACE (Fig. 2A; see Materials and methods). The three forms predict three different HLH-14 proteins, each containing a unique N terminus, but all containing the same bHLH domain and the same novel C terminus (Fig. 2B).

Both hlh-14 mutations are lesions in the bHLH domain (Fig. 2C). hlh-14(gm34) is a missense mutation, changing a conserved leucine to a phenylalanine in the N-terminal helix. hlh-14(ju243) is a nonsense mutation in the C-terminal helix, changing a glutamine codon to an ochre stop codon. Several observations suggest that both hlh-14 alleles severely reduce or eliminate gene function. First, both alleles are recessive, generate similar phenotypes and fail to complement one another (data not shown). Second, the deficiency madDf4 fails to complement both hlh-14(gm34) and hlh-14(ju243); hemizygous hlh-14/madDf4 and homozygous hlh-14 mutants display similar defects (Table 1). Third, reduction of hlh-14 function by RNA interference (RNAi) generates similar, although weaker, phenotypes as the hlh-14 mutations (Fig. 1C,D; Tables 2–4). Finally, the hlh-14 molecular lesions are predicted to reduce or eliminate hlh-14 function. The hlh-14(gm34) leucine to phenylalanine missense mutation alters a highly conserved leucine (Fig. 2C). An identical leucine to phenylalanine mutation is found in the C. elegans bHLH loss-of-function mutant lin-32(e1926) (Zhao and Emmons, 1995). The hlh-14(ju243) nonsense mutation is predicted to eliminate the C terminus of the second helix, which contains highly conserved residues (Fig. 2C).

HLH-14::GFP is expressed in the cells of the PVO/HSN/PHB neuroblast lineage

To determine which cells express HLH-14, we generated a full-length hlh-14::gfp translational fusion (see Materials and methods). hlh-14::gfp animals are rescued for the morphological defects and the larval lethality observed in hlh-14 mutants, indicating that this fusion is functional (data not shown). The hlh-14::gfp transgene expresses GFP in the nuclei of several cells in the developing embryo. Prior to morphogenesis, a horseshoe-shaped pattern of cells expresses HLH-14::GFP in the anterior embryo (data not shown). The expressing cells appear to be neuroblasts but have not been positively identified. During morphogenesis, HLH-14::GFP assumes a complicated, diverse pattern, and no HLH-14::GFP expression is detected in the cells of the PVO/HSN/PHB neuroblast lineage (see Results, Materials and methods, and Table 4).

Table 1. hlh-14 loss induces PHB neuron loss

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number of PHB neurons/side*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Wild type</td>
<td>0%</td>
</tr>
<tr>
<td>hlh-14(gm34)</td>
<td>100%</td>
</tr>
<tr>
<td>hlh-14(ju243)</td>
<td>100%</td>
</tr>
<tr>
<td>hlh-14(gm34)/madDf4</td>
<td>99%</td>
</tr>
<tr>
<td>ham-1(n1811)</td>
<td>3%</td>
</tr>
<tr>
<td>hlh-14(gm34); ham-1(n1811)</td>
<td>100%</td>
</tr>
<tr>
<td>ham-1(n1811)ced-3(n717)</td>
<td>0%</td>
</tr>
<tr>
<td>hlh-14(gm34); ham-1(n1811) ced-3(n717)</td>
<td>100%</td>
</tr>
</tbody>
</table>

*srh-6::gfp-expressing PHB neurons in the tails of L1 larvae.

srh-6::gfp is expressed in PHA and PHB. When hlh-14 mutants contained a single GFP-expressing cell/side, we scored this side as containing a single PHA, but lacking PHB (0 PHB neurons/side). Similarly, when ham-1 mutants contained an extra GFP-expressing cell/side, we scored this side as having two PHB neurons. These assumptions were confirmed using PHA- and PHB-specific markers (see Results, Materials and methods, and Table 4).
**C. elegans** HLH-14 promotes neurogenesis

**hlh-14** mutants are missing HSN motoneurons and PHB sensory neurons

In hermaphrodite larvae, a left/right bilaterally symmetric pair of HSN motoneurons is found at the middle of the animal, near the presumptive gonad. By Nomarski optics, we only rarely found the HSNs in **hlh-14** mutant worms; when we did find them, they were displaced posteriorly, having failed to complete their normal migratory routes (data not shown). We corroborated this observation using an HSN reporter, *unc-86::gfp* (Gitai et al., 2003). *unc-86* encodes a POU homeodomain protein, and this promoter fusion always expresses GFP in the HSNs of wild-type larvae (Fig. 4A). By contrast, we never saw HSNs expressing GFP in **hlh-14** (gm34); *unc-86::gfp* larvae (Fig. 4B; Table 2). Staining the HSNs with an anti-serotonin antibody (see Materials and methods) also revealed the loss of HSNs in **hlh-14** mutants (Table 2). On the rare occasions this antibody did detect the HSNs in **hlh-14** mutants, they had failed to migrate properly (data not shown). We conclude that **hlh-14** mutants are either missing their HSN neurons, or at best, they produce profoundly defective HSN neurons. In either case, **hlh-14** appears important for HSN development.

The PHB sensory neuron is the sister cell of the HSN. As **hlh-14** mutants lack HSNs, we hypothesized that they might also lack PHBs. To test this possibility, we used the reporter *srb-6::gfp*, which expresses GFP in both the PHA and PHB phasmid neurons, sensory neurons that are located in the tail. *srb-6* encodes a seven transmembrane receptor protein.
expressed in several sensory neurons (Troemel et al., 1995). Wild-type srb-6::gfp animals always had two GFP-expressing neurons on each side of the tail, one PHA and one PHB (Fig. 4C; Table 1). By contrast, hlh-14; srb-6::gfp larvae almost always had only a single GFP-expressing neuron per side, consistent with hlh-14 mutants missing their PHB neurons (Fig. 4D, Table 1). To eliminate the possibility that hlh-14 mutants lacked PHAs and not PHBs, we used more specific markers (data not shown). With the PHB-specific neuropeptide-like promoter fusion nlp-1::gfp (Li et al., 1999), we found that hlh-14::gfp is expressed in the daughters of the left HSN/PHB neuroblast (arrows analogous to H). (K,L) HLH-14::GFP is expressed in the daughters of the PHB neuroblast, the anterior PVQ neurons (closed arrows) and their posterior sisters, which are fated to die (open arrows). Scale bar: 5 μm. (M) Cell lineage depicting the PVQ/HSN/PHB neuroblast and its descendants.

**hlh-14 mutations are epistatic to mutations that disrupt the HSN/PHB neuroblast division**

hlh-14 mutants have the opposite neuronal phenotype as ham-1 mutants. ham-1 encodes a novel protein involved in executing the asymmetric divisions of neuroblasts (Guenter and Garriga, 1996). In ham-1 mutants, the HSN/PHB neuroblast divides symmetrically, inappropriately generating two HSN/PHB precursor cells, and subsequently, extra HSN and PHB neurons. This phenotype can be seen using srb-6::gfp, where PHB neuron duplications generate extra GFP-expressing phasmid neurons about 25% of the time in ham-1 mutants (Table 1). In ham-1 ced-3 double mutants, the phenotype is more striking (Table 1). Concurrent removal of the novel protein HAM-1 and the caspase CED-3, which is

### Table 2. hlh-14 loss induces HSN neuron loss

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number of HSN neurons/side</th>
<th>Genotype</th>
<th>Number of HSN neurons/side</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type adults*</td>
<td>0%</td>
<td>100%</td>
<td>100</td>
</tr>
<tr>
<td>*HSNs stained with an anti-serotonin antibody in adults.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hlh-14 (gm34) adults*</td>
<td>83%</td>
<td>17%</td>
<td>23</td>
</tr>
<tr>
<td>*HSNs expressing unc-86::gfp in L1 larvae.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hlh-14 (RNAi) adults*</td>
<td>27%</td>
<td>73%</td>
<td>180</td>
</tr>
<tr>
<td>Wild-type larvae†</td>
<td>0%</td>
<td>100%</td>
<td>66</td>
</tr>
<tr>
<td>*It is possible that additional HSN neurons did express unc-86::gfp in hlh-14 mutant larvae, but were completely migration defective. These HSNs would have been located in the tail among other neurons expressing unc-86::gfp and would not have been detected and counted. Supporting this possibility, many of the HSNs detected by anti-serotonin staining were located the tail, having failed to migrate.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hlh-14 (gm34) larvae†</td>
<td>100%†</td>
<td>0%‡</td>
<td>28</td>
</tr>
<tr>
<td>hlh-14 (RNAi) larvae†</td>
<td>45%†</td>
<td>55%‡</td>
<td>130</td>
</tr>
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</table>
necessary for normal programmed cell death (Ellis and Horvitz, 1986; Yuan et al., 1993), can increase the penetrance of extra PHB neurons to over 90% (Guenther and Garriga, 1996).

We reasoned that if hlh-14 mutations were affecting the overall ability to determine neuronal fate, hlh-14 should be epistatic to ham-1. Indeed this is the case, as hlh-14; ham-1 double mutants were always missing their PHB neurons (Table 1). To rule out the possibility that the effects we saw in hlh-14 mutants were simply due to inappropriate programmed cell death, we examined hlh-14; ham-1 ced-3 triple mutants. As with hlh-14 single mutants, these triply mutant animals were always missing their PHB neurons (Table 1). We conclude that hlh-14 mutations are epistatic to mutations that alter the HSN/PHB neuroblast division. Furthermore, hlh-14 mutants are not missing neurons because of inappropriate programmed cell death. Therefore, hlh-14 appears to be a vital factor in determining the HSN/PHB neuroblast fate, or the fate of a cell that generates the HSN/PHB neuroblast lineage. Alternatively, in hlh-14 mutants, PHB neuroblast division may simply occur at an inappropriate time.

**hlh-14 mutants are missing PVQ neurons**

Considering the HLH-14::GFP expression pattern, we hypothesized that the PVQ neurons might be missing or defective in hlh-14 mutants. Analysis of the tails of hlh-14 mutants by Nomarski optics suggested that the PVQs were missing (data not shown). To test this hypothesis directly, we used the PVQ-specific GFP reporter, sra-6::gfp (Troemel et al., 1995). Wild-type sra-6::gfp animals always had PVQ neurons expressing GFP (Fig. 4E; Table 3). By contrast, sra-6::gfp; hlh-14(gm34) animals never had PVQ neurons expressing GFP (Fig. 4F; Table 3).

**hlh-14 mutants have a cell division defect in the PVQ/HSN/PHB neuroblast lineage**

The preceding data are consistent with HLH-14 acting to promote the PVQ, HSN and PHB fates. HLH-14 could act directly in these neurons. However, as HLH-14 is an A-S family member, it could play a proneural role, acting earlier to ensure the proper execution of the entire PVQ/HSN/PHB neuroblast lineage. To address this possibility, we directly observed the cell division patterns of hlh-14(gm34) mutant embryos.

We examined two PVQ/HSN/PHB neuroblast lineages in hlh-14(gm34) embryos. In wild-type embryonic lineages (Fig. 5A), the ABpl/rappp cell divides to generate the PVQ neuroblast and the hyp7/T blast cell around 170 minutes after fertilization (Sulston et al., 1983). This division occurred at the right time in each hlh-14(gm34) mutant lineage. Approximately 230 minutes after fertilization in wild-type embryos, the PVQ/HSN/PHB neuroblast divides to generate the PVQ neuroblast and the HSN/PHB neuroblast (Fig. 5A). This division also appeared to occur normally in hlh-14(gm34) embryos. Approximately 280 minutes after fertilization in wild-type embryos, the HSN/PHB neuroblast

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number of PVQ neurons/side*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Wild type</td>
<td>0%</td>
</tr>
<tr>
<td>hlh-14(gm34)</td>
<td>100%</td>
</tr>
<tr>
<td>hlh-14(RNAi)</td>
<td>91%</td>
</tr>
</tbody>
</table>

*sra-6::gfp-expressing cells in the tails of L1 larvae.
Fig. 5. Cell lineage defects in hlh-14 mutants. (A) In wild-type embryos, the ABpl/rapp cell divides to generate the PVQ/HSN/PHB neuroblast and the hyp7/T blast cell. The PVQ/HSN/PHB neuroblast subsequently undergoes a series of divisions, ultimately generating the PVQ, HSN and PHB neurons. The hyp7/T blast cell divides only once during embryogenesis, generating a hyp7 cell and the T blast cell, a seam cell that divides postembryonically. (B) In hlh-14 mutants, the ABpl/rapp cell divides at the appropriate time. However, the presumptive PVQ/HSN/PHB neuroblast does not undergo a series of divisions to generate the PVQ, HSN and PHB neurons. Instead, it divides just once, like its sister cell, the hyp7/T blast cell. In hlh-14 mutants, it is possible that the PVQ/HSN/PHB neuroblast is transformed into a hyp7/T blast-like cell, a model shown in this lineage. (C) A wild-type L1 larva containing a C50B6.8::gfp reporter construct, which expresses GFP in the ten hypodermal seam cells, H0-H2, V1-V6 and T (Mounsey et al., 2002). (D) An hlh-14 mutant L1 larva expressing the C50B6.8::gfp reporter. In addition to expressing GFP in the ten hypodermal seam cells, this larva expresses GFP in an extra cell in the tail, presumably a T-like cell (arrow). In 38% of the of hlh-14 mutants, we observed this ectopic GFP expression (n=60 sides scored). We never observed this extra cell in wild-type animals bearing C50B6.8::gfp.

In hlh-14 mutants, the PVQ/HSN/PHB neuroblast may be transformed into its sister cell

We questioned why the HSN/PHB neuroblast and the PVQ neuroblast both failed to divide in hlh-14 mutants. They may have withdrawn from the cell cycle prematurely, or they may have inappropriately assumed the fates of other cells. Considering this latter possibility, we noted that in hlh-14 mutant embryos, the division pattern of the presumptive PVQ/HSN/PHB neuroblast was similar to the normal division pattern of its sister cell, the hyp7/T blast cell (Fig. 5A,B). We hypothesized that the PVQ/HSN/PHB neuroblast may adopt the fate of its sister cell in hlh-14 mutants. Such a cell fate transformation would signify a defect in asymmetric cell division, which is defined as the process by which sister cells adopt distinct fates (Horvitz and Herskowitz, 1992).

If the PVQ/HSN/PHB neuroblast were transformed into an extra hyp7/T blast cell, then hlh-14 mutants should have extra T cells, a type of dermal blast cell in the tail. To test whether hlh-14 mutants have extra T cells, we used a C50B6.8::gfp reporter. C50B6.8 is a C. elegans gene that is expressed in the ten hypodermal seam cells, including the T cells (Mounsey et al., 2002) (Fig. 5C). C50B6.8 encodes a protein with a domain similar to the ligand binding domains of nuclear hormone receptors (Mounsey et al., 2002). With this GFP reporter, we observed that hlh-14 mutants often have extra GFP-expressing cells in the tail, presumably extra T (or T-like) cells (Fig. 5D). This observation is consistent with the PVQ/HSN/PHB neuroblast assuming a hyp7/T blast cell fate in hlh-14 mutants. Notably, these supernumerary T-like cells are smaller than normal T cells (Fig. 5D). They are also located slightly medial to the seam cells, which is consistent with the positions of the presumptive PVQ/HSN/PHB neuroblast daughter cells in hlh-14 mutants. Collectively, our observations indicate that the PVQ/HSN/PHB neuroblasts can adopt characteristics of hyp7/T blast cells in hlh-14 mutants.

HLH-14 determines cell fate in the descendants of the PVQ/HSN/PHB neuroblasts

HLH-14 appears to determine PVQ/HSN/PHB neuroblast fate, a proneural characteristic. Yet HLH-14::GFP is expressed not only in the PVQ/HSN/PHB neuroblast but also in its
descendants. In addition to HLH-14 acting like a proneural factor, we wondered whether HLH-14 might also act like a neural differentiation bHLH factor and determine the fates of cells late in this neuroblast lineage.

To examine this possibility, we took advantage of the partial loss-of-function phenotype of *hlh-14(RNAi)*. *hlh-14(RNAi)* treatment does not cause a complete loss of PVQ, HSN or PHB neurons (Tables 2-4). Therefore, we were able to test whether the loss of one neuron type was correlated with the loss of another neuron type in *hlh-14(RNAi)* animals. A correlation between HSN loss and PVQ loss, for example, could indicate a defect in their common precursor, the PVQ/HSN/PHB neuroblast. A lack of correlation could indicate a role for HLH-14 later in the cell lineage, perhaps in the neurons themselves.

We first looked for a correlation between HSN and PHB loss in *hlh-14(RNAi)* animals. We subjected worms bearing the *nlp-1::gfp* transgene, which expresses GFP in the PHB neurons, to *hlh-14(RNAi)* treatment. We isolated F1 progeny as adults and double stained them with anti-serotonin and anti-GFP antibodies to examine the HSNs and PHBs. A large majority of the time, HSN loss (or presence) correlated with PHB loss (or presence; 69/78 sides scored correlated). Occasionally, however, one cell or the other was lost (9/78 sides scored). Additionally, several of the HSN neurons that were detected in *hlh-14(RNAi)* animals were not fully migrated, and some had axon outgrowth defects (data not shown). Together, these results indicate that in addition to determining the fates of HSN and PHB precursors, HLH-14 plays roles in determining the fates of the HSN and PHB neurons themselves.

In a similar experiment, we looked for a correlation between HSN and PVQ loss in *sra-6::gfp; hlh-14(RNAi)* worms. We found that HSN loss is not at all correlated with PVQ loss in these animals (21/54 sides scored correlated). The PVQ neurons are lost significantly more often than the HSN neurons (Tables 2, 3), accounting for most of the differences we observe. The PVQ neuroblast or PVQ neuron appear to be especially sensitive to *hlh-14* loss, by our assay. We conclude that although *hlh-14* may play a role in determining PVQ/HSN/PHB neuroblast fate, it also appears to determine the fates of the descendants of the neuroblast.

**Loss of *hlh-2* function can cause neuron loss and exacerbate *hlh-14(RNAi)* neuron loss**

Neural bHLH proteins often regulate transcription by forming heterodimers with the E/Daughterless (DA) proteins, a ubiquitously expressed family of bHLH proteins. Therefore, we wondered whether the *C. elegans* E/DA family member, HLH-2, might function with HLH-14 in the PVQ/HSN/PHB neuroblast lineage. Indeed, previous work demonstrated that HLH-2 is expressed in this lineage (Krause et al., 1997).

To date, no strong loss-of-function *hlh-2* mutants exist or have been reported, so we used RNAi treatment to see what effects *hlh-2* loss could exert on PVQ, HSN and PHB neuron development. Consistent with previous studies, *hlh-2(RNAi)* treatment caused nearly complete embryonic lethality (Kamath et al., 2003; Krause et al., 1997). However, a small percentage of animals escaped this lethality. Like *hlh-14* mutants, these *hlh-2(RNAi)* escapers had gross phenotypes, including posterior morphological defects and larval lethality (data not shown). Most relevant to our study, they often lacked PVQ, HSN and PHB neurons (Table 4).

**Table 4. *hlh-2* loss induces PVQ, HSN, and PHB neuron loss**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>PVQ*</th>
<th>HSN†</th>
<th>PHB‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td><em>hlh-2(RNAi)</em></td>
<td>25%</td>
<td>23%</td>
<td>50%</td>
</tr>
<tr>
<td><em>hlh-2(bx115)</em></td>
<td>N/D</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td><em>hlh-14(RNAi)</em></td>
<td>9%</td>
<td>55%</td>
<td>51%</td>
</tr>
<tr>
<td><em>hlh-2(bx115); hlh-14(RNAi)</em></td>
<td>N/D</td>
<td>43%</td>
<td>35%</td>
</tr>
</tbody>
</table>

* N/D, not determined. Because *hlh-2* and *kyIs39(sra-6::gfp)* both reside on LG I, we were unable to construct the strains needed to collect these data. *sra-6::gfp*-expressing cells in the tails of L1 larvae. *unc-86::gfp*-expressing HSN neurons in L1 larvae. Some HSNs may not have been counted by this assay. See Table 2 for explanation.
* nlpl-::gfp-expressing cells in the tails of L1 larvae.
† *p*<0.05 using a two-sample Z test.
‡ *p*<0.0001 using a two-sample Z test.

Partial loss-of-function *hlh-2* mutations generate no phenotypes on their own, but a prior study demonstrated that they can exacerbate the male tail ray loss phenotype of hypomorphic *lin-32* mutants; HLH-2 and LIN-32 act together to execute neuronal fates in the ray lineage (Portman and Emmons, 2000). We wondered if the mutation, *hlh-2(bx115)*, could also enhance *hlh-14(RNAi)*, which produces a partial loss-of-function phenotype (Fig. 1C,D; Tables 2-4). Although *hlh-2(bx115)* mutants have a normal number of HSN and PHB neurons, *hlh-2(bx115); hlh-14(RNAi)* animals lack more HSN and PHB neurons than *hlh-14(RNAi)* animals, showing that a weak *hlh-2* mutant can enhance partial *hlh-14* loss (Table 4).

**Discussion**

We have described a role in neuronal development for a *C. elegans* A-S bHLH family member, HLH-14. *hlh-14* mutants lack three lineally related neurons: the PVQ interneuron, the HSN motoneuron and the PHB sensory neuron. Based on GFP reporter data and cell lineage analyses, we hypothesize that these neurons are missing because their common precursor, the PVQ/HSN/PHB neuroblast, fails to differentiate properly, and instead adopts characteristics of its sister cell. It also appears that HLH-14 acts in conjunction with the *C. elegans* E/Daughterless homolog, HLH-2, in regulating neuronal fate in this cell lineage.

**hlh-14** and other Achaete-Scute family genes: similarities and differences

Aside from its conserved sequence, there are a number of similarities between *hlh-14* and previously characterized A-S genes. The most obvious similarity is that *hlh-14* mutants lack neurons. A-S family members in *Drosophila* specify external sense organs. In the absence of A-S genes, neuronal cell types needed for the function of these organs are lost (Garcia-Bellido, 1979; Gonzalez et al., 1989; Villares and Cabrera, 1987). Vertebrate A-S family members generate a wide variety of neuronal precursors, including the progenitors of the cerebral cortex and progenitors in the ventral telencephalon (Casarosa et al., 1999; Guillemot et al., 1993; Horton et al., 1999). In this study, we have clearly established that *hlh-14*...
function is required for normal PVQ, HSN, and PHB neuron development. Yet a close look at the types of neurons specified by hlh-14 reveals an important difference between hlh-14 and other A-S genes. While Drosophila and mammalian A-S genes appear to specify neuroblast lineages dedicated to generating neuronal cells of a particular type or coordinated function, hlh-14 specifies a neuroblast lineage dedicated to generating three disparate types of neuron: an interneuron (PVQ), a motoneuron (HSN) and a sensory neuron (PHB). There is no known coordinated function that these three neurons perform. Why is hlh-14 less specific than fellow A-S family members in this regard? One possibility is that C. elegans must adapt the function of neural bHLH genes such as hlh-14 in order to generate its diverse collection of 302 neurons (of 118 distinct types) out of only 959 somatic cells. Such adaptation may allow hlh-14 to specify lineally related, yet functionally distinct, collections of neurons.

A second similarity between hlh-14 and other A-S-like genes is the genetic interaction between hlh-14 and hlh-2, the C. elegans E/DA homolog. In Drosophila, heterodimers between DA and A-S family members are essential for neurogenesis (Cabrera and Alonso, 1991). In C. elegans, the A-S factor HLH-3 can bind E/DA HLH-2 in vitro, and the expression patterns of HLH-3 and HLH-2 overlap in a number of neuronal lineages (Krause et al., 1997; Thellman et al., 2003). Taken together, four facts suggest that HLH-14 and HLH-2 act together in the PVQ/HSN/PHB lineage to regulate neuronal development. First, A-S proteins, as well as other types of bHLH proteins, are known to interact physically with E/DA family members to form functional heterodimers in a number of organisms (Cabrera and Alonso, 1991; Johnson et al., 1992; Krause et al., 1998; Massari and Murre, 1999). Second, both HLH-14 and HLH-2 are expressed in the PVQ/HSN/PHB lineage (this study) (Krause et al., 1997). Third, loss of function of either gene results in the loss of neurons in this lineage. Fourth, a weak hlh-2 mutant can enhance the partial neuronal loss defects of hlh-14(RNAi).

Another similarity that HLH-14 shares with certain A-S family members is that it possesses proneural characteristics. Not only is hlh-14 necessary for neuron development, but also it is expressed early in neurogenesis. We see hlh-14::gfp expressed in the PVQ/HSN/PHB neuroblast, the first cell in this lineage solely dedicated to generating neurons.

**Individual neural bHLH factors can assume multiple roles in C. elegans**

Even though hlh-14 has proneural characteristics, a notable difference between hlh-14 and some A-S genes is that hlh-14 is not strictly proneural. Numerous pieces of evidence point to this conclusion. First, hlh-14::gfp expression is not restricted to early neuroblasts. We observe hlh-14::gfp expression in the lineal descendants of the PVQ/HSN/PHB neuroblast, including the PVQ neuron, a postmitotic cell. Second, hlh-14 mutants do not display wholesale neuron loss for all the neurons we examined. For example, the HSNs are not always lost. Furthermore, of the HSNs that are generated, many fail to migrate and project their axons properly. Finally, HSN loss is not perfectly correlated with PHB and PVQ loss in hlh-14(RNAi) animals. Collectively, these results demonstrate that hlh-14 plays important roles in the descendants of the PVQ/HSN/PHB neuroblast, not just the neuroblast itself.

There are other A-S genes that are not strictly proneural. For example, the Drosophila gene asense has proneural characteristics; its ectopic expression induces the generation of ectopic sense organs (Dominguez and Campuzano, 1993). However, unlike other proneural factors, asense is not normally expressed early in neuroblast lineages. It is expressed later, in the precursors of sensory organ cells (Dominguez and Campuzano, 1993; Gonzalez et al., 1989). Among A-S genes that have been characterized, hlh-14 appears unique in the sense that it is expressed early in a lineage to establish neuroblast fate, but nevertheless persists in subsequent generations to execute the fates of neuronal precursors and neurons.

This characteristic of hlh-14 is shared by other C. elegans neural bHLH factors. For example, the C. elegans Atonal homolog, LIN-32, both establishes ray neuroblast fate and helps execute the fates of cells at multiple steps in the ray lineage (Portman and Emmons, 2000; Zhao and Emmons, 1995). The C. elegans NeuroD bHLH homolog, CND-1, is expressed in both neuronal precursors and postmitotic neurons and appears to be important to keep these precursors from withdrawing from their lineages (Hallam et al., 2000). The only other neural bHLH that has been described in any detail in C. elegans is the A-S protein HLH-3. It has not been reported how HLH-3 promotes neurogenesis, but embryonic expression of an hlh-3::gfp transgene is extensive and shows considerable overlap with HLH-2 expression in neuronal lineages (Krause et al., 1997). Therefore, it seems plausible that HLH-3 could affect both early and late events in neuronal development.

It is unclear why some C. elegans bHLH factors act at several steps in neuronal lineages, seemingly unlike their Drosophila or vertebrate counterparts. It is not because nematodes have fewer neural bHLH proteins at their disposal; indeed, there are five A-S-like genes in C. elegans, but only four in Drosophila, and three in mouse (Ledent et al., 2002). The fact that individual C. elegans bHLH factors appear sufficient to drive both early and late events in neural development contrasts with the progressive determination model formulated from studies in Drosophila and vertebrates (Dambly-Chaudiere and Verwoert, 1998; Gnhysen and Dambly-Chaudiere, 1985; Jan and Jan, 1994). According to this model, neuronal lineages consist of cells whose fates become more restricted with each generation. A sequential cascade of bHLH factors helps drive this process, with proneural factors defining neuroblast lineages and signaling through Notch to promote the expression of differentiation bHLH factors that promote later developmental events. In C. elegans, however, a single neural bHLH protein appears capable of functioning in each of these steps, at least in some cell lineages.

**C. elegans bHLH mutations can disrupt asymmetric cell divisions**

An additional difference between hlh-14 and other A-S family members comes from analyzing Hlh-14 mutant phenotypes. The loss of neurons is expected for the loss of an A-S family member like hlh-14. What is unexpected is the inappropriate duplication of a lineally related sister cell. In hlh-14 mutants, there appear to be duplications of the hyp7/T blast cell at the
expense of its sister cell, the PVQ/HSN/PHB neuroblast. While these duplications are not completely penetrant, they do occur with reasonable frequency, as assayed by the presence of extra T-like cells in hlh-14 mutants (Fig. 5D). The incomplete penetrance may be due to incomplete or partial transformations of PVQ/HSN/PHB neuroblast fate.

Asymmetric cell division is defined as any division in which a mother cell gives rise to two daughter cells of distinct fates (Horvitz and Herskowitz, 1992). It is a fundamental mechanism employed by metazoans to generate cellular diversity. The vast majority of C. elegans non-germline cell divisions are asymmetric (Kimble and Hirsh, 1979; Sulston and Horvitz, 1977; Sulston et al., 1983). Normally, ABpl/rapp divides asymmetrically, generating the PVQ/HSN/PHB neuroblast and the hyp7/T blast cell. Our data suggest that this asymmetric division is disrupted in hlh-14 mutants because the PVQ/HSN/PHB neuroblast can be partially transformed into its sister cell.

The disruption of an asymmetric cell division is not unprecedented for the loss of a neural bHLH factor in C. elegans. This phenomenon is what one observes in the V5.pa cell lineage in lin-32 mutants and in the NSM cell lineage in hlh-3 mutants. In wild-type animals, the V5.pa cell becomes a neuroblast called the postedeird neuroblast. However, in lin-32 mutants, lineage analysis shows that V5.pa can assume the fate of its sister cell, the V5.pp hypodermal blast cell (Zhao and Emmons, 1995). In hlh-3 mutants, the sister cell of the NSM neuron can forego a programmed cell death fate and assume an NSM-like fate instead (Thellmann et al., 2003).

Why might some nematode asymmetric divisions become symmetric upon losing a bHLH factor? The answer is unclear, but it seems instructive to consider the cellular context in which neurons are generated in different organisms. In Drosophila, proneural genes are first expressed in the neuroectoderm in order to select neuronal precursors. Without proneural activity, neuroblast lineages are not selected, resulting in an absence of neuronal organs and excess dermal cells. In vertebrates, proneural genes are first expressed in neuroepithelial cells. Proneural expression induces the selection of neuronal precursors that then delaminate from the neuroepithelium and divide a finite number of times to generate neuronal cell types. Some neuroepithelial cells that are not selected to be neuroblasts delaminate and generate glia instead. Loss of proneural function can lead to a loss of neurons, but extra glia in vertebrates.

By contrast, C. elegans does not select most of its neuroblast lineages from populations of dermal cells. Instead, C. elegans works within the context of its cell lineage, which generates only 959 somatic cells in the adult (Kimble and Hirsh, 1979; Sulston and Horvitz, 1977; Sulston et al., 1983). Considering this, and considering our work, it seems possible that neural bHLH factors can act like a switch between neuroblast and ectodermal or epithelial/glia cell fates. The difference is that in flies and vertebrates cell fate is regulated spatially, while in C. elegans, cell fate may be distributed asymmetrically at mitosis. As a result, loss of a neural bHLH factor can result in the disruption of an asymmetric cell division.

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