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The *lin-11* LIM homeobox gene specifies olfactory and chemosensory neuron fates in *C. elegans*

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SUMMARY

Chemosensory neuron diversity in *C. elegans* arises from the action of transcription factors that specify different aspects of sensory neuron fate. In the AWB and AWA olfactory neurons, the LIM homeobox gene *lim-4* and the nuclear hormone receptor gene *odr-7* are required to confer AWB and AWA-specific characteristics respectively, and to repress an AWC olfactory neuron-like default fate. Here, we show that AWA neuron fate is also regulated by a member of the LIM homeobox gene family, *lin-11*. *lin-11* regulates AWA olfactory neuron differentiation by initiating expression of *odr-7*, which then autoregulates to maintain expression. *lin-11* also regulates the fate of the ASG chemosensory neurons, which are the lineal sisters of the AWA neurons. We show that *lin-11* is expressed dynamically in the AWA and ASG neurons, and that misexpression of *lin-11* is sufficient to promote an ASG, but not an AWA fate, in a subset of neuron types. Our results suggest that differential temporal regulation of *lin-11*, presumably together with its interaction with asymmetrically segregated factors, results in the generation of the distinct AWA and ASG sensory neuron types. We propose that a LIM code may be an important contributor to the generation of functional diversity in a subset of olfactory and chemosensory neurons in *C. elegans*.

Key words: *lin-11*, LIM homeobox, Chemosensory neurons, Fate specification, *Caenorhabditis elegans*

INTRODUCTION

In the olfactory and gustatory systems of vertebrates and invertebrates, diversity of sensory neuron function allows animals to fully experience the complexity of their chemical environment, and to make behavioral and developmental decisions accordingly. The distinct sensory functions of each olfactory and gustatory neuron type are defined by the expression of one or more members of the large olfactory or taste receptor gene families, in addition to the differential expression of other signaling genes (Adler et al., 2000; Chess et al., 1994; Clyne et al., 1999; Hatt and Ache, 1994; Meyer et al., 2000; Nef et al., 1992; Ressler et al., 1993; Strotmann et al., 1992; Vassar et al., 1993; Vosshall et al., 1999). The developmental mechanisms that generate this functional complexity are largely unknown.

The nematode *C. elegans* senses its chemical environment using a small number of relatively well-characterized chemosensory neurons. In total, the functions of 11 pairs of chemosensory neurons have been defined by laser ablation studies, showing that each neuron type responds to a distinct but partly overlapping set of chemicals (Bargmann and Mori, 1997; Troemel, 1999). Volatile odorants are sensed by the bilaterally symmetric AWA, AWB and AWC olfactory neuron types (Bargmann et al., 1993; Troemel et al., 1997). Aqueous attractants are sensed primarily by the ASE neuron type and pheromones by the ADF, ASI, ASG and ASJ neuron types (Bargmann and Horvitz, 1991a; Bargmann and Horvitz, 1991b; Schackwitz et al., 1996). Similar to vertebrates and *Drosophila*, the distinct response profile of each neuron type is defined by the expression of specific signaling molecules such as olfactory receptors, G proteins and channels (Prasad and Reed, 1999; Troemel, 1999).

The LIM subfamily of homeobox genes has been implicated in generating functional diversity in the nervous system. This class of transcriptional regulators contains two metal-binding LIM domains that mediate protein-protein interactions, and a DNA-binding homeodomain (Hobert and Westphal, 2000). LIM homeobox genes define a ‘combinatorial code’ for the specification of different classes of motorneurons in the central nervous system of vertebrates and *Drosophila* (Appel et al., 1995; Thor et al., 1999; Tsuchida et al., 1994). In the spinal cord of vertebrates, motorneuron subtypes can be distinguished on the basis of the positions of their soma, their axonal trajectory, their target choice and their patterns of gene expression. Distinct classes of postmitotic motorneurons express unique spatiotemporal combinations of LIM homeodomain proteins, leading to the proposal that these LIM

homeodomain proteins, along with other transcription factors, provide these neurons with their unique functional identities (Appel et al., 1995; Thor et al., 1999; Tsuchida et al., 1994). Support for this model has been obtained from knockout and misexpression experiments. In mice, the *Lhx3*/*Lhx4* LIM homeobox genes specify the distinct ventrally extending axonal trajectories of motorneurons (Sharma et al., 1998). In *Lhx3*/*Lhx4* double mutant mice, ventrally projecting motorneurons instead adopt the fate of motorneurons that project dorsally. Conversely, ectopic expression of *Lhx3* in the dorsally projecting motorneurons is sufficient to switch their trajectories to those of the ventral motorneurons (Sharma et al., 1998). Similarly, in *Drosophila*, the *lim3* and *islet* LIM homeobox genes are required to specify the distinctive identities and axonal trajectories of subsets of motorneurons (Thor et al., 1999; Thor and Thomas, 1997).

In *C. elegans*, members of the LIM homeobox gene family are expressed in largely non-overlapping sets of cell types, indicating that a combinatorial LIM code probably does not function in their specification (Hobert and Westphal, 2000). However, LIM homeobox genes regulate the development and differentiation of several neuron types in *C. elegans*, suggesting that these genes are important components of the suite of transcription factors that confers a unique identity to each neuron type. Several LIM homeobox genes have been implicated in sensory neuron function. The *mec-3* and *lim-4* LIM homeobox genes appear to play broad roles in the specification of the mechanosensory and the AWB olfactory neurons. In *mec-3* mutants, the six mechanosensory neurons are generated but fail to acquire differentiated features (Chalfie and Sulston, 1981; Way and Chalfie, 1988). In *lim-4* mutants, the AWB olfactory neurons fail to acquire any AWB-specific characteristics, but instead adopt an AWC-like default developmental state (Sagasti et al., 1999). The *ceh-14* and *lim-6* LIM homeobox genes regulate a more restricted subset of the features of the differentiated AFD thermosensory and the ASE chemosensory neurons, respectively (Cassata et al., 2000; Hobert et al., 1999). These findings indicate that LIM homeobox genes play crucial roles in the differentiation of a subset of sensory neuron types in *C. elegans*, and raise the question of whether members of this gene family are involved in the specification of sensory identity of additional chemosensory neurons.

We show that the LIM homeobox gene *lin-11* is required to specify the sensory identities of the AWA olfactory neurons, and their lineal sisters, the ASG chemosensory neurons. *lin-11* is a founding member of the LIM homeobox gene family, and was first identified on the basis of its role in regulating asymmetric cell divisions in vulval cell lineages (Ferguson and Horvitz, 1985; Ferguson and Horvitz, 1987; Freyd et al., 1990). Subsequent work has shown that *lin-11* also plays a role in the differentiation of the VC ventral cord motorneurons, and the AIZ interneurons (Hobert et al., 1998) which are a major component of the chemosensory and thermosensory circuits (White et al., 1986). In this study we show that in *lin-11* mutants, the AWA neurons lose most AWA-specific characteristics and instead adopt an AWC-like default state. *lin-11* functions by regulating expression of the AWA-specific *odr-7* nuclear hormone receptor gene, which has been previously implicated in promoting AWA-specific characteristics and repressing the AWC fate (Sagasti et al., 1999; Sengupta et al., 1994). *lin-11* also regulates the differentiation of the ASG neurons, such that in *lin-11* mutants, ASG-specific genes are not expressed. We show that the distinct functions of *lin-11* in the two neuron types are partly determined by the dynamic temporal expression pattern of *lin-11*. Moreover, we show that misexpression of *lin-11* is sufficient to cause ectopic expression of ASG but not AWA-specific genes. Our results provide evidence for hitherto undescribed functions of *lin-11*, and suggest that a LIM code may be an important contributor to the generation of functional diversity in the sensory neurons of *C. elegans*.

MATERIALS AND METHODS

Strains and constructs

Animals were grown under standard conditions (Brenner, 1974). Strains were obtained from the *Caenorhabditis* Genetics Center unless noted otherwise. Strains carrying the following stably integrated fusion genes were provided as indicated: *pkIs582* (*gpa-5*::GFP) (G. Jansen; Jansen et al., 1999); *ntIs1* (*gcy-5*::GFP) (S. Lockery; Yu et al., 1997); *oyIs17* (*gcy-8*::GFP) (J. Satterlee; Yu et al., 1997); *kyIs111* (*tax-2*::GFP), *kyIs128* (*str-3*::GFP), *kyIs104* (*str-1*::GFP), *kyIs140* (*str-2*::GFP), *oyIs14* (*sra-6*::GFP), *otIs24* (*sre-1*::GFP), *oyIs34* (*T08G3.3*::GFP), *kyIs37* (*odr-10*::GFP), *kyIs5* (*ceh-23*::GFP) and *kyIs29* (*glr-1*::GFP) (C. Bargmann; Coburn and Bargmann, 1996; Maricq et al., 1995; Peckol et al., 1999; Sagasti et al., 1999; Sengupta et al., 1996; Troemel et al., 1995; Troemel et al., 1997; Troemel et al., 1999; Zallen et al., 1999); *gmIs12* (*srb-6*::GFP) (G. Garriga; Troemel et al., 1995). The *gcy-10/odr-1*::RFP plasmid was provided by C. Bargmann (L'Etoile and Bargmann, 2000; Yu et al., 1997).

ops-1::GFP was constructed by amplifying approx. 2.0 kb of *T13A10.13* promoter sequences and fusing in frame to GFP. The *T13A10.13* predicted ORF shows the highest homology to the vertebrate rhodopsin genes, and is expressed in the ASG and ADL neurons in the head. *ops-1*::RFP was constructed by replacing GFP sequences with those encoding RFP (Matz et al., 1999). *lim-6*::GFP is a fusion of a 5.4 kb genomic fragment containing approx. 1.9 kb of promoter and all coding sequences of *lim-6* fused in frame to GFP. The expression in AWA and ASG observed with this fusion gene is repressed in a full-length *lim-6*::GFP rescuing construct (Hobert et al., 1999). These fusion genes were chromosomally integrated to generate *oyIs26* (*ops-1*::GFP) and *mgIs20* (*lim-6*::GFP).

osm-6::lin-11 and *odr-3::lin-11* were constructed by first replacing the GFP sequences in the expression vector pPD95.77 with the *lin-11* cDNA yk452F7. *osm-6* and *odr-3* promoter sequences were then inserted upstream (Collet et al., 1998; Roayaie et al., 1998). To make the GFP-tagged full-length *lin-11* construct, 3.3 kb of upstream promoter sequences, and all coding sequences of *lin-11* up to the STOP codon were amplified in two pieces and cloned in frame upstream of GFP in the expression vector pPD95.77.

Germline transformations

Germline transformations were performed using standard techniques (Mello and Fire, 1995). Coinjection markers used were the dominant marker pRF4 *rol-6(su1006)* at 100 ng/μl, and *ofm-1*::GFP at 50 ng/μl (Miyabayashi et al., 1999). All other plasmids were injected at 30 ng/μl.

Isolation of *odr-7(oy9)*

The *odr-7(oy9)* allele was isolated from the F2 progeny of a mutagenized strain carrying integrated copies of an *odr-7*::GFP fusion gene. *odr-7(oy9)* failed to complement the *odr-7(ky4)* null allele. To identify the molecular lesion in the *oy9* allele, *odr-7* genomic sequences were amplified from mutant animals in two independent

reactions, and the products were directly sequenced. The *odr-7(oy9)* strain was outcrossed a total of three times prior to characterization.

Generation of ODR-7 antibodies and immunocytochemistry

Sequences coding for residues 1-315 of ODR-7 were fused to GST coding sequences in the vector pGex3X. The purified fusion protein was injected into rabbits to generate polyclonal antibodies (CalTag Laboratories). Antibodies were used without affinity purification to stain adults using the Finney-Ruvkun protocol (Finney and Ruvkun, 1990). Embryos were stained using a modified protocol from Chin-Sang et al. (Chin-Sang et al., 1999). ODR-7 antibodies were used at a 1:20 dilution. Secondary antibodies were used as described previously (Sarafi-Reinach and Sengupta, 2000).

Microscopy

Animals were examined using a Zeiss Axioskop microscope with epifluorescence. Images were captured using a CCD digital camera (Hamamatsu) and PCI digital frame grabber. Images were analyzed and pseudocolored using OpenLab (Improvision) and Adobe Photoshop software.

RESULTS

lin-11 regulates gene expression in the AWA olfactory neurons

The LIM homeobox genes *lim-4*, *lim-6* and *ceh-14* regulate different aspects of fate and function of the AWB olfactory, the ASE chemosensory and the AFD thermosensory neurons respectively (Cassata et al., 2000; Hobert et al., 1999; Sagasti et al., 1999). To determine if members of the LIM homeobox family also play a role in the functional specification of the AWA olfactory neurons, we examined expression of the AWA-specific *odr-7* nuclear hormone receptor gene in animals carrying null mutations in the LIM homeobox genes *mec-3*, *ttx-3*, *ceh-14*, *lim-4*, *lim-6* and *lin-11*. *odr-7* has previously been shown to be required for all known AWA functions (Sengupta et al., 1994). As shown in Table 1, we find that expression of *odr-7* is altered in *lin-11* mutants, whereas expression in other LIM homeobox gene mutants is unaffected (data not shown). Approximately 80% *of lin-11(n389)* null mutants show loss of expression of an *odr-7*::GFP fusion gene in at least one AWA neuron. To confirm the observed loss of *odr-7* expression, we stained wild-type and *lin-11* mutant adult animals with polyclonal antibodies against ODR-7 (see Materials and Methods). This antibody is specific for ODR-7, since no staining is observed in *odr-7(ky4)* putative null mutants (data not shown). Antibody staining of *lin-11(n389)* mutants confirms the loss of ODR-7 expression observed with the *odr-7*::GFP marker (Fig. 1; Table 1).

Loss of *odr-7* expression could result either from the inability of *lin-11* mutants to maintain *odr-7* expression into adulthood, or from a defect in initiation of *odr-7* expression. To distinguish between these possibilities, we examined *odr-7*::GFP expression in young *lin-11(n389)* L1 larvae. As shown in Table 1, *odr-7* expression is also lost in larvae, suggesting that *lin-11* plays a role in initiating *odr-7* expression.

To determine the extent to which AWA-specific gene expression is affected in *lin-11* mutants, we examined the expression of two additional AWA-specific genes, the olfactory receptor gene *odr-10* and the G-protein α subunit gene *gpa-5* (Jansen et al., 1999; Sengupta et al., 1996). Expression of both genes is also reduced in *lin-11* mutants (Table 1), suggesting that *lin-11* regulates the expression of several, if not all, AWA-

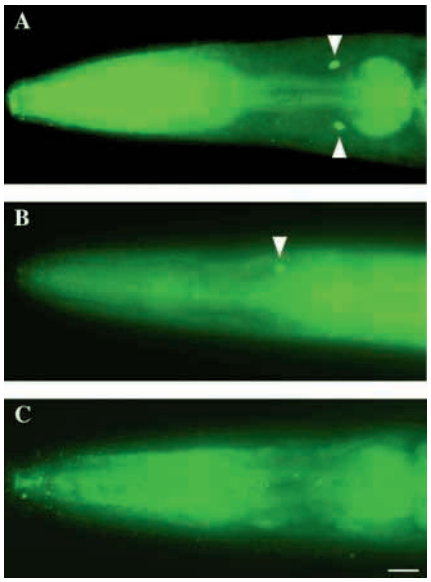
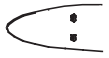


Fig. 1. ODR-7 expression is lost in *lin-11(n389)* mutants. Wild-type (A) and *lin-11(n389)* (B,C) animals stained with anti-ODR-7 antibodies show loss of ODR-7 expression in one (B) or both (C) AWA neurons in *lin-11* mutants (top down view). Arrowheads point to ODR-7 expression. Anterior is at left. Scale, 10 μm.

Table 1. Expression of AWA-specific genes is lost in *lin-11* mutants

		% of animals with expression pattern		
Strain	*n*			
Adults				
odr-7::GFP	112	100	0	0
lin-11(n389); odr-7::GFP	314	21.7	51.0	27.4
Wild-type (ODR-7 Ab)	318	99.4	0	0.6
lin-11(n566) (ODR-7 Ab)	144	52.1	41.7	6.3
lin-11(n389) (ODR-7 Ab)	134	20.1	43.3	36.6
odr-10::GFP	103	98.1	1.0	1.0
lin-11(n389); odr-10::GFP*	522	23.2	52.5	22.0
gpa-5::GFP	266	99.6	0.4	0
lin-11(n389); gpa-5::GFP‡	347	9.8	47.3	42.4
unc-86(n846) (ODR-7 Ab)	125	97.6	1.6	0.8
ttx-3(mg158) (ODR-7 Ab)	110	100	0	0
L1 Larvae				
odr-7::GFP	178	100	0	0
lin-11(n389); odr-7::GFP§	215	37.7	45.1	16.7

*1.0% of animals showed expression in two cells on one side and one on the other. 1.1% of animals showed expression in two cells on one side and none on the other.

‡0.3% of animals showed expression in two cells on one side and one on the other. 0.3% of animals showed expression in two cells on one side and none on the other.

Since *gpa-5*::GFP is expressed at low levels in wild-type animals, a slight decrease in *gpa-5*::GFP expression in *lin-11* mutants is scored as loss of expression, as compared to similar decreases in the expression of *odr-7*::GFP or *odr-10*::GFP.

§0.5% of animals showed expression in two cells on one side and one on the other.

Where applicable, the expression of integrated transgenes was examined.

specific genes. We were unable to determine whether loss of AWA-specific gene expression in *lin-11* mutants is correlated with a loss of AWA-mediated olfactory functions, owing to both the uncoordinated backward locomotion exhibited by *lin-11* mutants (Freyd, 1991), and defects in the differentiation of the AIZ postsynaptic partners of the AWA neurons (Hobert et al., 1998). It is interesting to note, however, that even in *lin-11(n389)* null mutants, the loss of expression of AWA markers is incompletely penetrant, suggesting that *lin-11* acts together with another unidentified gene(s) to regulate AWA cell fate.

Mutations affecting fate and function of the AIY and AIZ interneurons have no effect on AWA-specific gene expression

The major postsynaptic partners of the AWA neurons are the AIY and AIZ interneurons (White et al., 1986). *lin-11* is expressed in the AIZ interneurons and has been shown to be required for their function (Hobert et al., 1998). However, expression of *lin-11* in the AWA neurons had not previously been described. Therefore, it is possible that the defect in AWA gene expression results from the loss of *lin-11* function in the AIZ interneurons, with *lin-11* acting non cell-autonomously to regulate AWA cell fate. To address this issue, we examined ODR-7 expression in mutants in which the fate and function of the AIY and AIZ interneurons are affected. In *unc-86* mutants, the AIZ neurons are not generated because of a lineage defect (Chalfie et al., 1981), while in *ttx-3* mutants, the AIY neurons fail to differentiate correctly (Altun-Gultekin et al, 2001; Hobert et al., 1997). However, we find that ODR-7 expression is unaffected in both mutants (Table 1). ODR-7 expression is also unaffected in animals mutant in components of general synaptic transmission such as *snt-1* and *unc-13* (data not shown; Ahmed et al., 1992; Nguyen et al., 1995; Nonet et al., 1993). These results indicate that *lin-11* does not regulate AWA cell fate via a retrograde signaling mechanism from its AIZ and AIY postsynaptic targets.

A functional *lin-11*::GFP expression construct is expressed in several sensory neurons, including the AWA and ASG neurons and in the AVA interneurons

The expression patterns of *lin-11*::reporter fusion genes have been described (Freyd, 1991; Hobert et al., 1998; Newman et al., 1999). *lin-11* expression was observed in uterine and vulval cells and in several neuron types, including the ADL and ADF sensory neurons and the AIZ interneurons (Freyd, 1991; Hobert et al., 1998; Newman et al., 1999). However, the fusion genes used did not contain all the genomic sequences of *lin-11*, leaving open the possibility that sequences contained within the remaining introns direct expression in additional cell types. Moreover, since the minimal genomic sequences required for *lin-11* function were not defined by rescue of *lin-11* mutant phenotypes, the complete promoter sequences required for *lin-11* activity remained undetermined. To examine the expression of *lin-11* in more detail, we generated a full length GFP-tagged *lin-11* fusion gene containing all identified exons and introns, in addition to 3.3 kb of upstream promoter sequences (Fig. 2A). This fusion gene was stably integrated into the genome, and was used to examine rescue of *lin-11* mutant phenotypes. As shown in Table 2, both the *odr-7* loss-of-expression phenotype and

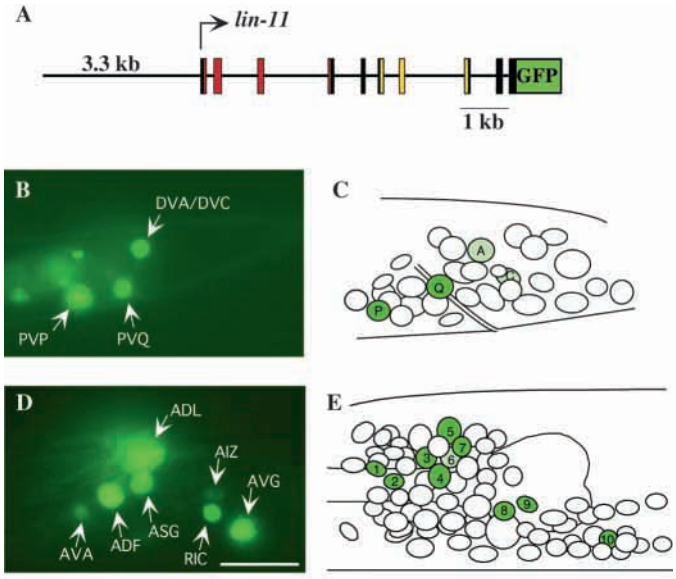


Fig. 2. Neuronal expression of *lin-11*::GFP. (A) Schematic of the *lin-11*::GFP fusion gene, which includes 3.3 kb of promoter sequences and all exons and introns of *lin-11*. GFP is fused in frame prior to the STOP codon in the last exon. Exons encoding the two LIM domains are in red; exons encoding the homeodomain are in yellow. (B,C) Expression in the lumbar ganglion. (C) Schematic of the region shown in B. P, PVP; Q, PVQ; A, DVA; C, DVC; we cannot unambiguously identify whether the expressing cell is DVA or DVC. (D,E) Expression in head neurons. (E) Schematic of head neuron expression shown in D, including neurons out of the plane of focus. 1, AVA; 2, AVE; 3, ADF; 4, ASH; 5, ADL; 6, AWA (where expression is seen embryonically - see text); 7, ASG; 8, RIC; 9, AIZ; 10, AVG. Expression is also observed in additional unidentified cells (see text). Anterior is at left. Scale, 10 μm.

Table 2. Rescue of *lin-11(n389)* phenotypes by *lin-11*::GFP*

	ODR-7 expression			Egg-laying behavior		
Strain	% wild-type	% mutant‡	*n*	% wild-type	% mutant‡	*n*
lin-11(n389)	18.6	81.4§	70	0	100	56
lin-11(n389); lin-11::GFP	97.9	2.1¶	95	67.5	32.5	80
lin-11::GFP	99.2	0.8**	118	90.7	9.3	97

**lin-11*::GFP was stably integrated into the genome.

‡Mutant for ODR-7 expression is defined as loss of anti-ODR-7 antibody staining in one or both AWA neurons. Mutant for egg-laying is defined as animals that fail to lay eggs.

§1.4% expressed ODR-7 ectopically on one side.

¶1.1% expressed ODR-7 in two neurons on one side, and none on the other.

**This animal showed expression in two neurons on one side, and one on the other.

egg-laying defect of *lin-11* mutants are rescued by this transgene, indicating that its expression pattern probably reflects the expression pattern of the endogenous gene.

Expression of *lin-11*::GFP is first observed after the time when most postmitotic neurons are born (at approx. 300 minutes postfertilization), suggesting that *lin-11* plays a role in regulating differentiation, but not generation, of these neurons. In larvae, the LIN-11::GFP fusion protein is localized to the nuclei of multiple neurons in the head and lumbar ganglia (Fig. 2B-E). In addition, *lin-11* expression is observed in uterine and vulval cells, as well as in the VC motor neurons as previously described (data not shown; Freyd, 1991; Hobert et al., 1998).

We characterized the neuronal expression of *lin-11* in further detail. In the lumbar ganglia, *lin-11* expression is observed in the PVPL/R and PVQ neurons, and in an additional unpaired neuron that we tentatively identified as DVC or DVA (Fig. 2B,C). In contrast to shorter *lin-11*::GFP fusion genes (Hobert et al., 1998), the rescuing *lin-11*::GFP fusion gene is not expressed in the PHA sensory neurons. In the head, as previously reported using shorter reporter fusions, we observed *lin-11* expression in the sensory neurons ADF and ADL, in the interneurons AIZ, RIC and AVG, and in another neuron type tentatively identified as either AVH or AVJ (Fig. 2D,E) (Freyd, 1991; Hobert et al., 1998). However, we also observed expression in a number of additional neurons using the full-length *lin-11*::GFP fusion gene. *lin-11* expression was observed in the AVA and AVE interneurons (Fig. 2D,E); the AVA interneurons are the primary command interneurons for the circuit that drives backward locomotion (Chalfie et al., 1985). Expression in AVA might provide a rationale for the uncoordinated backward locomotion of *lin-11* mutants (see below). We also detected faint and occasional expression in the ASH polymodal sensory neurons.

Interestingly, we observed *lin-11* expression in the AWA neurons in embryos and young larvae, but not in later stages. As shown in Fig. 3A-C, expression of *lin-11* in the AWA neurons is observed consistently in three-fold embryos, where expression of *lin-11* overlaps with that of ODR-7 (32/32 AWA neurons examined). Expression decreases by hatching such that in L1 larvae, expression in the AWA neurons is fainter and observed only occasionally, and is absent in later larvae and adults (Fig. 3D). We also observed *lin-11* expression strongly and consistently in the ASG chemosensory neurons (Fig. 3E), which are the lineal sisters of the AWA neurons (Sulston et al., 1983). Expression in the ASG neurons is observed throughout postembryonic development. Thus, *lin-11* expression is regulated differentially and in a dynamic manner in two neuron types (AWA and ASG) that result from the asymmetric division of a precursor neuroblast. We also detected *lin-11* expression in a few other non-sensory neurons in the head and tail that we did not identify definitively.

lin-11 initiates *odr-7* expression which then autoregulates to maintain expression in the AWA neurons

In *odr-7* mutants, all known aspects of differentiated AWA fate are lost (Sengupta et al., 1994). *odr-7* is expressed throughout postembryonic development in the AWA neurons (Sengupta et al., 1994), suggesting that continuous *odr-7* expression is required to maintain AWA cell fate. However, since *lin-11* is expressed only during early development in the AWA neurons, *lin-11* is likely required to initiate but not maintain *odr-7* expression. To determine whether ODR-7 maintains its own expression by autoregulation, we examined the expression of GFP driven by the *odr-7* promoter in an *odr-7* null mutant. The *odr-7(oy9)* allele is likely to be null, based on both the nature of its molecular lesion (Q231 to STOP in exon 2) resulting in a predicted truncated protein product, and a lack of detectable ODR-7 protein upon staining with anti-ODR-7 antibodies (data not shown). We find that expression of *odr-7*::GFP is detected in approx. 96% of young L1 larvae in *odr-7* null mutants (n>100), but is abolished in later larvae and adult animals (only 1% express *odr-7*::GFP; n>100), indicating that ODR-7 autoregulates to maintain its expression in the AWA neurons.

LIN-11 regulates AWA cell fate primarily by regulating *odr-7*

In *odr-7* null mutants, approx. 9% of animals retain expression

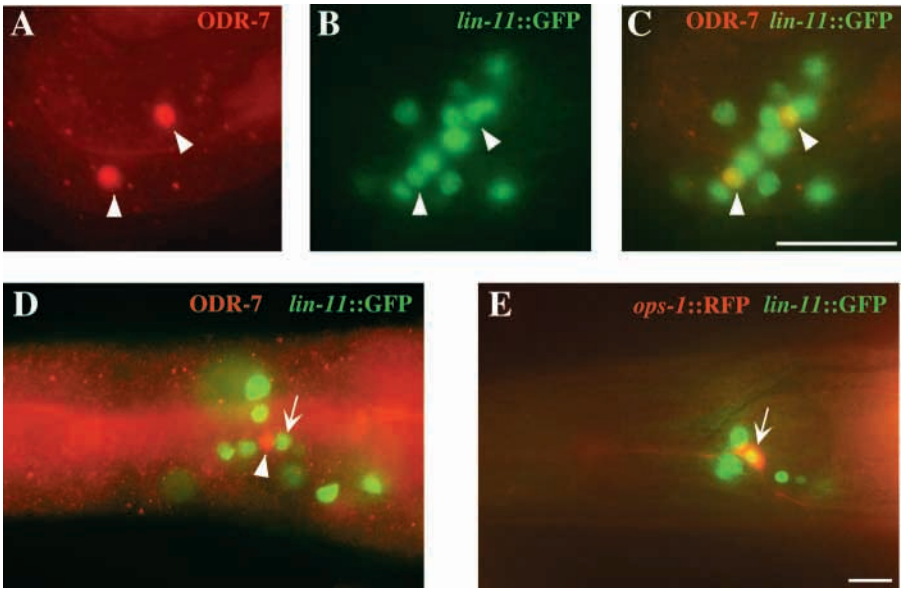


Fig. 3. *lin-11* is expressed dynamically in the AWA and ASG neurons. (A) Anti-ODR-7 antibody staining (red) of a three-fold embryo expressing stably integrated *lin-11*::GFP. (B) Same embryo as in A, visualizing *lin-11*::GFP expression (green). (C) Merge of A and B, showing *lin-11* expression in the AWA neurons. (A-C) Arrowheads indicate AWA neurons. Scale, 10 μm. (D) Lateral view of an adult animal expressing ODR-7 (red) in AWA (arrowhead) and *lin-11*::GFP (green) in other head neurons including ASG (arrow). *lin-11* is not expressed in AWA in adults. (E) Lateral view of an adult expressing *lin-11*::GFP (green) in head neurons including ASG (arrow), which also expresses the ASG marker *ops-1*::RFP (red). (D,E) Anterior is at left. Scale, 10 μm.

Table 3. LIN-11 regulates *odr-10*::GFP expression by regulating *odr-7*

Strain	*n*	% of animals with *odr-10*::GFP expression in 2 AWA	1 AWA	0 AWA
odr-10::GFP*	103	98.1	1.0	0
lin-11(n389); odr-10::GFP‡	107	28.0	47.7	21.5
odr-10::GFP; *odr-7(ky4)*§	108	1.9	7.4	90.7
lin-11(n389); *odr-10*::GFP; *odr-7(ky4)*§	112	5.4	11.6	83.0

*1.0% showed expression in two neurons on one side, and one on the other.
‡0.9% showed expression in two neurons on one side, and one on the other. 1.9% showed expression in two neurons on one side, and none on the other.
§These strains also contain *unc-9(e101)*.
The expression of integrated transgenes was examined in all cases.

of an *odr-10*::GFP fusion gene in at least one AWA neuron (Table 3; Sengupta et al., 1996), raising the possibility that ODR-7 acts together with another unidentified gene product(s) to specify AWA fate. LIN-11 could specify AWA fate by regulating the expression of only *odr-7*, or could regulate additional genes. To address this issue, we examined the expression of an *odr-10*::GFP fusion gene in a *lin-11; odr-7* double mutant. If LIN-11 acts by regulating genes in addition to *odr-7* in the AWA neurons, we might expect to observe a stronger *odr-10*::GFP expression defect in *lin-11; odr-7* double mutant animals than in *odr-7* single mutants. However, as shown in Table 3, the *odr-10*::GFP expression defect is not enhanced in *lin-11; odr-7* double mutants. Thus, LIN-11 appears to promote AWA cell fate primarily by regulating *odr-7* expression.

The AWA neurons adopt partial AWC-like fate in *lin-11* mutants

The AWA and AWB olfactory neurons have been shown to share a common AWC-like default developmental potential (Sagasti et al., 1999). This potential is modified by the expression of *odr-7* and *lim-4* respectively to result in the generation of three distinct olfactory neuron types. Thus, in *odr-7* mutants, the AWA neurons adopt partial AWC-like fate and express the AWC-specific olfactory receptor gene *str-2* (Sagasti et al., 1999). Since LIN-11 regulates *odr-7* expression, we might expect that the AWA neurons would also adopt AWC fate in *lin-11* mutants. We find that approximately 33% of *lin-11* mutants express *str-2*::GFP ectopically in at least one AWA neuron (Fig. 4; Table 4). We also examined the expression of additional AWC markers, including the guanylyl cyclase gene *gcy-10/odr-1* and the cyclic nucleotide channel subunit gene *tax-2* (Coburn and Bargmann, 1996; L'Etoile and Bargmann, 2000; Yu et al., 1997). Although we failed to observe expression of *gcy-10/odr-1* in the AWA neurons (data not shown), approx. 12% of *lin-11(n389)* mutants ectopically express *tax-2*::GFP in at least one AWA neuron (*n*=65). Thus, the AWA neurons appear to adopt partial AWC-like characteristics in *lin-11* mutants. However, the extent of fate conversion is clearly incomplete, based on both incomplete expression of AWC-specific markers, as well as incomplete transformation of AWA ciliary morphology. The AWA, AWB and AWC olfactory neurons can be morphologically distinguished by their distinctive specialized ciliary endings (Perkins et al., 1986; Ward et al., 1975). In *lim-4* mutants, the cilia of the AWB neurons resemble those of the AWC neurons (Sagasti et al., 1999). However, in *lin-11* mutants, the cilia of the AWA neurons acquire a morphology intermediate between those of AWA and AWC (data not shown).

A higher percentage of *odr-7* mutants than *lin-11* mutants exhibit ectopic expression of *str-2*::GFP in at least one AWA neuron (78% versus 33%; Table 4). This difference may be partly attributed to the incomplete loss of *odr-7* expression in

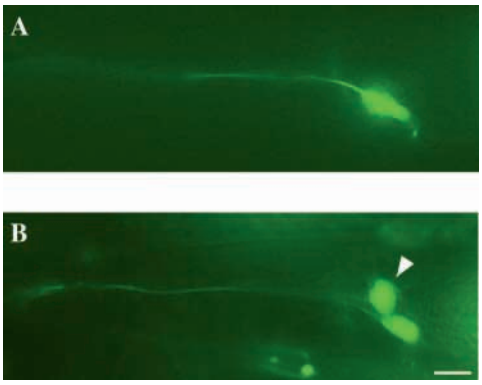
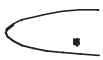
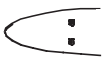


Fig. 4. The AWA neurons express the AWC marker *str-2*::GFP in *lin-11(n389)* mutants. (A) Wild-type animals express *str-2*::GFP in one AWC neuron. (B) In *lin-11(n389)* mutants, the AWA neurons (arrowhead) can also express *str*-2::GFP. Lateral views, anterior is at left. Scale, 10 μm.

Table 4. The AWA neurons express the AWC-specific marker *str-2* in *lin-11* mutants

Strain	*n*	% of animals with *str-2*::GFP expression pattern 			
str-2::GFP	106	98.1	0	1.9	0
lin-11(n389); str-2::GFP*,‡	114	66.7	18.4	10.5	3.5
str-2::GFP; *odr-7(ky4)*§	107	22.4	29.0	14.0	34.6
lin-11(n389); *str-2*::GFP; *odr-7(ky4)* §,¶,**	148	47.3	26.4	16.9	8.8

*0.9% of animals showed expression in two cells per side.
‡71.5% of ectopic expression was in neurons identified as AWA; occasional expression was also seen in ASG, ADL, and ADF (*n*=123 identified).
§These strains also contain *unc-9(e101)*.
¶0.7% of animals showed expression in three cells on one side and one on the other.
**71% of ectopic expression was in neurons identified as AWA; occasional expression was also seen in ADF (*n*=17 identified).
Expression of an integrated *str-2*::GFP transgene was examined.

lin-11 mutants (Table 1). However, this fails to account fully for the difference observed. Instead, this difference may arise partly from the fact that *str-2* is a marker for only one of two alternative AWC cell fates. *str-2* is expressed stochastically in only one AWC neuron in wild-type animals, and this expression is regulated by interneuronal calcium signaling between the two AWC neurons (Sagasti et al., 2001; Troemel et al., 1999). In *odr-7* mutants, it is possible that the AWA neurons have acquired an intermediate AWC fate that does not always carry out this signaling correctly, such that 35% of *odr-7* null mutants express *str-2* in both AWA neurons (Table 4). Since approx. 37% of *lin-11* mutants lack *odr-7* expression in both AWA neurons similar to *odr-7* null mutants (Table 1), we might expect that 35% of these animals (or a total of 13% of *lin-11* mutants) would also express *str-2* in both AWA neurons. However, as shown in Table 4, only approx. 3.5% of *lin-11* mutants misexpress *str-2* in both AWA neurons. It is possible that in *lin-11* mutants, bilateral *str-2* expression may be regulated similarly to its regulation in the AWC neurons. This ability to correctly signal the *str-2* 'ON/OFF' decision may represent a more complete transformation towards the AWC fate in *lin-11* mutants as compared to *odr-7* mutants. Consistent with this, we find that the number of animals expressing *str-2* in both AWA neurons in a *lin-11; odr-7* double mutant is similar to that in *lin-11* single mutants (Table 4). These results suggest that although *lin-11* appears to act primarily through *odr-7* to promote AWA fate, *lin-11* may regulate genes in addition to *odr-7* to modulate the default AWC fate in the AWA neurons.

lin-11 regulates gene expression in the ASG lineal sisters of the AWA neurons

Since *lin-11* is also expressed in the ASG lineal sisters of the AWA neurons, we examined whether ASG cell fate specification requires *lin-11* function, by examining expression of ASG markers in *lin-11* mutants. Markers examined include *ops-1*::GFP (see Materials and Methods), *unc-30*::GFP (expressed in the ASG neurons in the head; Jin et al., 1994), and a truncated *lim-6*::GFP fusion gene that is expressed in the AWA and ASG neurons (see Materials and Methods). In all cases, we observed loss of ASG-specific gene expression in *lin-11* mutants (Table 5). The loss of ASG marker expression is also incompletely penetrant in *lin-11* null mutants, suggesting that *lin-11* functions with other gene(s) in the ASG neurons to regulate ASG fate.

We have previously shown that in *unc-130* mutants, the ASG neurons adopt AWA fate, and that these neurons express the underlying default *str-2* 'ON' AWC fate only upon loss of both *unc-130* and *odr-7* gene function (Sarafi-Reinach and Sengupta, 2000). Consistent with this, we find that few *lin-11(n389)* mutant animals express *str-2*::GFP in ASG (<6%; *n*=123), indicating that loss of LIN-11 function alone is not sufficient for adoption of the *str-2*-expressing AWC fate in the ASG neurons.

Since LIN-11 regulates gene expression in both the AWA and ASG neurons, we wished to determine whether loss of gene expression in either an AWA or ASG neuron is correlated with a loss of gene expression in its lineal sister. We examined loss of *ops-1*::GFP expression in animals costained with

Table 5. Expression of ASG-specific genes is lost in *lin-11* mutants

Strain	% of animals with ASG marker expression: Normal	Reduced/absent in at least one ASG neuron	*n*
ops-1::GFP	100	0	104
lin-11(n389); ops-1::GFP	20.9	79.1	115
Ex[*unc-30*::GFP] Line 1*	51.9	48.1	106
Ex[*unc-30*::GFP] Line 2	48.0	52.0	100
Ex[*unc-30*::GFP]; *lin-11(n389)* Line 1‡	0	100	105
Ex[*unc-30*::GFP]; *lin-11(n389)* Line 2	1.6	98.4	63
lim-6::GFP§	100	0	110
lin-11(n389); lim-6::GFP	23.3	76.7	103

*2% of these animals showed expression in 2 ASG on one side, and one on the other.

‡Lines generated in *lin-11* are independent of those generated in wild-type animals.

§This *lim-6*::GFP fusion gene is expressed strongly in ASG and faintly in AWA (see Materials and Methods).

The expression of stably integrated marker::GFP fusion genes was examined, with the exception of *unc-30*::GFP.

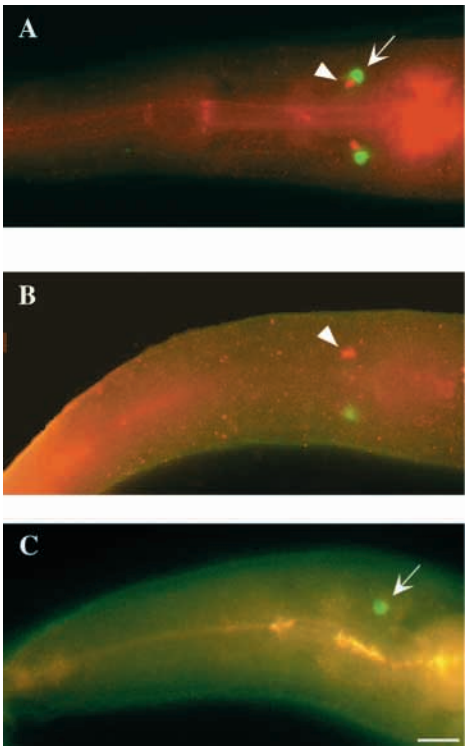


Fig. 5. Loss of ODR-7 expression does not always correlate with loss of ASG marker expression in *lin-11(n389)* mutants. (A) Wild-type worms expressing the integrated ASG marker *ops-1*::GFP (green, arrow) stained using anti-ODR-7 antibodies (red, arrowhead). ODR-7 and *ops-1*::GFP are both visible. (B,C) *lin-11(n389)* mutants expressing *ops-1*::GFP (green) stained with anti-ODR-7 antibodies (red). B shows an animal in which ODR-7 expression (arrowhead), but not *ops-1*::GFP expression, is seen on one side. On the opposite side, *ops-1*::GFP expression is visible; ODR-7 expression is out of the plane of focus. C shows an animal in which *ops-1*::GFP expression (arrow), but not ODR-7 expression, is seen on one side. The other side lacks both ODR-7 and *ops-1*::GFP expression. Anterior is at left; top down views. Scale, 10 μm.

antibodies against ODR-7. As shown in Fig. 5, we find that loss of ASG-specific gene expression does not strictly correlate with loss of expression of AWA-specific markers. This result suggests that a defect in fate specification of an AWA or ASG neuron does not result in a concomitant defect in the differentiation of its lineal sister.

lin-11 function is required for ectopic expression of AWA fate in *unc-130* mutants

In *unc-130* mutants, the ASG neurons ectopically express *odr-7* and fail to express ASG-specific markers (Sarafi-Reinach and Sengupta, 2000). *unc-130* is expressed in the precursors to the AWA and ASG neurons, and encodes a predicted forkhead domain transcription factor (Nash et al., 2000; Sarafi-Reinach and Sengupta, 2000). We have previously proposed that *unc-130* is required for the correct expression and/or segregation of cell fate determinants in the AWA/ASG precursors. Since *lin-11* is initially expressed in both the AWA and ASG neurons, asymmetric division of the precursors likely results in the differential segregation of other components that specify the distinct AWA and ASG neuron types. Although we have shown that expression of *odr-7* is regulated by LIN-11 in the AWA neurons, it is possible that in *unc-130* mutants, *odr-7* expression in the ASG neurons does not require LIN-11. To address this, we examined the expression of *odr-7* in *lin-11; unc-130* double mutants. If expression of *odr-7* is independent of *lin-11* in the ASG neurons, we might expect to observe a small number of animals expressing *odr-7* in both the AWA and ASG neurons in *lin-11; unc-130* double mutants. However, as shown in Table 6, *lin-11; unc-130* double mutants exhibit a defect at least as severe as *lin-11* single mutants, with no animals expressing *odr-7* ectopically. This result demonstrates that misexpression of *odr-7* in the ASG neurons in *unc-130* mutants requires *lin-11* function.

Ectopic expression of *lin-11* is sufficient to drive ASG- but not AWA-specific gene expression in a subset of neurons

We have shown that *lin-11* is necessary for the expression of both AWA and ASG neuronal fates. To determine whether *lin-11* is also sufficient for either fate, we first misexpressed *lin-11* using the *odr-3* promoter. *odr-3* drives expression strongly in the AWC neurons, and weakly in four other neuron types (Roayaie et al., 1998). Misexpression of *lim-4* in the AWC neurons using the *odr-3* promoter is sufficient for fate conversion of the AWC neurons to AWB fate (Sagasti et al., 1999). However, we find that misexpression of *lin-11* in the AWC neurons does not result in the ectopic expression of either *odr-7* or *ops-1*::GFP in the AWC neurons (data not shown), indicating that in the AWC neurons, *lin-11* expression from the *odr-3* promoter is not sufficient to drive either AWA or ASG cell fate.

To determine whether *lin-11* misexpression in neurons other than AWC is sufficient for expression of AWA or ASG fate, we misexpressed *lin-11* using the *osm-6* promoter. *osm-6* drives expression in most ciliated neurons, including all chemosensory neurons (Collet et al., 1998). We first examined expression of the *ops-1*::GFP gene in transgenic animals carrying the *osm-6::lin-11* construct. As shown in Fig. 6, we observed misexpression of *ops-1*::GFP in a small subset of ciliated neurons in the head ganglia. The majority of these neurons are in the anterior ganglia, which contain sensory neurons of largely unknown function (White et al., 1986). In addition, we occasionally identified faint expression of *ops-1*::GFP in the AWA and ASJ chemosensory neurons. Expression of *lin-11* from the *osm-6* promoter rescues the *ops-1*::GFP expression defect in the ASG neurons in *lin-11* mutants (data not shown). *ops-1*::GFP is expressed in both the ASG and ADL neurons. Since *lin-11* is also expressed in the ADL neurons, it is possible that misexpression of *lin-11* drives ectopic expression of ADL and not ASG fate. To address this, we examined the expression of additional ASG markers (*unc-30*::GFP and *lim-6*::GFP), and an ADL marker (*sre-1*::GFP; Troemel et al., 1995) in transgenic animals carrying the *osm-6::lin-11* construct. Both ASG markers, but not the ADL marker, were expressed ectopically (data not shown). However, clearly not all neurons in which *osm-6* is expressed adopt the ASG fate. This result indicates that *lin-11* is sufficient for ASG fate expression in some, but not all ciliated neurons. Interestingly, the ectopic expression of ASG fate does not appear to be dependent on *unc-130* function in most cell types, since we continued to observe misexpression of *ops-1*::GFP in *unc-130* mutants (not shown).

We also determined whether *osm-6::lin-11* is sufficient to

Table 6. *lin-11* is required for expression of ODR-7 in the ASG neurons in *unc-130* mutants

		% of animals with ODR-7 Ab staining pattern		
Strain	*n*	Wild-type	Ectopic in at least one neuron	Loss in at least one neuron
Wild-type	110	100	0	0
*unc-130(ev505)**	105	29.5	56.2	9.6
lin-11(n389)	54	9.2	0	90.7
lin-11(n389); unc-130(ev505)	84	1.2	0	98.8

*4.8% showed ectopic expression on one side and loss on the other.

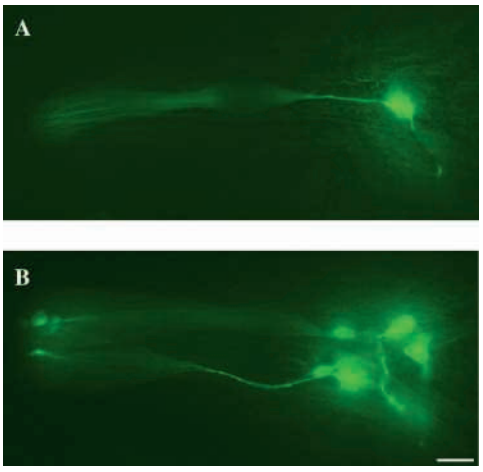


Fig. 6. Misexpression of *lin-11* is sufficient to promote ASG marker expression in a subset of neurons. (A) In wild-type animals, *ops-1*::GFP is visible in an ASG neuron (lateral view). (B) In transgenic animals carrying *osm-6*::*lin-11*, expression of an integrated *ops-1*::GFP fusion gene is visible in additional ciliated neurons. Anterior is at left. Scale, 10 μm.

promote AWA cell fate by examining the expression of *odr-7* in animals carrying this transgene. We find that *odr-7* is not expressed ectopically upon misexpression of *lin-11*. Instead, misexpression of *lin-11* resulted in the occasional loss of *odr-7* expression in the AWA neurons (4 independent lines; range of loss in at least one AWA neuron 14-63%; $n>50$ for each line). Expression driven by the *osm-6* promoter appears to peak at L1 larval stages but is maintained into adulthood (Collet et al., 1998). We suggest that the prolonged expression of *lin-11* in the AWA neurons from the *osm-6* promoter results in the occasional alteration of AWA fate and loss of AWA-specific gene expression. Since misexpression of *osm-6::lin-11* results in only occasional weak expression of ASG-specific markers in the AWA neurons (see above), this suggests that temporal misregulation of *lin-11* expression in the AWA neurons is only partly sufficient to promote ASG fate.

Role of *lin-11* in additional neuron types

In addition to the AWA and ASG neurons, *lin-11* is also expressed in additional sensory neurons including the ADF, ADL and ASH sensory neurons. To determine whether *lin-11* is necessary for the specification of these neurons, we examined the expression of markers for each neuron type, including genes encoding putative olfactory receptors, guanylyl cyclases and transcription factors. We also examined the ability of *lin-11* animals to correctly fill with the lipophilic dye DiI. We find that gene expression in the neurons ADL, ADF, ASH, AWB and ASE is largely unaffected in *lin-11* mutants, as is the overall pattern of DiI filling (Table 7).

We also examined gene expression in the AVA interneurons. Ablation of the AVA interneurons results in animals that are unable to move backwards normally (Chalfie et al., 1985; Zheng et al., 1999). The paired-type homeodomain gene *unc-42* is expressed in the AVA interneurons, and regulates expression of a subset of genes required for AVA function, including the ionotropic glutamate receptor genes *glr-1, glr-4* and *glr-5* (Baran et al, 1999; Brockie et al., 2001; Hart et al., 1995; Maricq et al., 1995). To determine the role of *lin-11* in the AVA interneurons, we examined expression of a *glr-1*::GFP fusion gene in the AVA interneurons in *lin-11* mutants. We find that *glr-1* expression in these neurons is unaffected (Table 7). Since UNC-42 directs *glr-1* expression in the AVA interneurons, this result suggests that LIN-11 does not regulate *unc-42* in these neurons, and that *lin-11* may function downstream or in a parallel pathway. We examined *lin-11*::GFP expression in *unc-42(e419)* mutants and found that *lin-11* expression in the AVA interneurons is unaltered in *unc-42* mutants (17/17 animals). Several other NMDA and non-NMDA glutamate receptors are also expressed in the AVA interneurons and may be required for AVA interneuron function (Brockie et al., 2001; Zheng et al., 1999). It is possible that *lin-11* regulates a subset of these genes to modulate AVA function, and hence locomotion.

Table 7. *lin-11* mutants do not exhibit gene expression defects in most other sensory neurons

		% with normal expression in		
Neurons	Marker	Wild-type	*lin-11(n389)*	*n*
ASH	*sra-6*::GFP	100	100	>100
ADL	DiI	100	100	>30
	ceh-23::GFP	97.0	96.1	>30
	sre-1::GFP	100	97.0	>67
ADF	*T08G3.3*::GFP	100	92.6*	>100
	srb-6::GFP	98.3	96.7	>57
ASER	*gcy-5*::GFP	100	100	>100
ASEL	*lim-6prom*::GFP‡	100	100	>100
AWB	*str-1*::GFP	100	100	>100
ASI	*str-3*::GFP	100	99.2§	>100
AFD	*gcy-8*::GFP	100	98.3§	>100
AVA	*glr-1*::GFP	ND	100	20¶

*5.7% of these showed two expressing cells on one side, and one on the other; 0.8% had one on one side and none on the other; 0.8% had two per side.

‡Hobert et al., 1999.

§The remainder of these had two expressing cells on one side, and one on the other.

¶AVA neurons in these animals were identified by position.

The expression of stably integrated marker::GFP fusion genes was examined in all cases (see Materials and Methods).

DISCUSSION

We have shown that the LIM homeobox gene *lin-11* is necessary for the functional specification of the AWA olfactory and ASG chemosensory neurons. In the AWA neurons, LIN-11 functions by regulating expression of the transcription factor *odr-7*. In addition, *lin-11* is sufficient in certain cellular contexts to promote ASG but not AWA fate. *lin-11* is expressed postmitotically and dynamically in both cell types. Our findings suggest that the temporal dynamic regulation of *lin-11* in these two neuron types, coupled with its interaction with other cell type-specific factors, result in the specification of the distinct AWA and ASG chemosensory neurons.

A model for *lin-11* function in the specification of the AWA and ASG neurons

Temporally regulated transcription factor cascades are critical mediators of neuronal cell fate specification. During *Drosophila* neurogenesis, sequential expression of the transcription factors Hunchback (Hb), POU-homeodomain proteins 1 and 2 (Pdm; also known as *nubbin*; nub), and Castor (Cas) in the CNS neuroblasts regulates neuroblast identity and sublineage generation (Brody and Odenwald, 2000; Kambadur et al., 1998). This sequential Hb→Pdm→Cas transition appears to be regulated in a neuroblast-intrinsic manner, in the absence of cell-cell communication. In the AWA neurons, a similar temporally regulated cascade of transcription factors is required for fate specification. In *C. elegans*, expression of most LIM homeobox genes appears to be maintained throughout the postmitotic life of neurons (Hobert and Westphal, 2000). However, results from this work show that *lin-11* is expressed only during a brief temporal window, shortly after the birth of the AWA neurons. This expression is sufficient to activate *odr-7*, which then autoregulates to maintain its expression and the expression of AWA-specific characteristics. Our data suggest that forced prolonged expression of *lin-11* in the AWA neurons may be partly sufficient to alter AWA fate, indicating that strict temporal control of *lin-11* expression is critical for correct AWA fate specification. In vertebrates, the expression pattern of LIM homeobox genes is also regulated temporally, and is an important component in determining cell type identity. *Isl1* is

first expressed early in all motorneurons, while expression of other LIM homeobox genes follows in a temporally stereotyped manner (Appel et al., 1995; Ericson et al., 1992; Pfaff et al., 1996; Tsuchida et al., 1994; Varela-Echavarria et al., 1996). Expression of the *Lhx3* and *Lhx4* genes has also been shown to be dynamic (Sharma et al., 1998). It will be interesting to investigate whether other LIM homeobox genes in *C. elegans* are also regulated dynamically.

What are the mechanisms by which *lin-11* expression is down-regulated in the AWA, but not in the ASG neurons? Maintenance of expression in one, but not the other sibling cell may arise from cell-cell interactions. Notch signaling between the two daughters of the pIIA sensory neuron progenitors in *Drosophila* has been shown to be required for the autorepressive and autoactivating functions of Suppressor of Hairless (Su(H)) in each of the two daughter cells, resulting in high levels of Su(H) in one, but not the other cell (Barolo et al., 2000). We have shown that a defect in specification of an AWA or ASG neuron does not result in a fate defect in the sibling neuron, suggesting that cell-cell signaling between the AWA and ASG neurons may not be the primary mediator of regulation of *lin-11* expression. Instead, we suggest that this difference in the temporal pattern of *lin-11* expression arises from the asymmetric segregation of factors to the AWA and ASG neurons. We have previously shown that the forkhead domain transcription factor UNC-130 plays an important role in the asymmetric division giving rise to the AWA and ASG neurons (Sarafi-Reinach and Sengupta, 2000). We propose that UNC-130 function in the AWA/ASG precursors is necessary for correct segregation of factors required for modulation of LIN-11 function in these two neurons. These factors could function to maintain *lin-11* expression in the ASG neurons, or to repress *lin-11* expression in the AWA neurons later in development. It is also possible that LIN-11 is autoregulatory, and functions along with these factors to maintain or repress its expression.

We also propose that UNC-130 regulates the asymmetric segregation of factors that work together with LIN-11 to regulate either AWA- or ASG-specific gene expression (Fig. 7). In *unc-130* mutants, incorrect segregation of factors to the ASG neurons may result both in the downregulation of *lin-11* expression and promotion of AWA-specific gene expression, thereby converting the ASG neurons to an AWA fate. These factors are likely to be different for each of the AWA and ASG cell types (X for AWA; Y for ASG in Fig. 7). The functions of LIM homeobox genes have been shown to be modified by interaction with a number of different proteins, including members of the paired-type homeodomain family, POU-homeodomain proteins and other LIM homeobox proteins (Bach et al., 1997; Bach et al., 1995; Feuerstein et al., 1994; Jurata et al., 1998; Schmeichel and Beckerle, 1994; Xue et al., 1993). It is unlikely that *lin-11* works with other LIM homeobox genes in the AWA and ASG neurons, since none of the six additional identified LIM homeobox genes in *C. elegans* is expressed in the AWA or ASG neurons, and mutations in these genes do not affect *odr-7* expression (Hobert and Westphal, 2000; this work).

It has been noted previously that *lin-11* is expressed in cell types that make direct physical connections with each other (Hobert et al., 1998; Newman et al., 1999). For instance, *lin-11* is expressed in the ADL neurons that synapse onto the AIZ interneurons (this study; Hobert et al, 1998). Similarly, *lin-11* is expressed in the VC and PVQ synaptic pairs (this study; Hobert et al, 1998). *lin-11* has also been shown to be expressed in components of the developing hermaphrodite egg-laying machinery, including the innervating VC motorneurons, uterine cells and vulval cells that must contact each other correctly in order to assemble a functional egg-laying system (Freyd, 1991; Hobert et al., 1998; Newman et al., 1999). Results in this study extend these observations, since we found that *lin-11* is expressed in the AWA-AIZ and ASH-AVA sensory neuron-postsynaptic interneuron pairs. We speculate that *lin-11* may regulate the expression of genes required for homophilic adhesion in members of each connected circuit. Intriguingly, in the uterine cells, but not in the VC neurons (O. H., unpublished), *lin-11* has been shown to regulate the *cdh-3* gene, encoding a member of the cadherin superfamily implicated in cell-cell adhesion (Newman et al., 1999; Pettitt et al., 1996). Similar coordinate expression of transcription factors has been noted in the sensory-motor circuit in the vertebrate spinal cord, where it has been shown that expression of the same ETS gene by different motorneuron pools and their corresponding sensory afferents may be required for functional synaptic connections (Arber et al., 2000; Lin et al., 1998).

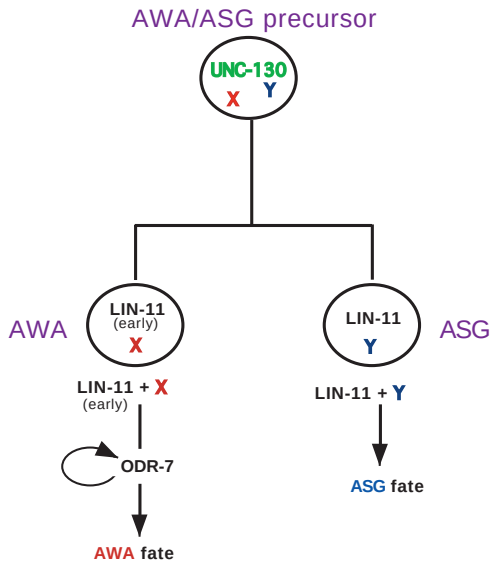


Fig. 7. Proposed model of the roles of LIN-11 in AWA and ASG specification. The forkhead domain transcription factor UNC-130 may function in the AWA/ASG precursors to asymmetrically allocate the unidentified determinants X and Y to the AWA and ASG neurons respectively. LIN-11 is expressed early in AWA, where it may act with X to initiate ODR-7 expression. ODR-7 then autoregulates as LIN-11 expression decreases. LIN-11 is expressed throughout adulthood in ASG, where it may act with Y to establish an ASG fate.

A LIM code for olfactory neurons?

The *C. elegans* genome is predicted to encode a total of seven LIM homeobox genes. Of these, five are expressed in largely non-overlapping sets of chemosensory neurons, raising the possibility that a LIM code (although non-combinatorial), may function in some chemosensory neurons for the specification of their functional identities. *lin-11* is expressed in the AWA, ASG, ASH, ADF and ADL chemosensory neurons (this study;

Freyd, 1991; Hobert et al., 1998); *lim-4* is expressed in the AWB olfactory neurons; *ceh-14* is expressed in the AFD thermosensory neurons (Cassata et al., 2000); *lim-6* is expressed in the ASE chemosensory neurons (Hobert et al., 1999); and *ttx-3* is expressed in the ASI and ADL chemosensory neurons (Altun-Gultekin et al., 2001). The ADL neurons are the only sensory neuron type that expresses more than one LIM homeobox gene. In addition, each of these LIM homeobox genes is also expressed in a number of other neuronal and non-neuronal cell types.

These LIM homeobox genes appear to specify different aspects of neuronal cell fate in different sensory cell types. Sensory functions and gene expression in the ADL neurons are largely unaltered even in *lin-11; ttx-3* double mutants (Altun-Gultekin et al., 2001). In *ceh-14* mutants, the AFD neurons have defective function, and somewhat defective ciliary morphology, but the overall pattern of differentiation, including gene expression, appears unaltered (Cassata et al., 2000). However, similar to the *mec-3* gene in the mechanosensory neurons, the *lin-11* and *lim-4* genes appear to specify overall aspects of the functional identities of the AWA, ASG and AWB chemosensory neurons. Thus, in the mechanosensory and some chemosensory neurons, LIM homeobox genes act to promote most, if not all, cell type-specific characteristics, and in the case of the olfactory neurons, to repress a default AWC-like developmental state. Misexpression of *lim-4*, but not *lin-11* confers AWB fate to the AWC neurons (Sagasti et al., 1999; T. M. and P. S., unpublished), suggesting that LIN-11 and LIM-4 have distinct protein-specific functions, as opposed to similar functions distinguished only by expression in different cellular contexts (Li and Noll, 1994; O'Keefe et al., 1998). Thus, although LIM homeobox genes appear to play different roles in different sensory cell types, we suggest that a LIM code is an important determinant of final differentiated identities of a subset of sensory neurons.

The distinct functional identity of a cell type is defined by the unique complement of transcription factors expressed in it at defined times. This study shows that LIM homeobox genes are crucial components of these combinatorial transcription factor profiles, and in a subset of sensory neurons are sufficient to confer most, if not all cell type-specific characteristics. In other sensory neurons, LIM homeodomain proteins function together with other transcription factors to confer cell fate. These factors are expected to be expressed in specific subsets of sensory neurons in a lineage-dependent manner. For example, while *unc-130* is clearly required to repress the AWA fate and promote ASG fate in the ASG neurons, it is not required for ASG-specific gene expression when *lin-11* is ectopically expressed in other neuron types, suggesting that factors that work with *lin-11* can be regulated by independent signals in different lineages. The fate of a single cell is determined by combinatorial logic integrating multiple developmental signaling modules, with the output at the level of gene regulation (Flores et al., 2000; Ghazi and VijayRaghavan, 2000; Halfon et al., 2000; Mitani et al., 1993; Wray, 1998; Xu et al., 2000; Yuh et al., 1998). It is the combined and precisely coordinated expression of transcription factors that allows a neuron to adopt an identity that distinguishes it from other neuron types. This study has led to the identification of additional components of the developmental cascades that result in the generation of two specific sensory neurons. In the future, it will be important to identify the complete complement of genes and regulatory modules that work together in specific combinations to generate the many different neuron types that constitute the nervous system.

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