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Developmental Biology 289 (2006) 229 – 242

DEVELOPMENTAL BIOLOGY

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The *C. elegans* histone deacetylase HDA-1 is required for cell migration and axon pathfinding

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Received for publication 1 July 2005, revised 13 October 2005, accepted 18 October 2005

Abstract

Histone proteins play integral roles in chromatin structure and function. Histones are subject to several types of posttranslational modifications, including acetylation, which can produce transcriptional activation. The converse, histone deacetylation, is mediated by histone deacetylases (HDACs) and often is associated with transcriptional silencing. We identified a new mutation, *cw2*, in the *Caenorhabditis elegans hda-1* gene, which encodes a histone deacetylase. Previous studies showed that a mutation in *hda-1*, *e1795*, or reduction of *hda-1* RNA by RNAi causes defective vulval and gonadal development leading to sterility. The *hda-1(cw2)* mutation causes defective vulval development and reduced fertility, like *hda-1(e1795)*, albeit with reduced severity. Unlike the previously reported *hda-1* mutation, *hda-1(cw2)* mutants are viable as homozygotes, although many die as embryos or larvae, and are severely uncoordinated. Strikingly, in *hda-1(cw2)* mutants, axon pathfinding is defective; specific axons often appear to wander randomly or migrate in the wrong direction. In addition, the long range migrations of three neuron types and fasciculation of the ventral nerve cord are defective. Together, our studies define a new role for HDA-1 in nervous system development, and provide the first evidence for HDAC function in regulating neuronal axon guidance.

Keywords: Histone deacetylase; Cell migration; Axon pathfinding; *hda-1*

Introduction

Eukaryotic chromatin includes many DNA-associated proteins, which perform both structural and regulatory functions. For example, DNA is packaged into nucleosomes in association with the core histones H2A, H2B, H3, and H4. Recently, it has become clear that, in addition to their structural roles, histones also play regulatory roles. Histones can be modified post-translationally by phosphorylation, ubiquitination, methylation, and acetylation of specific residues, and these modifications can influence the expression of nearby genes (Kurdistani and Grunstein, 2003; Turner, 2002). Increased acetylation of specific residues of individual histones often is associated with transcriptional activation whereas decreased acetylation of histones often is associated with transcriptional silencing. Many transcriptional regulators act by recruiting protein complexes that locally alter the acetylation of histones. Recruitment of histone acetyl transferases (HATs) can lead to transcriptional activation whereas recruitment of histone deacetylases (HDACs) can lead to transcriptional repression.

The HDACs have been subdivided into four subfamilies based on sequence homology and cofactor requirement (Gregoretti et al., 2004), although previous phylogenetic analyses recognized three (de Ruijter et al., 2003; Fischle et al., 1999; Grozinger and Schreiber, 2002; Grozinger et al., 1999; Imai et al., 2000; Landry et al., 2000; Shore, 2000). Class I and II HDACs are similar proteins that share homology in their catalytic sites, although class II HDACs contain additional sequence homology not shared with class I HDACs. In addition, class I HDACs generally are ubiquitously expressed whereas class II HDACs may be more restricted (de Ruijter et al., 2003; Fischle et al., 1999; Grozinger et al., 1999). Class III HDACs differ structurally from other classes of HDACs and utilize nicotinamide adenine dinucleotide (NAD) as a cofactor (Imai et al., 2000). Class IV HDACs have a unique catalytic domain and are overall structurally distinct from class I and II HDACs (Gregoretti et al., 2004). *Saccharomyces cerevisiae* contain three class I, two class II,

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doi:10.1016/j.ydbio.2005.10.033

and five class III HDACs, but lack an apparent class IV HDAC (Grozinger and Schreiber, 2002; Gregoretti et al., 2004). Humans have at least four class I HDACs, six class II HDACs, seven class III HDACs, and a single class IV HDAC (Gregoretti et al., 2004; Marks et al., 2003).

Caenorhabditis elegans contains three class I, four class II, four class III HDACs, and one probable class IV HDAC (Gregoretti et al., 2004). HDA-1, a class I HDAC, is expressed ubiquitously in somatic and germline cells, and expression was detected throughout embryonic and postembryonic development (Dufourcq et al., 2002). Depletion of both maternal and zygotic HDA-1 causes embryonic lethality (Shi and Mello, 1998). Furthermore, zygotic depletion of HDA-1 by a mutation in the gene or by limited RNAi has been reported to specifically affect gonadogenesis and vulval development (Dufourcq et al., 2002; Shi and Mello, 1998). HDA-1 also acts with POP-1, a TCF/LEF transcription factor, and UNC-37, a Groucho homolog, to repress *end-1* expression (Calvo et al., 2001).

We serendipitously found a new mutation in *hda-1*. We find that HDA-1 is required for the related processes of cell migration and axon guidance. Directed cell migration is essential for metazoan development. In vertebrates, primordial germ cells, cardiac precursors, melanocytes, and neuronal growth cones often traverse long distances during development. Cell migration is also important for *C. elegans* development. Many cell types migrate long distances during *C. elegans* development (Sulston and Horvitz, 1977; Sulston et al., 1983). In a process related to cell migration, the migration of neuronal growth cones, which are specialized structures at the leading ends of axons and dendrites, establishes the connectivity of the nervous system.

Here, we report a role for *hda-1* in cell migration and axon guidance. We identified a new mutation, *cw2*, within the *hda-1* gene and found that unlike the previously reported *hda-1(e1795)* mutation, *hda-1(cw2)* mutants were viable as homozygotes, although viability and fertility were less than wild type. The *hda-1(cw2)* mutation also produced a severe and highly penetrant uncoordinated locomotion (Unc) phenotype. This prompted us to examine the nervous system, where we found severe defects in axon pathfinding and fasciculation in *hda-1(cw2)* mutants. These defects are semidominant, and are not restricted to *hda-1(cw2)* mutants; similar defects are seen in *hda-1(e1795)* heterozygotes. In addition, in *hda-1(cw2)* mutants, the long range migrations of three neuron types, CANs, HSNs, and QR descendants, are defective. Therefore, our analysis of *hda-1(cw2)* mutants reveals a new role for HDA-1 in nervous system development.

Materials and methods

C. elegans culture

Strains were grown at 20°C as described (Brenner, 1974). Besides the wild-type strain, N2, and the wild isolate, CB4856 (Hodgkin and Doniach, 1997), strains containing the following mutations, chromosomal balancers, and reporter transgenes were used: *dpy-4(e1166)* (Brenner, 1974), *dpy-11(e224)* (Brenner, 1974), *epi-1(gm94)* (previously called *fam-2(gm94)*) (Forrester and Garriga, 1997), *hda-1(cw2)*, *hda-1(e1795)* (Dufourcq et al., 2002), *otIs33[kal-1::gfp]* (Bulow et al., 2002), *nT1[unc-?(n754) let-? qIs50[myo-2::gfp, pes-10::gfp, F22B7.9::gfp]], szT1[lon-2(e678)], unc-40(n473), unc-73(gm123)* (Forrester and Garriga, 1997), *unc-115(e2225), kyIs121[unc-115::gfp lin-15(+)], kyIs114[unc-115::gfp lin-15(+)]* (Lundquist et al., 1998), *yDf8*, *qIs56[lag-2::gfp]*, *Ex[krp95::gfp]* (Signor et al., 1999), and *kyIs5[ceh-23::gfp]* (Zallen et al., 1999).

Genetic screen

A screen that was designed to isolate new mutations in the *epi-1* gene, which encodes a laminin subunit (Huang et al., 2003; Zhu et al., 1999), yielded the *hda-1(cw2)* mutation. N2 males were treated with ethyl methanesulfonate (EMS) as described (Brenner, 1974) and crossed with *epi-1(gm94) kyIs5[ceh-23-unc-76::gfp] dpy-4(e1166)* homozygous hermaphrodites. Cross progeny were screened for animals that displayed uncoordinated locomotion (Unc). Two Unc animals were identified. One of these segregated *epi-1* homozygotes, weakly Unc animals, and approximately 1/4 strongly Unc animals. This mutation was named *cw2*.

Cloning and DNA sequencing

We mapped *cw2* genetically to chromosome V; from *unc-23(e324)+/+ cw2* heterozygous parents, we selected and cultured individually *unc-23* homozygous mutant offspring. Of 18 *unc-23* homozygotes, five segregated *cw2* mutants indicating linkage to *unc-23*. Subsequent mapping showed that *cw2* was located between single nucleotide polymorphisms (SNPs) within the two adjacent cosmids R02D5 (snp pkP5086) and C53A5 (snp C53A5[1]).

R02D5 and C53A5 DNA was introduced into *cw2* mutant animals by microinjection (Mello et al., 1991) and assayed for rescue by assessing Unc in transgenic animals. Three of three C53A5 transgenic lines, but zero of three R02D5 transgenic lines rescued *cw2* mutants. Four different PCR products derived from C53A5 sequences were generated: LP1 (using primers LP1.F 5′ CGCCCAACTGGCGTCCATCC 3′ and LP1.R 5′ CCACGTAAGCCAA-TAACCCG 3′), LP2 (using primers LP2.F 5′ CCCCATCGCCCACGCGAGTAC 3′ and LP2.R 5′ GAATACTGGCGATGCACTTCCC 3′), LP3 (using primers LP3.F 5′ CGAAACGTTGGCTCCAAGTGG 3′ and LP3.R 5′ GCTCATTAA-CAAGGGCACTAC 3′), and LP4 (using primers LP4.F 5′ GCTCACGCACA-CACACCTTCTC 3′ and LP4.R 5′ CATCGCACACCTGCTTCGAAA 3′). Finally, we generated a 2.2 kb DNA fragment (PCR 5) that contained the predicted C53A5.3 gene (generated using primers C53A5.3F 5′ CCTGTCTTA-CACCTTCTCCTTC 3′ and C53A5.3′R 5′ CGACATAAACGATGTCAACTGC 3′), which corresponds to *hda-1*, and assessed rescue of the Unc phenotype. We found that a PCR fragment of 5.8 kb (LP3) rescued the phenotypes of *cw2* mutant animals. The 2.2 kb fragment containing C53A5.3 gene also rescued. The rescuing fragment is predicted to contain only a single gene, *hda-1*, which encodes a *C. elegans* histone deacetylase homolog.

We isolated the *hda-1* gene by PCR from *cw2* mutants using primers C53A5.3F and C53A5.3R (5′ GCACATTCTCCGTGGCCTTTC 3′) and C53A5.3′F (5′ CCAATCATGACAAAGGTTATGG 3′) to C53A5.3′R and determined the DNA sequence of three independent PCR products. In each sequenced product, we found that nucleotide 221 (where 1 is the A of the predicted ATG start codon) was mutated from a C to a T.

Genetic characterization of cw2

We examined the *hda-1(cw2)* mutation in trans to a chromosomal deficiency that deletes the entire *hda-1* locus. *hda-1(cw2)/+* heterozygous males were crossed with sperm-depleted *yDf8/nT1[unc-?(n754) let-? qIs50(myo-2::gfp, pes-10::gfp, F22B7.9::gfp)]*. Cross-progeny that lacked GFP expression (i.e. that lacked nT1) were *hda-1(cw2)/yDf8* or *yDf8/+*. Nearly half of the cross progeny were phenotypically indistinguishable from *hda-1(cw2)* homozygotes.

RNA characterization

RNA was purified using the Trizol reagent from Invitrogen according to the manufacturer's instructions and after electrophoresis, RNA was linked by ultraviolet light to positively charged Nylon Membrane (Roche). A gene

specific probe was generated by PCR using C53A5.3F and C53A5.3R primers to amplify an 800 bp region near the 5′ end of the *hda-1* gene with the PCR DIG Probe Synthesis kit according to the supplier's instructions (Roche). RT-PCR was performed using primers C53A5.3F and C53A5.3R and resulting products were sequenced.

Protein analysis

To examine the HDA-1 protein produced in *hda-1(cw2)*, *hda-1(e1795)*, and wild type, 30 worms were sonicated for 15 min at 60°C in a bath sonicator in sample buffer, then boiled for 3 min. Proteins were separated by sodium dodecyl sulfate, polyacrylamide gel electrophoresis and transferred to nitrocellulose. The resulting blot was incubated with 1/500 diluted anti-HDA1 (Santa Cruz Biotechnology, Inc., Dufourcq et al., 2002) or 1/10,000 diluted anti-GLH-1 (gift from L. Bender and S. Strome) rabbit polyclonal antibody, followed by goat anti-rabbit horse radish peroxidase-conjugated secondary antibody, and detected by chemiluminescence.

Phenotypic characterization

The extent of cell migration in wild-type, mutant, and transgenic animals was determined by comparing the positions of nuclei relative to non-migratory hypodermal nuclei using Nomarski optics. For ALM, BDU, CAN, and HSN cells that migrate embryonically, we scored the positions of the nuclei of these cells relative to non-migratory hypodermal V and P nuclei in newly hatched hermaphrodite larvae (L1). For the Q neuroblasts and their descendants, which migrate during the L1 stage, we scored the final positions of the Q descendant nuclei relative to the two daughter hypodermal nuclei Vn.a and Vn.p derived from V1-6 in mid-L1 stage hermaphrodites. Overall cellular morphology often was abnormal in *hda-1(cw2)* homozygotes, rendering approximately 50% of animals unscoreable.

To examine components of the nervous system, we used a combination of indirect immunofluorescence and green fluorescent protein (gfp) reporter transgenes. To examine HSN morphology, we used an anti-serotonin antibody (J. Steinbusch, Free University, Amsterdam) as described (McIntire et al., 1992). To examine HDA-1 distribution, we used a rabbit polyclonal antibody diluted 1/50 (Santa Cruz Biotechnology, Inc., Dufourcq et al., 2002), following the protocol of Finney and Ruvkun (1990). Fluorescein isothiocyanate (FITC)-conjugated secondary antibodies were obtained from Jackson Immunoresearch. Stained animals were examined using a Nikon E600 microscope with 60× objective with Photometrics CoolSnap CCD camera and Image Pro Express software. Images were manipulated in Adobe Photoshop.

Results

While attempting to isolate new mutations in the *C. elegans epi-1* gene, which encodes a laminin subunit (Huang et al., 2003; Zhu et al., 1999), we instead isolated a mutation, *cw2*, that interacts genetically with specific alleles of *epi-1* (Kean et al., in preparation). Homozygous *cw2* mutants often die as embryos or larvae (detailed below). Animals that escape the lethality generally are severely uncoordinated (Unc) and often sterile. Upon closer inspection, we observed the modest cell migration and severe axon guidance defects that made us interested in studying the gene further.

cw2 is a mutation in the hda-1 gene

To clone the gene that is responsible for the defects in *cw2* mutants, we first mapped *cw2* to a 76 kb region of chromosome V that is predicted to contain 12 genes (Fig. 1A). We next assessed the ability of R02D5 and C53A5 to rescue *cw2* mutant phenotypes. C53A5, but not R02D5 transgenic lines, rescued *cw2* mutants (Fig. 1A). To further refine the interval that contains the gene, we used PCR to generate fragments that contained subsets of the DNA between the polymorphisms. A single PCR fragment of 5.8 kb rescued the phenotypes of *cw2* mutant animals (Fig. 1A). A 2.2 kb fragment (PCR5) also rescued (Fig. 1A). The rescuing fragment is predicted to contain only a single gene, *hda-1*, which encodes a *C. elegans* histone deacetylase homolog.

HDA-1 is a predicted *C. elegans* class I HDAC. A mutation in *hda-1*, *e1795*, previously called *gon-10(e1795)*, has been reported to cause sterility and vulval development defects (Dufourcq et al., 2002) that are similar to, but more severe than, those of *hda-1(cw2)* mutants (Table 1).

hda-1(cw2) is a missense mutation

To confirm that *cw2* is an allele of *hda-1*, we sequenced *hda-1* from *cw2* mutants. We found a single mutation in *hda-1(cw2)* that is predicted to change nucleotide 221, the predicted first nucleotide of the second exon, from C to T, thereby changing amino acid 59 from arginine to cysteine. Alignment of this region from four class I HDACs shows that a basic amino acid, either arginine or lysine, is found at the analagous position (Fig. 1B) within the catalytic domain of the protein (Finnin et al., 1999). Similarly, *e1795* also mutates an amino acid within the catalytic domain of the deacetylase (Fig. 1B, Dufourcq et al., 2002).

Because *hda-1(cw2)* alters the first nucleotide of exon 2, and therefore might affect mRNA splicing, and to characterize the transcripts, we examined the *hda-1* mRNAs produced. The consensus 3′ splice site in *C. elegans* is UUUUCAG*R where R represents purine and * indicates the splice site (Blumenthal and Steward, 1997). Although in *C. elegans* purine is preferred at the mutated position, the preference is not absolute; pyrimidines are found in that position approximately 25% of the time, with no clear preference for C over U (Blumenthal and Steward, 1997). In *hda-1*, the sequence at the end of intron 1 is AUUUUAG*C whereas in *hda-1(cw2)* mutants, it is AUUUUAG*U. Therefore, we expected that the change would not alter *hda-1* mRNA splicing. To confirm that this was the case, we used Northern hybridization to examine *hda-1* mRNA produced using an *hda-1* specific probe. In both wild type and in *hda-1(cw2)* mutants, we detected *hda-1* mRNA of approximately 2100 bp in size (Fig. 1C). The *hda-1* mRNA in *hda-1(cw2)* mutants was not detectably altered in size or reduced in abundance. Rather, it reproducibly appeared substantially more abundant in mutants. Because the RNA blot would not have detected relatively minor alterations in mRNA size, we next asked whether the *hda-1(cw2)* mutation altered the mRNA produced by RT-PCR, using primers that flanked the mutation. In wild type, we detected a single RT-PCR product of 362 base pairs (not shown). In *hda-1(cw2)* mutants, the *hda-1* mRNA was indistinguishable in size and nucleotide sequence from wild type (not shown)

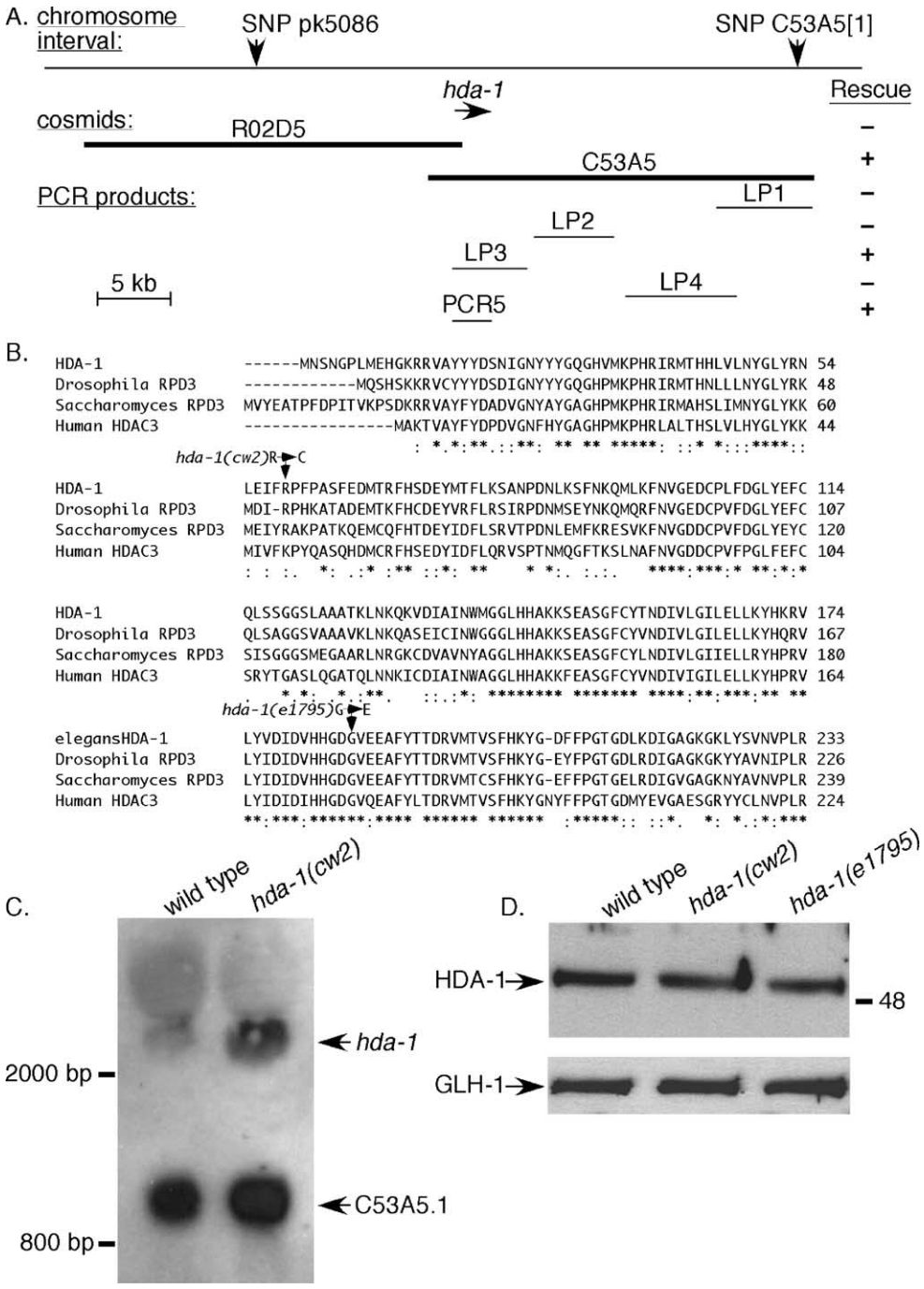

Fig. 1. (A) Cloning *hda-1(cw2)*. *cw2* mapped genetically to a small region on chromosome V, between SNPs pkP5086 and C53A5[1]. Lines below indicate cosmids (R02D5 and C53A5) or PCR products that were tested for their ability to rescue *cw2* mutant phenotypes. Column to the right indicates whether cosmid or PCR product rescued (+) or did not rescue (−) Unc. (B) Comparison of the N-terminal segment of HDA-1 that includes the mutations in *hda-1(cw2)* and *hda-1(e1795)* (arrows) with *Saccharomyces cerevisiae* and *Drosophila melanogaster* RPD3 and human HDAC3. Conserved residues are highlighted below the sequence. (C) RNA blot showing *hda-1* mRNA from wild type and *hda-1(cw2)* mutants. Blot was also hybridized to a C53A5.1 specific probe. Faint upper band appears to be ribosomal RNA. (D) HDA-1 protein from wild type, *hda-1(cw2)* and *hda-1(e1795)* mutants detected on protein blot using anti-HDA-1 specific antibodies. Bottom panel shows GLH-1 loading control. Number to the right denotes the position of protein size standard.

except for the substitution of U for C as noted above. These results confirm that *hda-1(cw2)* mutants do not accumulate an aberrantly spliced mRNA.

To further examine the effect of the mutation, we investigated by immunoblot the HDA-1 protein produced in *hda-1(cw2)* mutants (Fig. 1D). In extract prepared from wild type, we detected a single band of 50 kDa, similar to the predicted size of 52.1 kDa. In extract from *hda-1(cw2)* mutants, a protein indistinguishable in size and abundance from wild type was detected, as expected. The levels of HDA-1 protein in *hda-1(e1795)* mutants were also similar to those in wild type (Fig. 1D).

Table 1
Behavioral and morphological phenotypes of *hda*-1 mutants[a]

Strain	Emb[b]	Larval arrest[c]	Unc[d]	Muv[e]	Pvl[f]	Sterility[g]
Wild type	1.4 (1278)	0 (1278)	0 (100)	0 (50)	0 (50)	0 (35)
hda-1(cw2)	34.5 (794)	20.5 (794)	100 (100)	11.5 (26)	61.5 (26)	33.3 (75)
hda-1(cw2)[h] from *cw2/+*	nd	nd	85.7 (77)	4.5 (22)	54.5 (22)	4.5 (22)
hda-1(e1795)[h]	nd	nd	1.0 (110)	20.8 (48)	97.9 (48)	100 (48)

[a] Numbers are percentage of total that exhibit the phenotype. Numbers in parentheses are the total number examined. nd = not determined.
[b] Numbers are percent of embryos laid that failed to hatch.
[c] Numbers are percent of total embryos laid that hatched, but then arrested as first or second stage larvae.
[d] Numbers are percent of animals that display movement clearly distinguishable from wild type. Movement defects in *hda-1(cw2)* mutants ranged from paralyzed (>90% of animals that survive beyond early larval stages) to near-normal.
[e] Numbers are percent of animals that exhibit multiple vulvae.
[f] Numbers are percent of animals with abnormally protruding vulvae.
[g] Adult hermaphrodites with transparent, empty uterus were scored as being sterile.
[h] Phenotypes of homozygous mutant offspring of heterozygous parents were assessed.

We next investigated the localization of protein in *hda-1* mutants. As previously reported, HDA-1 in wild-type animals is detected in most or all cells of the body, including cells of the nervous system (Fig. 2, Dufourcq et al., 2002). The protein is concentrated in nuclei (Fig. 2, Dufourcq et al., 2002), but, unlike in the previous report, we also detect prominent staining within neuronal processes (Fig. 2). We believe that this staining represents HDA-1 protein, because this antibody only detects a single band on immunoblot (Fig. 1D). Furthermore, the staining is eliminated in *hda-1(e1795)* mutants, although we occasionally detect very low levels of staining (Fig. 2; not shown). In *hda-1(cw2)* mutants, the distribution of HDA-1 protein resembles that in wild type (Fig. 2). The observation that *hda-1(e1795)* mutants produce near wild-type levels of HDA-1 protein that is not detected in animals suggests that perhaps the epitope recognized by the antibody is masked in *hda-1(e1795)* mutants.

Mutation in hda-1 disrupts cell migration

To gain insights into HDA-1 function, we examined the phenotypes of *hda-1* mutants. *hda-1(cw2)* mutants often die as embryos or young larvae (Table 1). However, 45% of the embryos developed beyond the first or second larval stages. Of these, approximately one third were sterile. In addition, all *hda-1(cw2)* mutant animals that hatched displayed uncoordinated

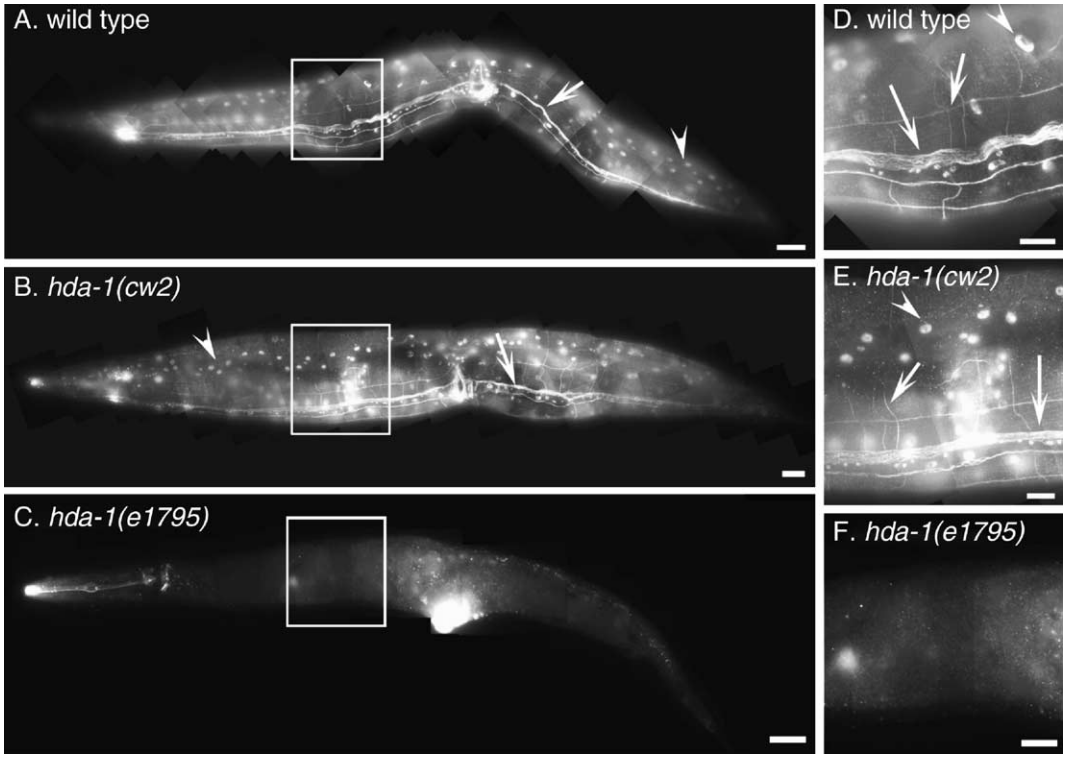

Fig. 2. Distribution of HDA-1 protein in wild type (A, D), *hda-1(cw2)* (B, E), and *hda-1(e1795)* (C, F) mutants. Panels D–F show close up of corresponding boxed regions in panels A–C. Anterior is to the left and dorsal is up. In both wild type and *hda-1(cw2)*, HDA-1 protein appears to localize to nuclei (arrowheads) and much of the nervous system (arrows). Long arrows denote the ventral nerve cord and short arrows indicate other neuronal processes. HDA-1 protein is undetectable in *hda-1(e1795).* Patches of staining seen in *hda-1(e1795)* are non-specific antibody clumping. Scale bars in panels A–C = 40 μm. Scale bars in panels D–F = 20 μm.

(Unc) locomotion (Table 1). The Unc locomotion of *hda-1(cw2)* mutants ranged from near-complete paralysis for most animals to occasional animals that were only slightly Unc; they were able to move almost as well as wild type. In contrast, *hda-1(e1795)* mutants were sterile but not Unc (Table 1, Dufourcq et al., 2002).

A number of cellular defects can disrupt locomotion, including defective nervous system development. Because *hda-1(cw2)* mutants are Unc, we wondered whether the morphology of the nervous system was normal. To begin to look at the nervous system, we first asked whether neuronal cell migrations were normal in *hda-1(cw2)* mutants. In wild-type animals, the ALMs and CANs migrate posteriorly to near the middle of the animal during embryogenesis (Sulston et al., 1983). The HSNs migrate from the tail anteriorly to the middle of the animal during embryogenesis, and BDU neurons migrate a short distance anteriorly (Sulston et al., 1983). QL and QR descendants migrate during the first larval stage (Sulston and Horvitz, 1977). The QL descendants migrate posteriorly, whereas the QR descendants migrate anteriorly.

We found that the migration of multiple neuronal cell types is disrupted in *hda-1(cw2)* mutants (Figs. 3 and 4, Table 2). CAN cells occasionally were misplaced anteriorly along their migratory route. Similarly, HSN cells also were misplaced posteriorly along their migratory routes or migrated a short distance anteriorly beyond their normal destinations. In addition, postembryonic QR descendant migrations were sometimes defective (Table 2). The migrations of BDU cells appeared unaffected in *hda-1(cw2)* mutants. In *hda-1(e1795)* mutants, we occasionally detected misplaced ALM, HSN, and QR descendant cells, showing that cell migration is also affected, albeit less severely than in *hda-1(cw2)* mutants. The observation that multiple cell migrations are defective in both *hda-1* mutants shows that HDA-1 is required for normal migration, perhaps to ensure proper levels of expression of a guidance cue or substrate required for cell migration.

HDA-1 is required for axon fasciculation and pathfinding

Although cell migration is defective in *hda-1* mutants, the effects are relatively modest. To determine whether the migrations of neuronal growth cones might also be defective, we first examined the morphology of the nervous system overall, using a *krp95::gfp* reporter transgene that is expressed in most if not all of the nervous system (Signor et al., 1999). In wild type, many neurons extend processes along the left and right ventral nerve cord in two tight bundles that are separated by a ridge of hypodermal tissue (White et al., 1986). In *hda-1(cw2)* mutants, the tight fasciculation often broke down. Neurons could be seen separating from the major bundle to extend individually or in smaller bundles (Fig. 5, Table 3). The cell bodies of VD and DD motorneurons are located in the ventral nerve cord, and extend axons along the lateral body wall to the dorsal nerve cord, where they turn to run along the anterior–posterior body axis. Axons usually leave the ventral nerve cord at near right angles in wild type. In *hda-1(cw2)* mutants, axons often left the ventral nerve cord at acute angles to extend to the dorsal nerve cord with less direct trajectories (Table 3).

We next looked at the projection of individual HSN axons to see whether they were affected. After migrating, each HSN sends an axon that extends ventrally along the lateral body wall to the ventral nerve cord, where it turns anteriorly and extends to the head (Garriga et al., 1993; White et al., 1986). The HSN axon defasciculates where it skirts the vulva and typically produces one or two branches that innervate the egg laying muscles (Garriga et al., 1993; White et al., 1986). The HSNs express the neurotransmitter serotonin in adults, and therefore the HSN axons can be seen easily by staining adult hermaphrodites with anti-serotonin antibodies (McIntire et al., 1992).

In *hda-1(cw2)* mutants, there are extensive HSN axon defects. Perhaps most strikingly, HSN axons often seemed to

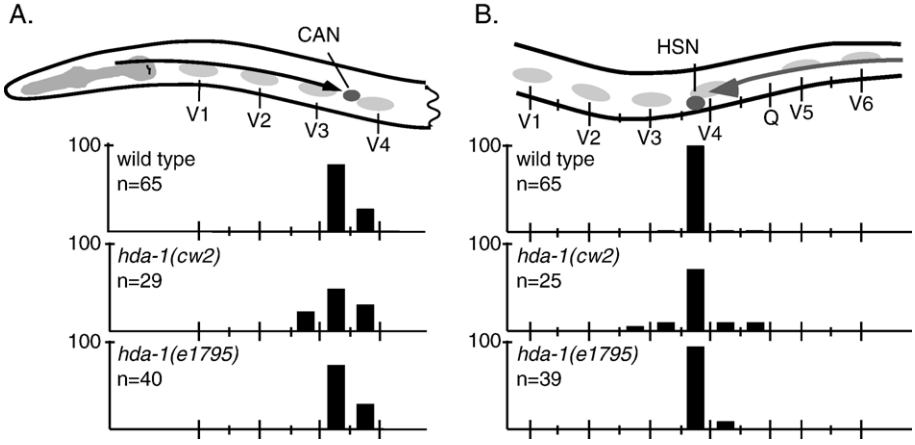

Fig. 3. (A) Cell migration in *hda-1* mutants. Anterior is to the left and dorsal is up. Light ovals and long hash marks below show V or Q cell nuclei (named) and short hash marks denote the location of P cell nuclei (not named). Y axis denotes percentage of cells at particular position along the longitudinal body axis. *n* = number of cells tallied. (A) CAN cell migration. Shown at the top is a schematic of the anterior section of an animal with the CAN cell (dark oval) and its migration route (arrow). Bars represent percentage of CAN cells located at that position along the anterior–posterior axis of L1 larvae as assessed by Nomarski optics. (B) HSN cell migration. Shown at the top is a schematic of the middle section of a hermaphrodite with the HSN cell (dark circle) and its migration route (arrow). Bars represent percentage of HSN cells located at that position along the anterior–posterior axis of L1 larvae as assessed by Nomarski optics.

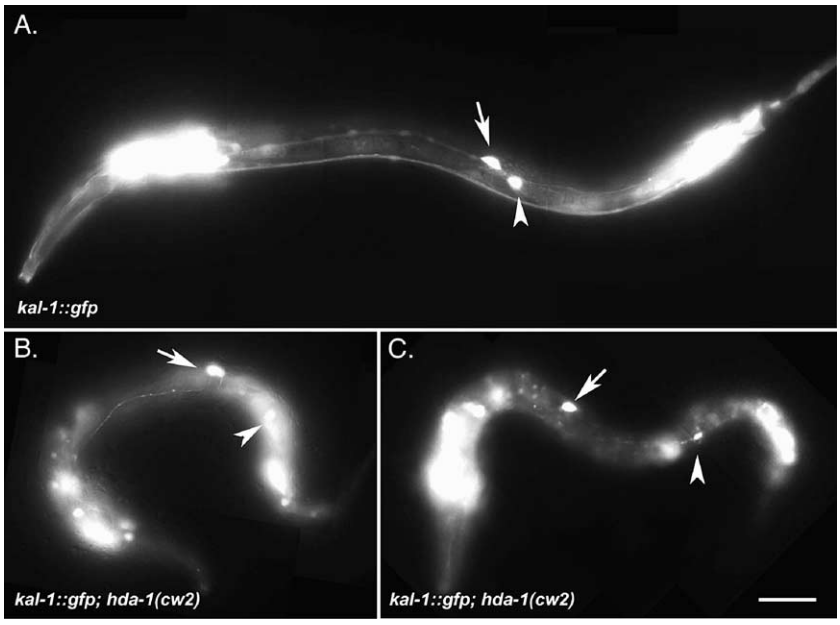

Fig. 4. CAN and HSN cell migrations are defective in *hda-1(cw2)* mutants. *kal-1::gfp* reporter transgene is utilized to illuminate CAN (arrow) and HSN (arrowhead) cells. Anterior is to the left and dorsal is up. (A) Wild-type worm showing CAN and HSN cells. (B) *hda-1(cw2)* mutant showing HSN cell that failed to migrate to its normal position. (C) *hda-1(cw2)* mutant showing CAN cell that failed to migrate to its normal position. Scale bar = 20 μm.

wander aimlessly along the lateral body wall, frequently circling a particular region or looping back upon themselves (Fig. 6, Table 3). HSN axons also often produced ectopic branches in *hda-1(cw2)* mutants (Table 3). Occasionally, the HSNs extended axons both anteriorly and posteriorly, or rarely, just posteriorly (Table 3). Lastly, many animals displayed a combination of defects, with single HSN axons looping along the lateral body wall and forming ectopic branches. The same defects are seen in *hda-1(e1795)* mutants, albeit with reduced penetrance, indicating that the nervous system defects are not specific to *hda-1(cw2)* mutants (Table 3). Furthermore, similar HSN axonal defects are seen in *hda-1(cw2)/+* and in *hda-1(e1795)/+* heterozygotes, indicating that both mutations are semidominant (Table 3).

Table 2
Cell migration defects in *hda*-1 mutants[a]

Strain	ALM[b]	BDU[c]	CAN[d]	HSN[e]		QR[f]
				Anterior	Posterior	
Wild type[g]	3.1 (65)	0 (65)	1.5 (65)	1.5 (65)	3.1 (65)	3.1 (32)
hda-1(cw2)	3.4 (29)	0 (27)	20.7 (29)	12.0 (25)	16.0 (25)	25.0 (32)
hda-1(e1795)	7.5 (40)	0 (40)	0 (40)	0 (39)	7.7 (39)	8.3 (36)

[a] Cell positions were assessed by Nomarski optics. ALM, BDU, CAN, and HSN positions were determined in newly hatched hermaphrodite larvae (L1). QR descendant positions were determined in older L1 stage hermaphrodites after their migrations were complete. Numbers are percentage of cells that failed to migrate to their normal position. Numbers in parentheses are number of cells scored.

[b] An ALM was scored as misplaced if its nucleus was posterior to the V3 nucleus.

[c] A BDU was scored as defective if its nucleus was posterior to the V1 nucleus.

[d] A CAN was scored as misplaced if its nucleus was anterior to the V3 nucleus.

[e] An HSN was scored as anteriorly misplaced if its nucleus was anterior to P5/6 and posteriorly misplaced if its nucleus was posterior to the V4 nucleus.

[f] A QR cell descendant was scored as defective if its nucleus was posterior to the V2.a nucleus. Because they occupy positions near each other, the data for SDQR and AVM were combined. The position of AQR, a third QR descendant, was not included because it migrates to a location near other nuclei with similar morphology.

[g] Some of these data have been reported elsewhere (Kim and Forrester, 2003). They are presented here for comparison.

hda-1(cw2) may reduce gene function

hda-1(e1795) is thought to represent a null mutation (Dufourcq et al., 2002), and yet it displays less severe nervous system defects than *hda-1(cw2)* mutants. By contrast, the *hda-1(cw2)* mutation causes more severe defects in cell migration, axon pathfinding, and locomotion but less severe defects in fertility and viability. *hda-1(cw2)* is viable as homozygous mutants, whereas *hda-1(e1795)* mutants are sterile and therefore cannot be maintained as homozygotes. A plausible explanation for the difference in phenotypes is that maternally provided HDA-1 is sufficient to partially rescue the nervous system defects but not the sterility. An alternate possibility is that *hda-1(cw2)* and *hda-1(e1795)* confer new functions on HDA-1, which produce the differences between the two mutations.

To determine whether *hda-1(cw2)* was likely to reduce or eliminate gene function, we generated animals in which *hda-1(cw2)* was in trans to a chromosomal deficiency, *yDf8*, that deletes the entire *hda-1* locus and surrounding loci. The phenotypes of *hda-1(cw2)/yDf8* animals were indistinguishable from those of *hda-1(cw2)* mutants alone (not shown), suggesting that *hda-1(cw2)* reduces or eliminates gene function. The *hda-1(cw2)/yDf8* animals that escape the lethality were severely Unc. Similarly, *hda-1(e1795)/Df* animals also are identical to *hda-1(e1795)* mutants (Dufourcq et al., 2002). An observation that mutant/deficiency produces a phenotype

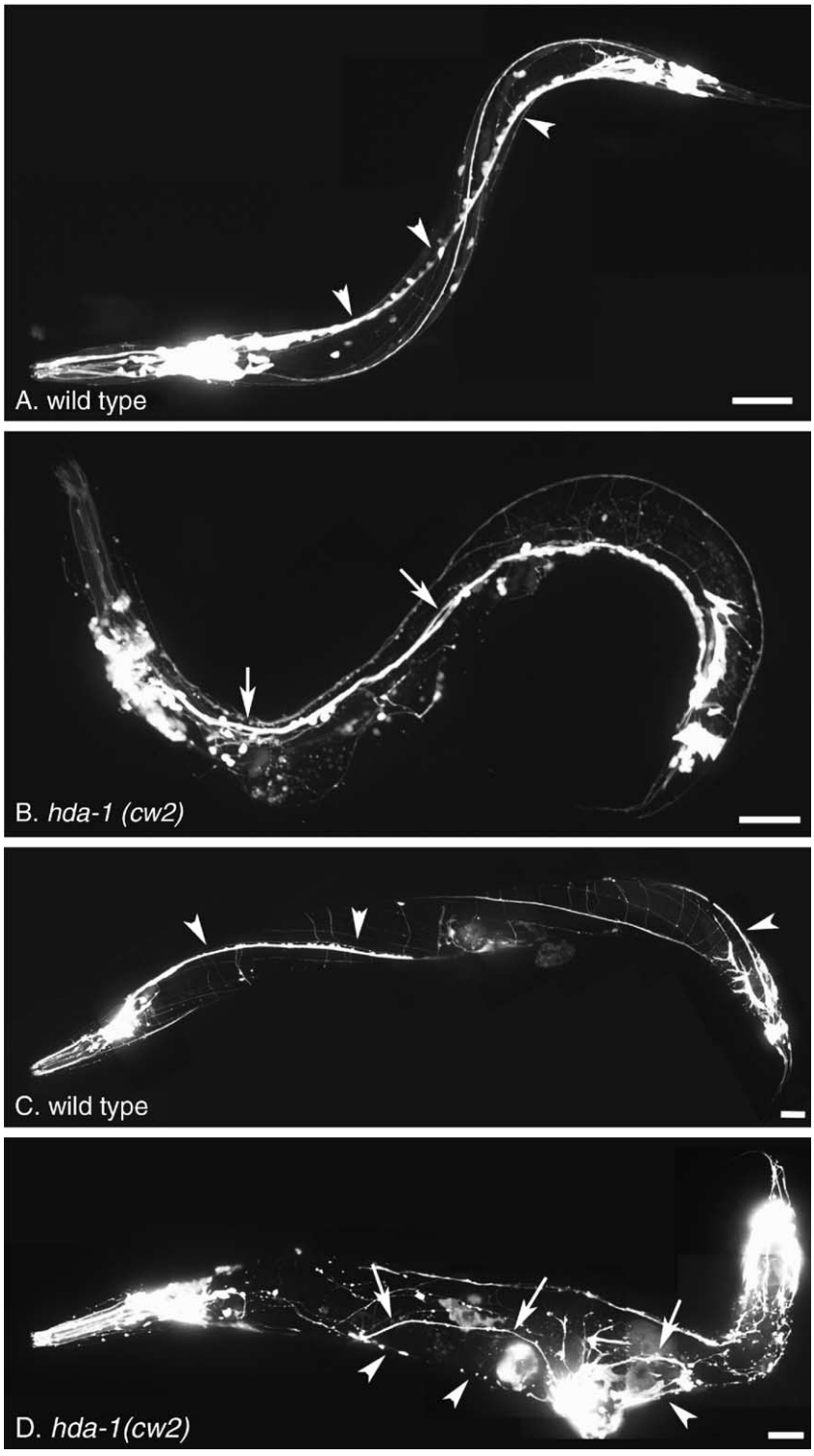

Fig. 5. Ventral nerve cord fasciculation in wild type (A, C) and *hda-1(cw2)* mutant (B, D). Anterior is to the left. Animals contain *krp95*::*gfp* transgene that expresses GFP throughout the nervous system. Spiral appearance results from presence of the *rol-6* cotransformation marker on the transgene. (A) Wild-type first stage larva showing tightly bundled ventral nerve cord (arrowheads). (B) *hda-1(cw2)* mutant first stage larva showing defasciculation of ventral nerve cord (arrows). (C) Wild-type adult showing tightly bundled ventral nerve cord (arrowheads). (D) *hda-1(cw2)* mutant adult showing extremely disorganized ventral nerve cord (arrowheads), with portions extending along lateral body wall (arrows). Scale bar in all panels indicates 20 μm.

identical to homozygous mutant often is interpreted as suggesting that the mutation reduces or eliminates gene function. Therefore, *hda-1(cw2)* is likely to reduce or eliminate function.

To directly assess whether maternally provided product rescued the nervous system defects, we compared offspring of homozygous mutant mothers to those derived from heterozygous mothers. Nervous system defects in homozygous *hda-*

Table 3
Axonal defects of *hda-1* mutants

Strain	HSN defects[a]				VNC defects[b]		
	Posterior axon[c]	Ectopic branches[d]	Wandering axon[e]	n[f]	Defasciculation[g]	Oblique angles[h]	n[f]
Wild type	0	2.5	2.5	81	0	5.0	40
hda-1(cw2)	22.4	30.8	30.8	143	36.7	20.0	30
hda-1(e1795)	14.3	33.3	14.3	21	nd	nd	nd
hda-1(cw2) from *cw2/+*	20.0	11.4	8.6	35	nd	nd	nd
hda-1(cw2)/hda-1(e1795)	3.7	29.6	29.6	27	nd	nd	nd
hda-1(cw2)/nT1	3.1	12.5	6.3	96	nd	nd	nd
hda-1(e1795)/nT1	8.2	22.4	4.1	49	nd	nd	nd
yDf8/nT1	19.0	19.0	19.0	42	nd	nd	nd
dpy-11(e224)/nT1	0	0	0	21	nd	nd	nd
hda-1(cw2); unc-115(e2225)/+[i]	11.3	27.5	12.5	80	nd	nd	nd
unc-115(e2225)	6.0	30.0	0	50	nd	nd	nd
unc-40(n473)/+; hda-1(cw2)[i]	23.9	19.6	10.9	46	nd	nd	nd
unc-40(n473)	43.9	19.5	14.6	41	nd	nd	nd
unc-73(gm123)/+; hda-1(cw2)[i]	18.0	32.8	16.4	61	nd	nd	nd

[a] HSN morphology was assessed by indirect immunofluorescence using anti-serotonin antibodies (see Materials and methods).
[b] Ventral Nerve Cord (VNC) defects were assessed in animals that contained the pan-neuronal GFP reporter *krp95::gfp* (Signor et al., 1999).
[c] The HSN axon in wild type extends anteriorly in the VNC. Numbers are percent of animals in which an HSN axon extended posteriorly. This included animals in which HSN produced a single axon that extended posteriorly and animals in which HSN produced an ectopic axon that extended posteriorly.
[d] HSN normally produces one or two small branches near the egg-laying muscles. In some *hda-1* mutants, we detected additional HSN axon branches. The numbers present the percentage of HSN axons that had ectopic branches. See Fig. 6 for an example.
[e] In wild type, the HSN axon defasciculates from the VNC to skirt the vulva. In some *hda-1* mutants, HSN axons sometimes failed to enter the VNC or entered but then exited the VNC. In these mutant animals, the HSN axon appeared to wander along the lateral body wall. See Fig. 6 for an example. The number represents the percent of HSN axons that appeared to wander along the lateral body wall.
[f] n = total number of HSNs or ventral nerve cords examined.
[g] The VNC is composed of two bundles of neurons. In some *hda-1* mutants, this organization was disrupted so that individual neuronal processes or bundles of processes separated from the VNC. Numbers are the percent of nerve cords that exhibited that defasciculation.
[h] Many axons extend from the VNC to the dorsal nerve cord (DNC). These axons typically exit the VNC at approximately 90° angles. Numbers are percent of axons that exited the VNC at angles < approximately 60°.
[i] Animals derived from *hda-1(cw2)/hda-1(cw2); unc-115(e2225)/szT1, unc-40(n473)/szT1; hda-1(cw2)/hda-1(cw2)* or *unc-73/tDf4 dpy-5(e61); hda-1(cw2)/hda-1(cw2)*. We expect 2/3 heterozygous and 1/3 homozygous for *unc-115*, *unc-40*, or *unc-73* (but few *unc-73* homozygotes survive to adulthood). We have not scored HSN axons in *unc-73* homozygotes because few animals reach adulthood (Forrester and Garriga, 1997).

1(cw2) mutants were less severe if the mother was heterozygous for the mutation, demonstrating that the nervous system defects were partially maternally rescued (Table 3). Similarly, the offspring of heterozygous mothers were less Unc than those derived from homozygous *hda-1(cw2)* mutant mothers (Table 1). Taken together, our data suggest that maternal HDA-1 is required for normal nervous system development. However, most homozygous *hda-1(cw2)* mutants derived from heterozygous mothers were Unc, whereas *hda-1(e1795)* animals were not. Therefore, maternal effect provides an incomplete explanation for the differences between the two mutations.

Reducing hda-1 gene dosage by half produces pathfinding defects

Because both *hda-1(cw2)* and *hda-1(e1795)* are likely to be loss-of-function mutations and yet are semidominant, we asked whether deleting one copy of the *hda-1* locus causes defects. We examined the HSN axons in *yDf8/nT1* animals by staining with anti-serotonin antibodies. Animals that lacked a single copy of the *hda-1* locus exhibited HSN axonal defects similar to those of animals heterozygous for either *hda-1* mutation (Table 3). These defects were not caused by the presence of the chromosomal balancer *nT1*, because HSN axons in an *nT1* containing strains were normal (Table 3). Because multiple genes in addition to *hda-1* are removed by *yDf8*, it is possible that other genes might be responsible for the phenotypes seen in *yDf8/nT1*. However, because *yDf8/nT1* animals displayed phenotypes similar to those of either *hda-1* mutant, the most likely interpretation is that loss of one copy of *hda-1* produces the pathfinding defects.

Reducing unc-115 gene dosage by half partially suppresses the uncoordinated locomotion of hda-1(cw2) mutants

Several *C. elegans* guidance cues, their receptors, and downstream effectors have been identified to function in the process of axon pathfinding. We wondered whether the HSN axon pathfinding defects and Unc locomotion might result from increased expression of one of these molecules in *hda-1(cw2)* mutants. UNC-115/abLim, an actin binding protein, is thought to function with Rac proteins in axon pathfinding by modifying the actin cytoskeleton (Lundquist et al., 1998; Struckoff and Lundquist, 2003). UNC-73/Trio is a guanine exchange factor (GEF) that functions in axon guidance (Steven et al., 1998). UNC-40/DCC, is a Netrin receptor that functions in dorso-ventral axon guidance (Chan et al., 1996). Mutations in *unc-73*, *unc-115*, and *unc-40* produce axon pathfinding defects (Hedgecock et al., 1990; Lundquist et al., 1998; Steven et al., 1998).

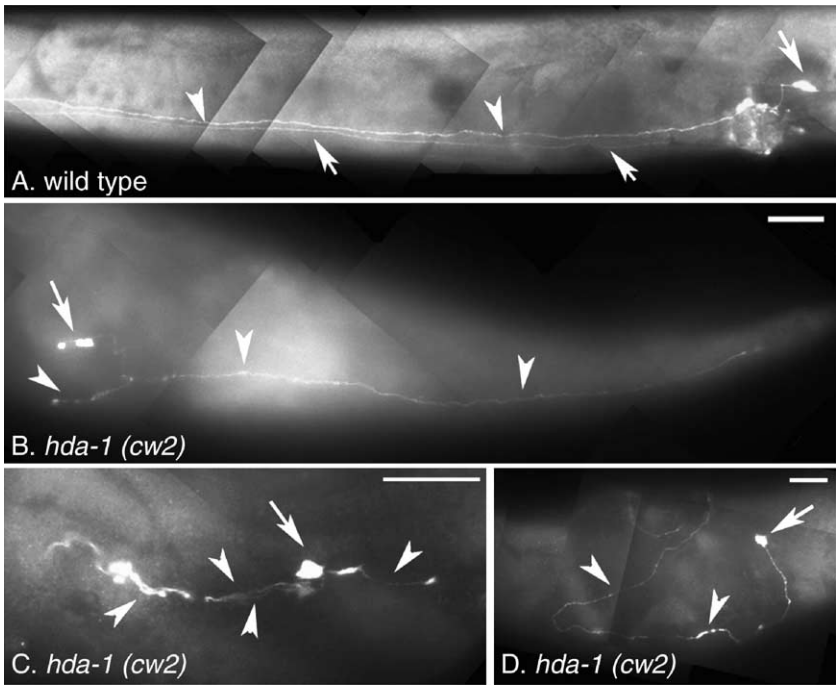

Fig. 6. HSN axon defects in *hda-1(cw2)* mutants. HSNs (arrow) and their axons (arrowheads) are detected by anti-serotonin antibody immunofluorescence in adult wild type (A) and *hda-1(cw2)* mutants (B–D). Anterior is to the left and dorsal is up. (A) Oblique view, shows left HSN cell and axon. In this view, the right HSN axon (short arrows) also is visible running parallel to the left HSN axon. (B) HSN axon extended ventrally to ventral nerve cord, where it branched to produce a short anterior axon and a longer posterior-projecting axon. (C) HSN axon failed to extend ventrally to VNC, but produced two axons, one that extended anteriorly and one that extended posteriorly along the lateral body wall. (D) HSN axon appeared to wander along the lateral body wall. Scale bar = 20 μm.

To determine whether axon guidance defects of *hda-1(cw2)* were suppressed by removal of one copy of *unc-115*, *unc-73*, or *unc-40*, we generated animals that were homozygous for *hda-1(cw2)* and heterozygous for either *unc-115*, *unc-73*, or *unc-40* mutations. We found that *unc-115*, but not *unc-40* or *unc-73*, partially suppressed multiple HSN axonal defects (Table 3). Both the production of posterior axons and the wandering axons were reduced by about half in *hda-1(cw2)/hda-1(cw2); unc-115/+* animals. In addition, *unc-115*, but not *unc-40* or *unc-73* partially suppressed the Unc defect of *hda-1(cw2)* mutants. In *hda-1(cw2)/hda-1(cw2); unc-115/+* animals, 23.2% of offspring were severely Unc, whereas 47.0% of the offspring of escaper *hda-1(cw2)/hda-1(cw2)* mutants were severely Unc. A possible explanation for these results might be that the *hda-1(cw2)* mutation derepresses *unc-115*. To begin to address this, we have examined the expression of two *unc-115*∷*gfp* reporter transgenes (Lundquist et al., 1998) in *hda-1* mutants. We do not see elevated GFP in either *hda-1(cw2)* or *hda-1(e1795)* mutants bearing either of the *unc-115*∷*gfp* transgenes, suggesting that the suppression does not result from a direct effect on *unc-115* expression. Our results suggest that a regulator of *unc-115* or another component of the pathway may be a target of *hda-1(cw2)* regulation.

hda-1(cw2) does not affect lag-2 gene expression

HDACs like HDA-1 are thought to repress gene expression by altering chromatin structure via histone deacetylation. We consistently detect elevated levels of *hda-1* and to a lesser extent C53A5.1 mRNAs in *hda-1(cw2)* mutants (Fig. 1C). Similarly, *hda-1(e1795)* causes *lag-2* to be derepressed (Fig. 7, Dufourcq et al., 2002). Normally, *lag-2* is expressed in two cells, the distal tip cells, in animals after hatching. In *hda-1(e1795)* mutants, *lag-2* expression is detected in many cell types. To ask whether *hda-1(cw2)* affected *lag-2* expression in a similar manner, we assessed GFP expression in an *hda-1(cw2)* mutant strain that also contained a fusion of *lag-2* regulatory sequences to *gfp*. Unlike *hda-1(e1795)*, significant ectopic expression of *lag-2*∷*gfp* was not detected in *hda-1(cw2)* mutants (Fig. 7).

Discussion

HDA-1 functions in nervous system development

In *hda-1(cw2)* and *hda-1(e1795)* mutants, neuronal axon pathfinding, ventral nerve cord fasciculation, and cell migration are defective. Given the highly abnormal trajectories of some HSN axons and defects within the ventral nerve cord, it is likely that the defects arise as a consequence of improper migration, rather than a secondary process such as adhesion to substrate. In support of this idea, similarly misguided axons also result from loss-of-function mutations in specific guidance cues or their receptors, such as *slt-1*/Slit or *sax-3*/Robo (Hao et al., 2001; Zallen et al., 1998) and from gain-of-function mutations in the Rho family member *mig-2* (Forrester and Garriga, 1997; Zipkin et al., 1997).

We also detect other nervous system defects. Motor-neuron axons that normally exit the VNC at near-right angles, instead frequently turn dorsally at more acute angles,

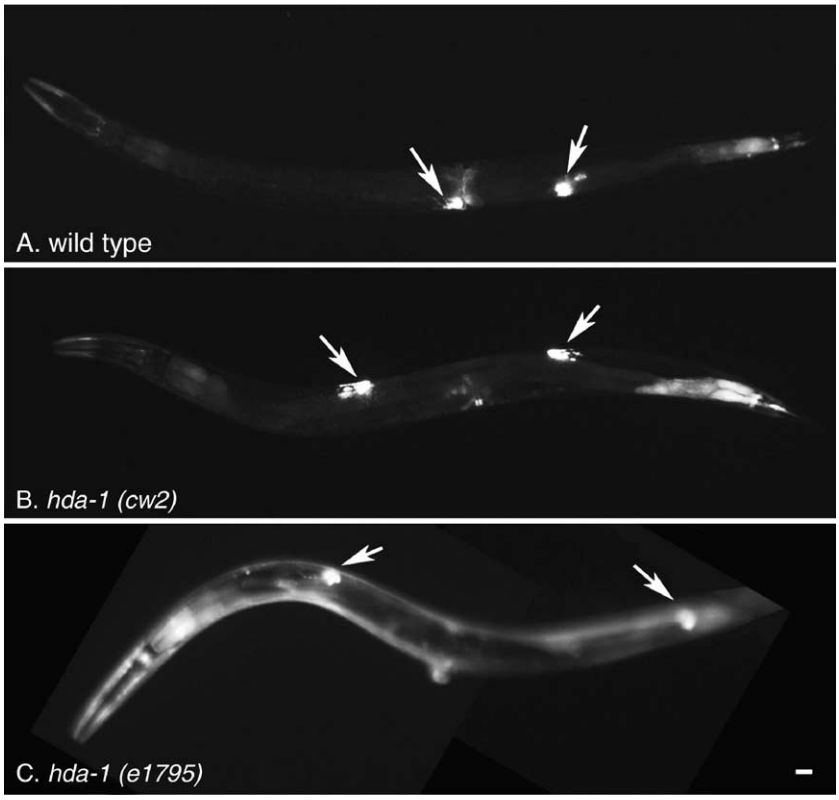

Fig. 7. *lag-2∷gfp* expression in *hda-1* mutants. Anterior is to the left and dorsal is up. All animals contain a *lag-2∷gfp* reporter transgene. (A) In wild type, GFP is most prominently detected within the distal tip cells (arrows). (B) In *hda-1(cw2)* mutants, GFP expression resembles that in wild type. (C) In *hda-1(e1795)* mutants, GFP expression spreads to many other cell types. Scale bar = 20 μm.

following a less direct trajectory to the dorsal nerve cord. The VNC is composed of two parallel bundles of neuronal processes, separated by a small ridge of hypodermal tissue (White et al., 1986). In *hda-1(cw2)* mutants, the processes of the VNC are poorly organized, with individual or small groups of axons frequently exiting and rejoining the ventral nerve cord. In addition, three neuron types, the CANs, HSNs, and QR descendants, fail to migrate to their proper position occasionally, although most do migrate fully. Our observations demonstrate that, in addition to its function in gonadogenesis and vulval development, *hda-1* is required for neuronal growth cone and cell migration. This is consistent with a recent report that identifies many extracellular matrix-encoding genes as targets of HDA-1 and shows that HDA-1 activity can affect cell invasiveness (Whetstine et al., 2005).

hda-1(cw2) reduces gene function

To interpret mutant phenotype, it is important to know the effect that the mutation produces on gene function. Although we cannot eliminate the possibility that *hda-1(cw2)* is a gain-of-function mutation, several observations argue that *hda-1(cw2)* reduces gene function. First, *hda-1(cw2)* is recessive for cell migration and Unc phenotypes, consistent with a reduction of gene function. Second, *hda-1(cw2)* in trans to a chromosomal deficiency that deletes the *hda-1* gene produces the same phenotype as homozygous *hda-1(cw2)* mutants, suggesting that the mutation reduces or eliminates gene function. Third, we find many of the same phenotypes in *hda-1(cw2)* and *hda-1(e1795)* mutants; both share defects in gonadal and vulval development and cell migration and HSN axon pathfinding defects, although with differing severity. Furthermore, both mutations semidominantly disrupt nervous system development as does *yDf8*, a chromosomal deletion that removes the entire *hda-1* locus. Because *hda-1(e1795)* is reported to be a null mutation (Dufourcq et al., 2002) and *yDf8* deletes the *hda-1* locus, and because both resemble *hda-1(cw2)* mutants phenotypically, the latter is likely to reduce function. The simplest interpretation of the data is that *hda-1(cw2)* reduces gene function.

hda-1(cw2) is predicted to change an arginine to cysteine at position 59. An arginine or lysine also is found at the corresponding position of other class I HDACs. Our analysis of *hda-1(cw2)* mutants is consistent with an arginine or lysine at this position being important for proper HDAC function. The mutation does not detectably alter the electrophoretic mobility or the abundance of HDA-1 protein. Furthermore, we detect no obvious difference in localization of HDA-1 in animals. Therefore, Arg59 is not required for protein stability or proper localization. Interestingly, *hda-1(cw2)* causes a dramatic increase in *hda-1* mRNA levels without producing a concomitant increase in protein levels. This suggests that either the additional mRNA fails to be translated or some posttranslational mechanism regulates protein abundance.

Models for HDA-1 function

Histone deacetylases are associated with transcriptional repression. Similarly, *C. elegans* HDA-1 has been shown to negatively regulate *end-1*, a gene that specifies endodermal fate (Zhu et al., 1997), and *lag-2*, a homolog of Delta/Serrate ligands for Notch receptors, expression (Calvo et al., 2001; Dufourcq et al., 2002; Henderson et al., 1994; Tax et al., 1994). Our results suggest that HDA-1 also may negatively regulate its own transcription and that of C53A5.1, an unrelated gene located on the same cosmid as *hda-1*; increased levels of both transcripts are detected in *hda-1(cw2)* mutants (Fig. 1C). Perhaps HDA-1 performs a similar regulatory function during nervous system development. In this model, the deregulation of a single or multiple gene(s) leads to the defects in cell migration and axon development seen in *hda-1* mutants.

In an attempt to identify possible targets of HDA-1 regulation, we reduced by half the wild-type copies of *unc-115*/abLim, *unc-40*/DCC, and *unc-73*/Trio, all involved in guidance or migration (Hedgecock et al., 1990; Lundquist et al., 1998; Steven et al., 1998), to determine whether axon guidance defects were suppressed. Reduction by half of *unc-115*, but not *unc-40* or *unc-73*, in *hda-1(cw2)* homozygotes partially suppresses the HSN axon defects, and results in an increased percentage of animals that escape the near-paralysis of most *hda-1(cw2)* mutants. A possible explanation of this result might be that *unc-115*, a regulator of *unc-115* expression or some component of the *unc-115* pathway, is derepressed in *hda-1(cw2)* mutants leading to its overexpression. Our observation that an *unc-115*∴*gfp* transgene is not detectably altered in expression by mutation in *hda-1* suggests that *unc-115* is not a direct target of *hda-1* regulation. Compromising the pathway by reducing *unc-115* by half restores the activity of the pathway to nearer normal, thereby suppressing the axon pathfinding defects and near-paralysis of *hda-1(cw2)* mutants. A recent report identifies a large number of extracellular matrix (ECM) genes as targets of *hda-1* repression (Whetstine et al., 2005). In light of this observation, perhaps the guidance and migration defects in *hda-1(cw2)* mutants result from elevated ECM components in a UNC-115-dependent manner. This may also explain why we isolated *hda-1(cw2)* from a screen for new *epi-1* alleles. Perhaps in *hda-1(cw2)*/+ animals, levels of ECM components are elevated and in this background, mutation of one copy of *epi-1*/laminin compromised nervous system development.

An alternative model for HDA-1 function in nervous system development is suggested by recent findings that histones are not the sole HDAC targets. Several HDACs have been shown to regulate the acetylation of target proteins other than histones, including tubulin, p53, and Hsp90 (Hubbert et al., 2002; Ito et al., 2002; Juan et al., 2000; Yao et al., 2001). An intriguing possibility is that perhaps HDAC regulates the deacetylation of some other protein, in which case it might play a more direct role in the processes of nervous system development that go awry in *hda-1(cw2)* mutants. In this model, the suppression of *hda-1* defects by *unc-115* might be due to HDA-1 regulating by acetylation a component of the UNC-115 pathway. Testing this model will require identification of the relevant targets of HDAC in nervous system development. In support of this model, the apparent high levels of HDA-1 protein detected in neuronal processes raise the possibility that an HDA-1 target is present within these processes.

Why do hda-1(cw2) and hda-1(e1795) phenotypes differ?

A partial explanation of the difference between *hda-1(cw2)* and *hda-1(e1795)* is that maternally provided product in *hda-1(e1795)* mutants derived from heterozygous mothers provides partial rescue. Consistent with this model, we find that locomotion and nervous system defects are less severe in *hda-1(cw2)* homozygotes derived from heterozygous mothers than those derived from homozygous mutant mothers. This is perhaps somewhat surprising in light of the observation that the HSN axon, for example, does not grow out until after the completion of embryogenesis, during larval development (Desai et al., 1988). Therefore, maternal HDA-1 or the modifications that it produces persist into larval stages.

However, maternal effect provides only a partial explanation; *hda-1(cw2)* homozygous mutant progeny of heterozygous mothers often are severely Unc, whereas *hda-1(e1795)* homozygotes are not. There are three plausible explanations for the differences. First, *hda-1(cw2)* could confer a new function on HDA-1. This seems unlikely in light of our observations that mutants in which *hda-1(cw2)* is placed in trans to a chromosomal deficiency, *yDf8*, which deletes the *hda-1* locus, are indistinguishable from homozygous *hda-1(cw2)* mutants. In addition, *hda-1(cw2)* and *hda-1(e1795)* produce similar defects, although with differing severity. Still, we cannot exclude the possibility that one or both are gain-of-function mutations. However, *hda-1(cw2)*, *hda-1(e1795)*, and *yDf8* dominantly produce the same HSN axon guidance defects, demonstrating that reducing *hda-1* by half disrupts HSN axon guidance and suggesting that all three similarly reduce, or in the case of *yDf8*, eliminate gene function. Second, *hda-1(cw2)* might be linked to a second mutation that produces Unc. However, the *hda-1(cw2)* mutation has been extensively outcrossed. Furthermore, recombinants identified during SNP mapping failed to separate Unc from the mutation in *hda-1*. Therefore, a second mutation would have to reside within the same 76 kb of DNA that includes *hda-1*. In addition, wild-type *hda-1* fully rescues the Unc phenotype of *hda-1(cw2)* mutants. And, *hda-1(e1795)* produces cell migration and axonal pathfinding defects, demonstrating that HDA-1 is required for normal nervous system development. Therefore, the possibility that a second, linked mutation causes the nervous system defects can be excluded. Third, perhaps *hda-1(e1795)* affects a subset of HDA-1 functions, but its nervous system function is not fully compromised. Identifying additional *hda-1* mutations including molecular nulls and examining resulting phenotypes will resolve this issue.

Acknowledgments

We thank the Caenorhabditis Genetics Center, Yang Shi and Erik Lundquist for providing strains used in this work. We thank Jennifer Kean, Megan Gavin and Christina MacLaren for their contributions to this project and Claire Walczak for critical reading of the manuscript and technical assistance. This work was supported by National Institutes of Health grant R01 HD37815.

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