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unc-8, a DEG/ENaC Family Member, Encodes a Subunit of a Candidate Mechanically Gated Channel That Modulates *C. elegans* Locomotion

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Summary

Mechanically gated ion channels are important modulators of coordinated movement, yet little is known of their molecular properties. We report that *C. elegans unc-8*, originally identified by gain-of-function mutations that induce neuronal swelling and severe uncoordination, encodes a DEG/ENaC family member homologous to subunits of a candidate mechanically gated ion channel. *unc-8* is expressed in several sensory neurons, interneurons, and motor neurons. *unc-8* null mutants exhibit previously unrecognized but striking defects in the amplitude and wavelength of sinusoidal tracks inscribed as they move through an E. coli lawn. We hypothesize that UNC-8 channels could modulate coordinated movement in response to body stretch. *del-1*, a second DEG/ENaC famly member coexpressed with *unc-8* in a subset of motor neurons, might also participate in a channel that contributes to nematode proprioception.

Introduction

Mechanical signaling, the process by which stretch or tension is transduced into cellular responses, plays critical roles in the function of virtually all cells (French, 1992; Sackin, 1995). Thus, it is striking that although mechanotransduction constitutes the basis of touch sensation, hearing, balance, and proprioception and influences cell volume regulation, fertilization, and differentiation, very little is known about the molecular properties of the proteins that mediate conversion of mechanical signals into appropriate biological activities. While it is established that specialized channels in both prokaryotes and eukaryotes respond to mechanical forces, only a single cloned gene, *E. coli mscL,* has been demonstrated to encode a mechanically gated ion channel (Sukharev et al., 1994). MscL oligomerizes to form a hexameric channel in the bacterial inner membrane (Blount et al., 1996), which, in the absence of any other proteins, can be gated by membrane stretch (Sukharev et al., 1994). Specialized mechanically gated eukaryotic ion channels, such as the elusive hair cell channel of the cochlea, require tension-generating interactions with proteins inside and outside the transducing cell in order to function (see Hudspeth, 1989; Pickles and Corey, 1992) and are likely to be more complex in their molecular composition. Clearly, isolation of the genes encoding tension-gated ion channels and their associated proteins will be required before detailed mechanisms of mechanotransduction can be elaborated.

A candidate eukaryotic mechanotransducing channel includes the *mec-4* and *mec-10* proteins required for function of six specialized neurons that mediate body touch sensitivity in *C. elegans* (reviewed in Chalfie, 1995; Corey and García-Añoveros, 1996; García-Añoveros and Corey, 1996; Driscoll and Kaplan, 1997). *mec-4* and *mec-10* are expressed almost exclusively in these six touch receptor neurons (Mitani et al., 1993; Huang and Chalfie, 1994) and encode similar proteins characterized by two transmembrane domains and a large extracellular domain that includes three Cys-rich regions (Huang and Chalfie, 1994; Lai et al., 1996). Specific *mec-4* and *mec-10* mutant alleles [*mec-4(u231*) and *mec-10(A673V)*] induce neuronal swelling and degeneration, a property that led to the designation of the *C. elegans* gene family as the degenerin family (Driscoll and Chalfie, 1991). *deg-1* (Chalfie and Wolinsky, 1990) and *unc-105* (Liu et al., 1996) are also members of this family. The *C. elegans* degenerins exhibit significant sequence similarity to subunits of the epithelial Na^+ channel (the ENaC family; Canessa et al., 1993, 1994; Chalfie et al., 1993), which plays a key role in Na^+ readsorption in the distal kidney, colon, and lung (reviewed in Palmer, 1992; Rossier et al., 1994). By analogy, MEC-4 and MEC-10 are thought to be ion channel subunits and, although the activity of the MEC-4/MEC-10 channel has not yet been assayed directly, experiments with chimeric nematode–rat proteins in transgenic *C. elegans* strains (Hong and Driscoll, 1994) and in an oocyte expression system (Waldmann et al., 1995) are consistent with such a model. Genetic analyses support the idea that MEC-4 and MEC-10 subunits are coassembled into a multimeric channel in the touch receptor neurons (Hong and Driscoll, 1994; Huang and Chalfie, 1994; Gu et al., 1996). Another gene required for body touch sensitivity, *mec-6*, is essential for the toxicity of *mec-4(u231)*, i.e., *mec-6* mutations block neurodegeneration induced by the dominant *mec-4(u231)* allele (Chalfie and Wolinsky, 1990). *mec-6* has been postulated to encode a subunit or channel-associated protein critical to the assembly and/or function of the mechanically gated channel, but its molecular identity is as yet unknown. Molecular analyses of additional genes required for touch transduction in *C. elegans* have led to the identification of extra- and intracellular proteins that may link the channel to the extracellular matrix and to a specialized cytoskeletal network in the touch receptor neurons to form a functional mechanotransducing complex (Huang et al., 1995; Du et al., 1996).

Several other *C. elegans* behaviors are likely to be influenced by mechanically gated ion channels. For example, an animal will back up in response to a head-on collision with an object in its path. The nose touch stimulus is sensed mainly by the ASH, FLP, and OLQ neurons (Kaplan and Horvitz, 1993). Head withdrawal in response to light touch on the side of the nose is mediated by the mechanosensory OLQ and IL1 neurons (Hart

et al., 1995). A response to harsh touch, such as a prod with a metal wire, detectable when the six touch receptor neurons are defective, is mediated by the PVD neurons (Way and Chalfie, 1989). It has also been proposed that stretch sensors in motor neuron processes influence the propagation of the sinusoidal body wave that characterizes *C. elegans* locomotion (see White et al., 1986). A pressing question that remains to be answered is whether degenerin family members encode channel subunits that contribute to distinct mechanosensitive behaviors in *C. elegans*.

The database compiled by the *C. elegans* Genome Sequencing Consortium includes a total of 15 degenerin-related genes at the time of this writing. Our characterization of these genes has led to the identification of two that are expressed in motor neurons and subsets of sensory neurons. One of these, R13A1_4, corresponds to the *unc-8* locus, which was identified by semidominant alleles that cause severe incoordination (Brenner, 1974; Park and Horvitz, 1986a) and transient neuronal swelling (Shreffler et al., 1995). *unc-8* is expressed in the ASH and FLP sensory neurons and in several classes of ventral cord motor neurons. A second degenerin, E02H4_1, defines a new gene (designated *del-1* for degenerin-like), expressed exclusively in the FLP sensory neurons and the VA and VB motor neurons (a subset of *unc-8*-expressing neurons that have been proposed to be stretch sensitive [White et al., 1986]). Interestingly, we find that tracks inscribed in an E. coli lawn by *unc-8* null mutants are significantly reduced in amplitude and wavelength, consistent with a role of *unc-8* in the modulation of coordinated locomotion. We propose that UNC-8 could participate in different mechanically gated channels in various cell types, and we discuss ways in which UNC-8 channels could respond to local body stretch to influence sinusoidal locomotion.

Results

Two Members of the *C. elegans* DEG/ENaC Superfamily Are Harbored in Cosmids R13A1 and E02H4

In a database search of sequences reported by the *C. elegans* Genome Sequencing Consortium (Wilson et al., 1994), we identified 11 novel predicted proteins with sequence similarity to the DEG/ENaC family members. Analysis of the expression patterns of nine of these degenerin genes revealed that a few are expressed specifically in the nervous system (N. T., and M. D., unpublished data). Here, we report characterization of the two degenerin genes harbored on cosmids R13A1 (R13A1_4) and E02H4 (E02H4_1), the primary sequences of which were confirmed by sequencing reverse transcription–polymerase chain reaction (RT–PCR) products and cDNA clones. R13A1_4 and E02H4_1 are 59% and 71% similar to MEC-4, respectively. Alignment of the amino acid sequences of R13A1_4 and E02H4_1 with characterized *C. elegans* degenerins established that these genes also encode proteins with two similarly positioned hydrophobic domains and three Cys-rich domains (Figure 1).

The R13A1_4 Degenerin Is Expressed in Specific *C. elegans* Motor Neurons, Interneurons, and Sensory Neurons

To determine the spatial and temporal expression pattern of the R13A1_4 degenerin, we constructed a reporter gene that included 1 kb of the putative 5′ regulatory region plus 48 N-terminal amino acids fused to the *E. coli lacZ* gene ($p_{R13A1-4}$*lacZ*) and stained transgenic animals harboring the reporter gene for β-galactosidase activity (Figure 2). Because we found extensive staining in the *C. elegans* nervous system, we performed cell identifications by characterizing progeny of ZB166, a transgenic line that harbors the reporter gene on an extrachromosomal array. The extrachromosomal array occasionally fails to segregate to both daughters of a cell division such that mosaic animals are generated within a population. Individual stained cells could be visualized in some of these mosaic animals, which enabled us to identify neurons by their process morphologies and positions relative to other cells (Sulston and Horvitz, 1977; Sulston et al., 1983; White et al., 1986). We also characterized the $p_{R13A1-4}$*lacZ* expression pattern of a strain in which the reporter construct was stably integrated into the genome (ZB167), which gave results consistent with those observed for mosaic animals.

$p_{R13A1-4}$*lacZ* activity is apparent in the embryo (Figure 2A) and is first detected during the 3-fold stage, about 300 min prior to hatching. This staining is likely to occur in the embryonically derived DA, DB, and/or DD motor neurons. β-galactosidase activity is observed in additional neurons later in development and peaks during the L2 stage when intense staining of sensory neurons, interneurons in the nerve ring, and motor neurons in the ventral nerve cord are apparent (Figure 2B). Strong staining persists into adulthood. Among the neurons that express $p_{R13A1-4}$*lacZ* are the ASH and FLP sensory neurons, the PVM touch neuron, the PVC, AVB, AVA, and AVD command interneurons, and the PDA, PDB, DA, DB, DD, VA, VB, and VD classes of motor neurons (examples are shown in Figures 2C–2E). Additional neurons situated either within or in the vicinity of the nerve ring express the R13A1_4 reporter gene. An identical expression profile was observed when a second fusion, including 2.5 kb upstream of the predicted initiation codon, was assayed (data not shown). In summary, our data suggest that R13A1_4 expression is neuron specific and restricted to about 45 cells.

Degenerin R13A1_4 Corresponds to *unc-8*

Because R13A1_4 is expressed widely in the nervous system, we consulted the *C. elegans* genetic map to identify genes in the neighborhood of cosmid R13A1 (right arm of Chromosome IV between *deb-1* and *egl-19*) known to affect locomotion. *unc-8*, which maps to this region and shares several genetic properties with degenerin family members (Shreffler et al., 1995), emerged as a strong candidate locus for R13A1_4. Since genetic characterization had shown that the uncoordinated phenotype of *unc-8(gf)* animals could be ameliorated by the addition of a wild-type copy of the gene (Park and Horvitz, 1986a; Shreffler et al., 1995), we first looked for evidence that R13A1_4 corresponds to the

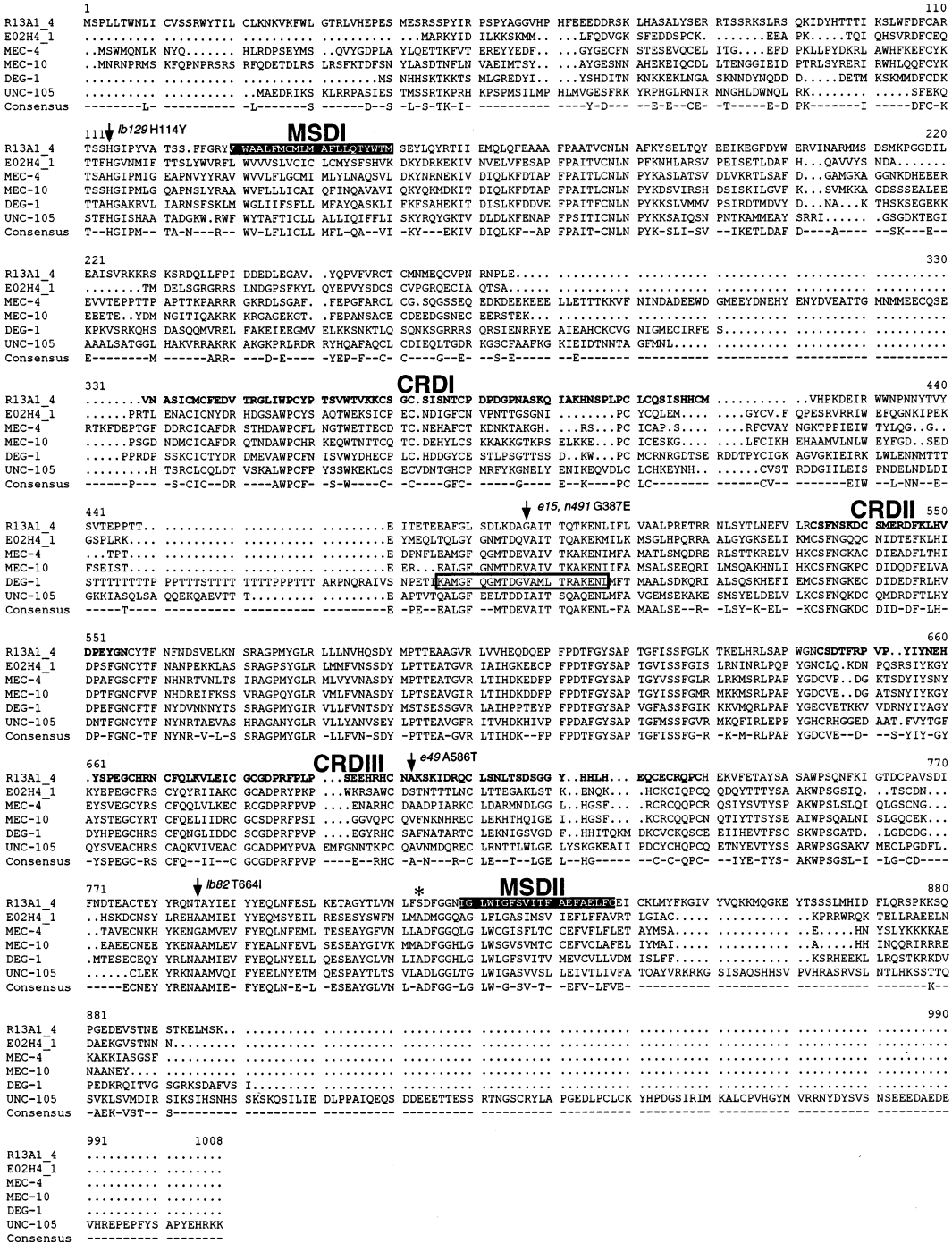


Figure 1. R13A1_4 and E02H4_1 Are New Degenerin Family Members. Shown Is a Sequence Alignment of R13A1_4, E02H4_1, and Characterized Members of the *C. elegans* Degenerin Family

Primary sequences of R13A1_4 and E02H4_1 were verified by sequencing cDNAs or RT–PCR products. Note that the open reading frames that we determined differed in places from those predicted by the Genefinder program. Alignment was performed using the PILEUP program of the Genetics Computer Group sequence analysis package (Needleman and Wunsch, 1970). The consensus line highlights residues common to >50% of the *C. elegans* family members. Degenerin domains indicated are predicted membrane-spanning domains MSDI and MSDII (black box), Cys-rich domains CRDI, CRDII, and CRDIII (bold), and the extracellular region implicated in channel closing (enclosed white box; García-Añoveros et al., 1995). The site of toxic amino acid substitutions encoded by dominant *mec-4* and *deg-1* alleles is marked by an asterisk. The amino acid changes in semidominant *unc-8* alleles *e15*, *e49*, and *n491*, *trans*-suppressor *e15lb129,* and *trans*-enhancer *n491lb82* are indicated by arrows.

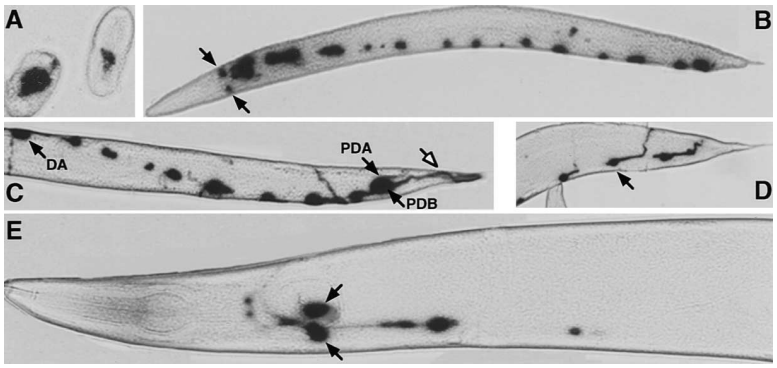


Figure 2. The Gene Encoding R13A1_4 Is Expressed in Specific *C. elegans* Sensory Neurons, Interneurons, and Motor Neurons

(A) Embryonic expression of $p_{R13A1-4}$*lacZ* in late stage ZB167 embryos.

(B) L2 stage ZB167: staining in the ventral nerve cord is apparent. Arrows indicate the ASH cell bodies.

(C–E) Representative neurons staining in ZB166 adults that are mosaic for the presence of the $p_{R13A1-4}$*lacZ* reporter construct. Note that a slight distortion in process morphology and positioning appears because transgenic animals express *rol-6(su1006)*, which causes the body to have a twisted configuration.

(C) Staining of PDA, PDB, and DA motor neuron processes helps confirm cell identities. PDA has a cell body situated in the preanal ganglion and a process that crosses the body and turns to run anteriorly; PDB has a cell body situated in the preanal ganglion that extends nearly to the end of the tail where it turns back to run anteriorly; the white arrow highlights the PDB process. DA motor neurons have cell bodies in the ventral cord; processes cross the body and extend anteriorly.

(D) DA and DB motor neurons in the ventral cord extend processes that cross the body and extend along the dorsal side.

(E) FLP sensory neuron with identifying processes that project anteriorly and encircle the pharynx. Arrowheads indicate FLP cell bodies. The more anterior faintly staining cells are the two ASH neurons; the more posterior darkly staining cell with an anteriorly directed process is a VA motor neuron.

unc-8 locus by introducing cosmid R13A1 into *unc-8(e15)* and assaying backward locomotion in transgenic lines. Approximately 80% of transgenic animals from three independent lines exhibited significantly improved backward locomotion compared to animals harboring the transformation marker alone (Figure 3A). Motor neuron swelling caused by *unc-8(gf)* mutations was also suppressed in transgenic lines (data not shown). These results suggest that the *unc-8* gene is encoded by sequences contained within cosmid R13A1.

We established that R13A1_4 corresponds to the *unc-8* locus in three ways. First, we identified restriction fragment length polymorphisms within the R13A1_4 coding region in three *unc-8* loss-of-function alleles derived from a strain in which transposition occurs at a high frequency. PCR amplification experiments revealed that two *unc-8(lf)* alleles, *e15lb143* and *e15lb147*, harbor Tc1 insertions within R13A1_4 sequences (Figure 3B). Another *unc-8* allele, *e15lb145*, harbors a deletion within R13A1_4 boundaries that removes CRDII and much of CRDIII.

In a second experiment, we introduced R13A1_4 sequences from the most severe *unc-8* allele *(n491)* into wild-type animals and tested transgenic animals for locomotory defects. Using a protocol for PCR amplification of long DNA fragments (Barnes, 1994; see Experimental Procedures), we purified sequences encoding R13A1_4 and approximately 2.5 kb 5′ to the predicted initiation codon from *unc-8(n491)* mutants and from wild-type animals. Amplified DNA was coinjected into wild-type animals with transformation marker *rol-6(su1006)*. We found that 4/5 lines transformed with DNA amplified from *unc-8(n491)* exhibited aberrant locomotion characteristic of *unc-8(n491)*/+ heterozygotes. In contrast, 5/5 lines transformed with R13A1_4 sequences derived from wild-type animals did not exhibit locomotion defects (Figure 3C). In other experiments, we found that introduction of similarly amplified sequences from *unc-8(e49)* into the N2 background also induced aberrant locomotion (6/6 lines, data not shown). We conclude that R13A1_4 is mutated in the *unc-8(n491)* and *unc-8(e49)* strains and that the dominant mutations are included within the sequences amplified.

Finally, we identified point mutations in five unusual *unc-8* alleles (amino acid changes indicated in Figure 1). Gain-of-function alleles *e15* and *n491,* which induce neuronal swelling (Shreffler et al., 1995), both encode substitution G387E situated within an extracellular domain previously implicated in channel closing (García-Añoveros et al., 1995). *e49*, a weaker semidominant allele that does not induce neuronal swelling, encodes substitution A586T within CRDIII. Allele *e15lb129* was isolated as an interallelic suppressor of *unc-8(e15)* that confers normal locomotion in homozygotes and has the interesting property that it fully suppresses the *e15* allele in *trans* heterozygotes (Shreffler et al., 1995). The *lb129* substitution (H114Y) alters a highly conserved residue in the intracellular N-terminal domain. Another intragenic revertant, allele *n491lb82*, does not confer locomotion defects when homozygous. However, *n491lb82*/+ heterozygotes are more severely uncoordinated than *n491*/+ heterozygotes (Shreffler et al., 1995). The *trans*-enhancing *lb82* substitution is T641I. Implications of these substitutions for UNC-8 channel function are considered in the Discussion.

unc-8 Null Mutants Are Defective in Sinusoidal Locomotion

Putative *unc-8* null alleles were identified as intragenic suppressor mutations of *n491* and *e15* (Park and Horvitz, 1986a; Shreffler et al., 1995). Despite the extensive expression of *unc-8* in the nervous system, null mutants do not exhibit obvious locomotion defects. That null mutants appear wild type in behavior has also been observed for the *deg-1* degenerin (Chalfie and Wolinsky, 1990), which is expressed in many *C. elegans* neurons and muscles (Hall et al., 1997), and for the *unc-105* degenerin expressed in muscle (Park and Horvitz, 1986b; Liu et al., 1996). The apparent absence of a phenotype in null mutants of these degenerins could result

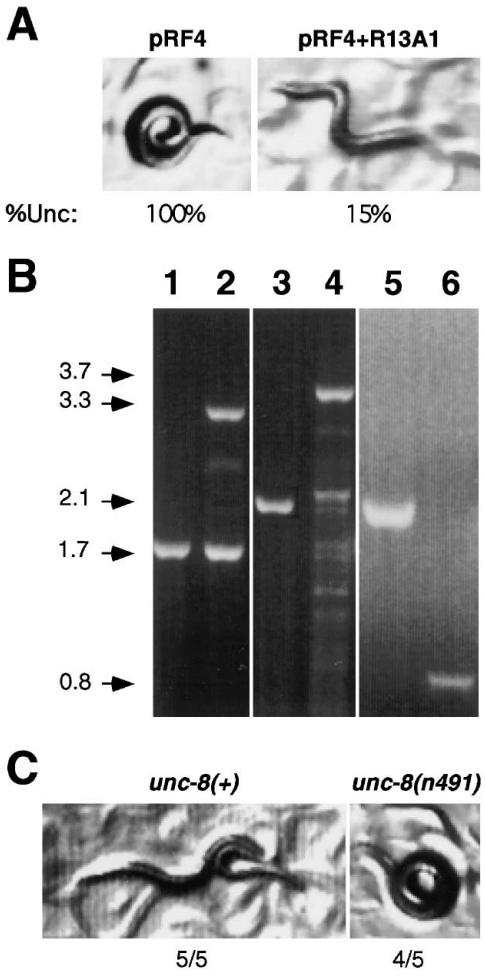


Figure 3. R13A1_4 Corresponds to the *unc-8* Gene

(A) Cosmid R13A1 rescues the Unc phenotype of *unc-8(e15)*. Transgenic lines harbor pRF4 (which encodes the dominant *rol-6[su1006]* allele that enables transformants to be identified by their roller phenotype) or pRF4 + cosmid R13A1 in the gain-of-function *unc-8(e15)* background; % value indicates the average number of transgenic animals that can back up rather than coil onto themselves when stimulated on the head (three lines scored for at least 100 rollers/line).

(B) *unc-8* alleles have DNA polymorphisms within R13A1_4 sequences. DNAs were amplified from *unc-8(e15)* revertants generated in the high frequency transposition background, *mut-2(r459)* (Shreffler et al., 1995). Lanes 1 [*unc-8(e15)*] and 2 [*unc-8(e15lb143)*], sequences between 9353 and 10980 (cosmid R13A1 annotation) are amplified; lanes 3 [*unc-8(e15)*] and 4 [*unc-8(e15lb147)*], sequences between 7441 and 9451 are amplified; lanes 5 [*unc-8(e15)*] and 6 [*unc-8(e15lb145)*], sequences between 10873 and 12865 are amplified. Note that *unc-8(e15)* bands were identical to wild-type N2 in all amplification experiments and that results depicted were reproduced in five independent PCR experiments; 1.6 kb insertions evident in alleles *unc-8(e15lb143)* and *unc-8(e15lb147)* were due to Tc1 elements as established by PCR experiments in which one Tc1-specific primer and one R13A1_4-specific primer were utilized (data not shown). The amplified band in lane 2 that comigrates with the *e15* band is most likely a product of somatic excision of the Tc1 element (see Discussion in Plasterk, 1995) and was not evident when a Tc1-specific primer was utilized.

(C) *unc-8(n491)* encodes a mutation within R13A1_4 sequences. Representative animals transformed with DNA from *unc-8(+)* or *unc-8(n491)*. Transgenic lines that harbor R13A1_4 sequences amplified from *unc-8(n491)* exhibit the uncoordinated phenotype characteristic of *unc-8(n491)/+* heterozygotes in 4/5 lines. 5/5 transgenic lines that harbor R13A1_4 amplified from wild-type animals do not exhibit locomotion defects.

because: 1) the degenerin may influence locomotion in a subtle manner that is difficult to detect, 2) the function of one degenerin may be redundantly encoded by another coexpressed family member, or 3) the degenerin might not be essential for cell function.

To distinguish among these possibilities, we first tested for subtle defects in the locomotion of *unc-8* null mutants by placing age-matched *unc-8* mutants and wild-type animals on agar plates coated with E. coli and comparing the tracks left in the bacterial lawn after animals moved through it. This analysis revealed a reproducible phenotype for 13/13 *unc-8* null mutants examined—the track pattern that mutant animals inscribe on bacterial lawns is significantly different from wild type (Figure 4).

To quantitate the locomotory defects in *unc-8* null mutants, we photographed tracks made by age-matched *unc-8* and wild-type animals and then measured the amplitude and the wavelength of the inscribed sinusoidal wave (Figure 4B). We find that the amplitude of the body wave is approximately 3.5-fold reduced in *unc-8(lf)* animals, and wavelength is reduced approximately 4-fold compared to wild type. The path phenotype appears fairly specific to *unc-8(lf)* mutants—for example, we do not observe path defects for *mec-4 (u253)* animals that are insensitive to body touch. We conclude that *unc-8(lf)* mutants are modestly defective in coordinated locomotion and that *unc-8* is required for maintenance of the wild-type pattern of sinusoidal motion.

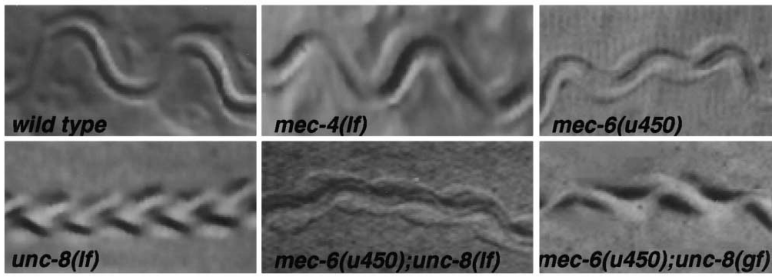
How might the UNC-8 degenerin contribute to coordinated locomotion? Given that UNC-8 is homologous to MEC-4 and MEC-10, which are likely to be subunits of a mechanically gated ion channel, we suggest that UNC-8 might be a subunit of a mechanically gated ion channel that modulates sinusoidal locomotion (see Discussion).

unc-8 is expressed in the ASH and FLP neurons, which function as mechanoreceptors in nose touch avoidance (backing up in response to head-on collisions; Kaplan and Horvitz, 1993). We observe only a slight reduction in nose touch sensitivity in the *unc-8* null mutant (data not shown) that does not approach the reduction that results when the ASH and FLP neurons are ablated with a laser microbeam (Kaplan and Horvitz, 1993). Thus, it appears that the UNC-8 degenerin does not contribute significantly to nose touch avoidance.

mec-6; unc-8(gf) Double Mutants Inscribe Tracks of Reduced Wavelength and Amplitude

mec-6 mutations suppress *unc-8(gf)*-induced uncoordination (Shreffler et al., 1995). To investigate the influence of *mec-6* mutations on locomotion in the background of *unc-8(gf)* alleles, we first tested whether *mec-6* mutants exhibit locomotion abnormalities (Figures 4A and 4B). We find that all of six *mec-6* mutants assayed inscribe somewhat irregular tracks in bacterial lawns. Overall, the amplitude and wavelength of the tracks are reduced; paths are also more noticeably meandering, seldom running in a straight trajectory for long. Interestingly, *mec-6(u450)*; *unc-8(gf)* double mutants traverse the lawn in

A



B

	Amplitude	Wavelength
Wild type	10.0 ± 0.2	10.0 ± 0.3
mec-4(lf)	9.2 ± 0.3	10.0 ± 0.4
mec-6(lf)	4.6 ± 0.9	5.0 ± 0.5
unc-8(lf)	2.7 ± 0.1	2.5 ± 0.1
mec-6(lf); unc-8(lf)	3.1 ± 0.5	3.4 ± 0.1
mec-6(lf);unc-8(gf)	3.8 ± 0.2	4.1 ± 0.2

Figure 4. Locomotion Abnormalities in *unc-8(lf)* and *mec-6* Mutants

(A) Track patterns inscribed by *unc-8(lf)* and other mutants. Tracks were carved into a bacterial lawn by L4 stage animals. *mec-4(lf)* mutant is the null allele *u253*, *mec-6(u450)* is a likely hypomorph, *unc-8(lf)* is deletion allele *e15lb145*, and *unc-8(gf)* is *n491*.

(B) Quantitation of track abnormalities in various mutant backgrounds. Mutants depicted are as in (A). Numbers cited are the average of 50 measurements per trial for 10 trials of L4 animals. Scores are reported ± standard deviation; p values for these measurements were <0.01. Track differences in the *unc-8* null background are not due to differences in body length (N2, .8 ± 0.1 mm, $p < 0.1$; *unc-8e15lb145*, .8 ± 0.1 mm, $p < 0.1$). Other strains assayed but not documented here [*unc-8(lf)*: *n491n1192*, *n491n1193*, *n491n1194*, *n491-n1195*, *n491n1196*, *e15lb55*, *e15lb56*, *e15lb-57*, *e15lb143*, *e15lb144*, *e15lb146*, *e15lb147*; *mec-6: bz20*, *bz22*, *bz23;* double mutants *mec-6(u450); unc-8(e15)*, *mec-6(bz20); unc-8 (e15)*, *mec-6(bz22); unc-8(e15)*, *mec-6(bz23); unc-8(e15), mec-6(bz20); unc-8(n491)*, *mec-6 (bz22); unc-8(n491), mec-6(bz23); unc-8 (n491), mec-6(lb126); unc-8(n491)*] behaved similarly to the prototypical animals shown.

a manner similar (although not identical) to that of *unc-8(lf)* mutants—amplitude and wavelength of the path are consistently reduced. The *mec-6(u450)*; *unc-8(e15lb-145)* double mutant (*e15lb145* is null) moves similarly to *unc-8(lf)* mutants, although the tracks of the double mutant are somewhat longer in wavelength and a bit more irregular than for *unc-8(lf)* alone. The interpretation of how *mec-6* interacts with the *unc-8* channel is somewhat complicated by the facts that *mec-6* appears to affect many degenerin channels in cells other than those that express *unc-8*, and available *mec-6* alleles may not be null (Chalfie and Wolinsky, 1990; Shreffler and Ublinsky, 1997; see also Discussion). Nonetheless, this behavioral assay is consistent with a model in which *mec-6* mutations suppress *unc-8(gf)* mutations by reducing activity of the mutant UNC-8 channel.

The E02H4_1 Degenerin Defines a New Gene, *del-1*, That Is Coexpressed with *unc-8* in the VA and VB Motor Neurons

The mammalian epithelial Na^+ channel includes at least three homologous subunits that must be coexpressed to reconstitute physiologic channel activity in oocytes (Canessa et al., 1994). To identify other degenerin channel subunits that might be coexpressed with *unc-8*, we surveyed the expression patterns of *C. elegans* family members currently reported by the *C. elegans* Genome Sequencing Consortium. We fused sequences including approximately 2.5 kb of putative 5′ regulatory regions and N-terminal coding sequences of each degenerin to the *E. coli lacZ* reporter gene. Transgenic lines were constructed and stained for β-galactosidase activity to identify expression patterns. Of 9 new degenerin family members thus surveyed, we identified only one, *del-1* (encoding E02H4_1), which is expressed broadly in the nervous system in a subset of cells that express *unc-8*. Specifically, $p_{del\text{-}1}$*lacZ* is expressed in the VA and VB motor neurons and the FLP sensory neurons (Figure 5A). Consistent with expression in the VA and VB ventral cord neurons, which are added to the nervous system during the L1 stage, $p_{del\text{-}1}$*lacZ* is expressed postembryonically. Similar to the expression pattern of *unc-8*, $p_{del\text{-}1}$*lacZ* activity peaks in the L2 stage (Figure 5A) and persists throughout adulthood at a reduced level (Figure 5B).

A common feature of neuronally expressed degenerins is that they can mutate to encode toxic proteins that can kill the cells in which they are synthesized (Chalfie and Wolinsky, 1990; Driscoll and Chalfie, 1991; Huang and Chalfie, 1994; García-Añoveros et al., 1995; Shreffler et al., 1995). We hypothesized that analogous *gf* mutations in the *del-1* degenerin would also be toxic. With an interest in quickly assessing whether any previously reported *C. elegans* mutants might harbor *gf* mutations in the *del-1* gene, we attempted to mimic the anticipated *gf* phenotype by constructing integrated lines in which expression of the toxic dominant allele, *mec-4(u231)*, was placed under the control of the *del-1* promoter [construct $p_{del\text{-}1}$*mec-4(u231)*]. As anticipated, transgenic animals that harbored an integrated $p_{del\text{-}1}$*mec-4(u231)* fusion (strain ZB171) were uncoordinated and exhibited vacuolated neurons in the ventral cord (Figure 5C). Movement in either the forward or backward direction was irregular, nonsinusoidal, and appeared to be executed with difficulty. Such defects are consistent with the disruption of the activities of the VA and VB classes of motor neurons that promote backward and forward locomotion, respectively (Chalfie et al., 1985). $p_{del\text{-}1}$*mec-4(u231)*-induced behavioral defects were suppressed by *mec-6(u450)* (Figure 5D), indicating that *mec-6* may be coexpressed with *del-1* in the VA and VB neurons.

del-1 maps to the right arm of the X chromosome

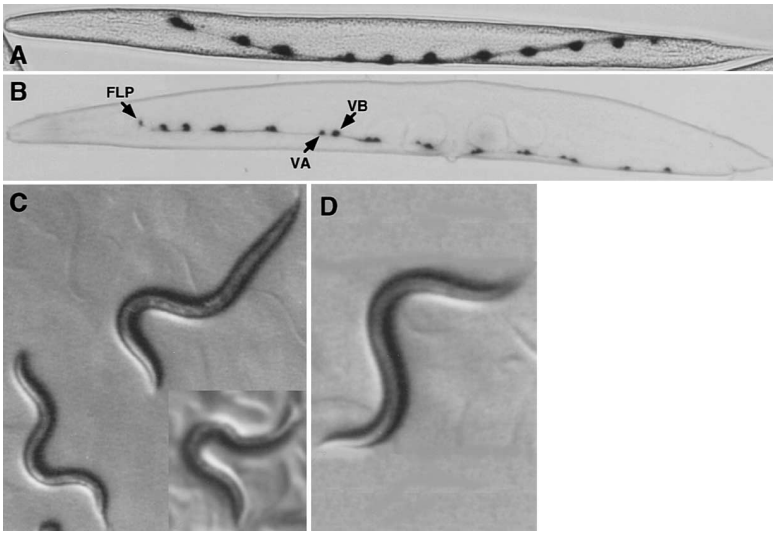


Figure 5. *del-1* Is Expressed in the FLPs and the VA and VB Motor Neurons

(A) Expression of p_{del-1}*lacZ* in strain ZB170, L2 stage. ZB170 genotype is *bzIs5* [p_{del-1} *lacZ* pRF4]. Reporter gene activity in VA and VB ventral cord neurons is apparent. The ventral cord is twisted because ZB170 is a roller.

(B) Expression of p_{del-1}*lacZ* in strain ZB170, adult stage. VA and VB ventral cord neurons stain less intensely than in the L2 stage but are still clearly evident.

(C) p_{del-1}*mec-4(u231)* induces uncoordinated locomotion in transgenic animals. Shown are animals from transgenic line ZB171 (genotype: *bzIs7*[p_{del-1}*mec-4(u231)*]). Attempted locomotion in either direction results in a somewhat flattened extension of the body extremities. When attempting to back up animals, adopt an "omega" posture similar to that of *unc-4* mutants, which lack VA neurons (White et al., 1992).

(D) *mec-6(u450)* suppresses the Unc phenotype conferred by *bzIs7*. Strain ZB172 shown is *mec-6(u450); bzIs7*. In the *mec-6* mutant background, p_{del-1}*mec-4(u231)* does not induce uncoordinated locomotion.

between *col-9* and *his-24*. Analysis of the *C. elegans* genetic map did not reveal any candidate *unc* mutants in this region that appeared likely to harbor mutations in the *del-1* gene. As is the case for *unc-8,* loss-of-function mutations in this gene might have locomotion defects that would not be readily detected in a genetic screen, and *gf* alleles would be identified at a very low frequency.

Given that the VA and VB motor neurons have been suggested to be stretch-sensitive (White et al., 1986; see Discussion) and that *del-1* is expressed nearly exclusively in these neurons, DEL-1 is a plausible candidate for a subunit that coassembles with UNC-8 (and possibly MEC-6) in the VA and VB neurons to form a mechanically activated ion channel that modulates sinusoidal locomotion.

Discussion

Stretch-sensitive coordination of involuntary muscle movement, for example as occurs in digestive processes and blood pressure regulation, is indispensable for the viability of most organisms. These and other mechanosensitive processes that transpire at the cellular or systems level are mediated by ion channels of unknown molecular nature. Exciting prospects for the understanding of molecular mechanisms of mechanosensitive channel activity came with the cloning of the gene encoding the mechanically gated MscL channel of E. coli (Sukharev et al., 1994) and with the genetic and molecular analyses in *C. elegans* that strongly implicated the products of two ion channel genes of the DEG/ENaC family, *mec-4* and *mec-10,* in touch transduction (Driscoll and Chalfie, 1991; Huang and Chalfie, 1994). Although it remains to be experimentally demonstrated that the MEC-4/MEC-10 channel is mechanically gated, a working hypothesis in the field is that the touch receptor channel and channels composed of related degenerins are specialized to be opened and closed by mechanical force. Here, we report characterization of two genes, *unc-8* (R13A1_4) and *del-1* (E02H4_1), which encode degenerin channel subunits expressed in *C. elegans* neurons. We find that *unc-8* null mutants exhibit a subtle locomotion defect—sinusoidal tracks inscribed when they traverse an E. coli lawn are reduced in amplitude and wavelength as compared to wild-type animals. Based on inferred expression patterns, *unc-8* behavioral defects, and neuronal morphologies that suggest roles in stretch reception, we propose a model of degenerin action in the locomotory nervous system in which UNC-8 (and, in some motor neurons, possibly DEL-1) is a component mechanically gated channel that may regulate locomotion in response to body stretch.

Degenerin Family Members *unc-8* and *del-1*

The *unc-8* and *del-1* proteins include all domains characteristic of degenerin family members and are likely to adopt similar transmembrane topologies (amino and carboxy termini situated inside the cell and a large extracellular domain that includes three Cys-rich regions; García-Añoveros et al., 1995; Lai et al., 1996). Neither degenerin has any primary sequence features that differ markedly from other *C. elegans* family members, although one somewhat atypical feature of UNC-8 is that it has a relatively long C-terminal domain that shares some primary sequence homology with the extended C-terminus of the UNC-105 degenerin.

Semidominant *unc-8* Mutations Underscore the Importance of an Extracellular Region in Regulation of Ion Transport

Genetic properties divide *unc-8(gf)* alleles into two classes. *unc-8(e49)* is mildly uncoordinated and does not cause neuronal swelling, whereas *unc-8(n491)* and *unc-8(e15)* are severely uncoordinated and induce swelling of neurons in the head and ventral cord that peaks in the L2 stage and then regresses (i.e., neurons do not die) (Shreffler et al., 1995). Interestingly, the amino acid change specified by *unc-8(n491)* and *unc-8(e15)* is

situated in the extracellular region corresponding to that implicated in channel closing by recessive *deg-1* mutations (García-Añoveros et al., 1995). Recessive allele *deg-1(u506)*, which induces swelling and death of *deg-1*-expressing neurons, encodes substitution A393T at a site corresponding to A377 in UNC-8. A similar substitution or a small deletion of the surrounding region engineered into *mec-4* also induces neurodegeneration (this region corresponds to UNC-8 residues 376–397). Thus, García-Añoveros et al. (1995) proposed that this domain may be part of an extracellular gate required for channel closing. The neuronal vacuolation phenotype seen in *e15* and *n491* mutants underscores that the integrity of this degenerin domain is required for negatively regulating channel transport. Why *unc-8*-expressing cells do not die in *e15* or *n491* mutants is not clear. Perhaps the insult delivered by the G387E mutant channel is not severe enough to be lethal, the affected neurons may be better able to compensate for osmotic imbalance than are other neuronal types, or a developmental change in gene expression may ameliorate toxicity.

Semidominant allele *e49* encodes substitution A586T, which affects a conserved residue situated within extracellular CRDIII. This site has not been previously identified as a mutation site in other characterized degenerin mutants (Driscoll and Chalfie, 1991; Huang and Chalfie, 1994; García-Añoveros et al., 1995; Shreffler et al., 1995; Liu et al., 1996), although it falls within a region where several recessive *mec-4(lf)* mutations are situated (K. Hong and M. D., unpublished data). Since CRDIII bears sequence similarity to a large family of protein interaction domains (N. T. and M. D., unpublished data), we suggest that the A586T substitution may disrupt association with another channel component or the extracellular matrix.

UNC-8 Is Likely to Participate in Different Heteromeric Channel Complexes in Different Groups of Neurons

The vertebrate ENaC channel (Canessa et al., 1993, 1994) and the *C. elegans* MEC-4/MEC-10 channel (Hong and Driscoll, 1994; Huang and Chalfie, 1994) are heteromeric complexes that include multiple DEG/ENaC subunits. It is interesting that spatial and temporal *unc-8* expression overlaps with that of other members of the degenerin gene family. *unc-8* is coexpressed with *deg-1* in the ASH neurons (Chalfie and Wolinsky, 1990; Hall et al., 1996), with *mec-10* and *del-1* in the FLP neurons (Huang and Chalfie, 1994), with *mec-4* and *mec-10* in the PVM neurons (Mitani et al., 1993; Huang and Chalfie, 1994) and with *del-1* in the VA and VB motor neurons. We hypothesize that UNC-8 might coassemble with other degenerins into different channels with distinct gating properties in different cell types.

Allele-specific interactions have been observed for certain *unc-8* mutations, suggesting that at least in some cells, more than one UNC-8 subunit is present in the multimeric channel complex (Shreffler et al., 1995). Allele *n491lb82* enhances the Unc phenotype when present in *trans* to a wild-type *unc-8* allele; *n491b82/+* heterozygotes are more severely uncoordinated than are *n491/+* heterozygotes. We find that the enhanced toxicity is caused by substitution T664I, which is in the extracellular domain close to MSDII. *unc-8(e15lb129)* fully suppresses the semidominant Unc phenotype of *e15* or *n491* in *trans*-heterozygotes but does not affect locomotion when homozygous. The *lb129* substitution (H114Y) affects a highly conserved residue in the intracellular N-terminal domain. It is intriguing that an amino acid change inside a cell can affect the extracellular candidate regulatory gate and can influence the activity of mutant subunits in *trans*. Perhaps the conserved N-terminal domain plays a role in regulating channel opening.

mec-6 Influences UNC-8 Channel Activity

Genetic interactions between the *mec-6* gene product and several members of the *C. elegans* degenerin family suggest that *mec-6* interacts directly or indirectly with degenerin channels of different subunit compositions in a large number of *C. elegans* cells. *mec-6* is required for *mec-4(u231)* and *mec-10*(A673V)-induced degeneration of the touch receptor neurons (Chalfie and Wolinsky, 1990; Huang and Chalfie, 1994). *mec-6* is needed for *deg-1(u38)*-induced degeneration of the PVCs and several other neurons (Chalfie and Wolinsky, 1990). We have also found that *mec-6(u450)* partially suppresses the hypercontraction of *unc-105(gf)* alleles (N. T., W. S., and M. D., unpublished data). Finally, our finding that genetic ablations mediated by $p_{R13A1-4}$*mec-4(u231)* depend on *mec-6* are consistent with genetic studies that indicate *mec-6* interacts with *unc-8* in ventral cord neurons (Shreffler et al., 1995). Although the biochemical activity of MEC-6 is unknown, our finding that *mec-6(u450)*; *unc-8(gf)* double mutants have locomotion defects similar to *unc-8* null mutants and lack swollen cord neurons seen in the *unc-8(gf)* background suggest that *mec-6(u450)* lowers or eliminates the activity of UNC-8-containing channels.

Given the predicted ubiquitous involvement of *mec-6* in degenerin channel activity, it seems somewhat paradoxical that *mec-6* mutants do not exhibit more severely defective locomotion—for example, rather than experiencing irregular locomotion, *mec-6* mutants might be expected to move with consistently reduced path amplitude characteristic of *unc-8(lf)* mutants. We think that part of the reason *mec-6* mutants may not be more severely uncoordinated is that existing *mec-6* alleles are weakly hypomorphic rather than null (Shreffler and Wolinsky, 1997; N. T., W. S., and M. D., unpublished data). In addition, since *mec-6* is required for the activities of different channels in different cells, the *mec-6* phenotype is expected to result from the summed effects of defective channels in several cell types, which could induce a different behavioral response than the absence of one channel class.

UNC-8-Mediated Regulation of Locomotion

How might UNC-8 channels influence coordinated locomotion? Given that UNC-8 is closely related to degenerin channels that have been strongly implicated as mechanosensitive channels, we speculate that locomotory defects in *unc-8(lf)* mutants could result from the absence of one or more classes of mechanically sensitive neuronal channels.

Which of the many neurons that express *unc-8* mediate the observed coordination of locomotion? It is not possible to distinguish the contributions of various interneurons and motor neurons to the subtle uncoordinated phenotype by cell ablation approaches, since animals are highly uncoordinated in the absence of these neurons (see Chalfie et al., 1985). It is also difficult to construct appropriate genetic mosaics to address this question because so many neurons of a given type are involved in locomotion. Furthermore, our analysis of *mec-4* in the touch receptor neurons has shown that overexpression of a single channel subunit partially interferes with normal channel function (Hong and Driscoll, 1994), and thus expression of *unc-8* in specific neuronal classes in transgenic animals is not likely to yield an interpretable answer to this question. Still, some inferences may be drawn from examination of locomotion of mutant animals and from neuroanatomy. For example, the DD and VD motor neurons, which express *unc-8*, are inhibitory neurons that use GABA as their neurotransmitter (McIntire et al., 1993a). *unc-25* mutants that fail to synthesize GABA have been noted to exhibit a markedly reduced wave amplitude when they move (McIntire et al., 1993a, 1993b), consistent with the idea that the DD and VD classes of motor neurons, in addition to their important role in cross-inhibition, may contribute to subtle modulation of sinusoidal locomotion.

Other motor neurons that express *unc-8* are intriguing candidates for stretch sensors. For example, the processes of the VA and VB motor neurons feature strikingly long undifferentiated regions that do not receive synaptic input and do not participate in neuromuscular junctions (White et al, 1986). It had been proposed (by R. L. Russell and L. Byerly; cited by White et al., 1986) that these process extensions (as well as undifferentiated processes in DA and DB motor neurons) are stretch-sensitive regions that help regulate propagation of the sinusoidal body wave. (Our observations suggest that the UNC-8-containing channel in these neurons is not essential for generation or propagation of the wave since in *unc-8* null mutants, the wave, although lessened in amplitude, still passes through the body length.) As an illustrative example of the manner in which we envision an UNC-8 channel could modulate locomotion, we discuss the VB motor neurons, which innervate ventral muscles and mediate forward locomotion (Chalfie et al., 1985; White et al., 1986) (Figure 6). Each of the 11 VB motor neurons distributed along the ventral cord stimulates a unique subset of ventral muscles. Neuromuscular junctions are usually situated close to the VB cell body; a lengthy extension of the process distal to the neuromuscular junctions is devoid of synaptic input, and we suggest that mechanically activated channels could be concentrated at these ends (Figure 6A). During locomotion, these mechanosensitive channels could be activated at sites along the body where the animal is maximally curved on the ventral side (Figure 6B), potentiating motor neuron excitation and enhancing the strength or duration of the pending anterior muscle contraction.

Although we explained details of the motor neuron stretch model using the VB motor neurons as an example, it should be emphasized that several motor neuron classes cooperate to effect coordinated locomotion, and the VB motor neurons cannot be the only neurons that contribute to the *unc-8(lf)* phenotype. If this were the case, the locomotion defect would primarily affect muscles on the ventral side, and the locomotion path would appear as an asymmetric wave (see discussion by Driscoll and Kaplan, 1997). Thus, if UNC-8 channels are in fact mechanically gated, UNC-8-containing channels in additional motor neurons must contribute to modulation of muscle contraction in response to localized body stretch. We also note that it is interesting that *del-1* is expressed nearly exclusively in the VA and VB motor neurons, which have distinctive undifferentiated distal processes. Coexpression with *unc-8* suggests that a heteromeric UNC-8/DEL-1 channel could assemble in these motor neuron types.

One prediction of the motor neuron stretch model is that degenerin channels should be localized to the undifferentiated neuronal ends postulated to be stretch sensitive. Transgenic lines harboring an UNC-8/GFP gene fusion that includes nearly all UNC-8 coding sequences exhibit fluorescence throughout the neuronal processes and cell bodies (similar to what is observed for MEC-4/GFP fusions in the touch receptor neurons; data not shown). Although our observations indicate that the UNC-8 fusion protein is transported to process ends, the precise distribution of the channel remains unknown because newly synthesized and transported proteins in high gene dosage transgenic lines are expected to label the cell body and body-proximal portions of the process.

Finally, although here we discuss in detail one hypothesis of how the UNC-8 channel could act in motor neurons to modulate locomotion, we emphasize that *unc-8* is also expressed in interneurons that regulate locomotion so that it is equally plausible that UNC-8 could act within these neurons to modulate coordinated movement. Additionally, although we speculate that the UNC-8 channel might be mechanically gated based on homology to *C. elegans* MEC-4 and MEC-10, we stress that direct evidence that any of these channels are mechanically gated remains to be obtained.

Degenerin Function in Mechanical Signaling Versus Maintenance of Osmotic Integrity

Because it has not yet been possible to demonstrate mechanical gating of the MEC-4/MEC-10 touch receptor channel or the UNC-8 channels using electrophysiological approaches, two models for the biological activities of degenerin channels have been considered. In the simplest model, the degenerin channel mediates mechanotransduction directly. The alternative model is that the degenerin channel acts indirectly to maintain a required osmotic balance within a neuron so that a mechanosensitive channel, yet to be identified, can function. In the case of the touch receptor channel, the absence of either MEC-4 or MEC-10 renders the mechanosensory neuron nonfunctional, making it impossible to distinguish between the two alternative hypotheses. The situation with the UNC-8 channel is different. It is clear from the phenotype of *unc-8* null mutants that the majority of neurons that express *unc-8* must remain functional

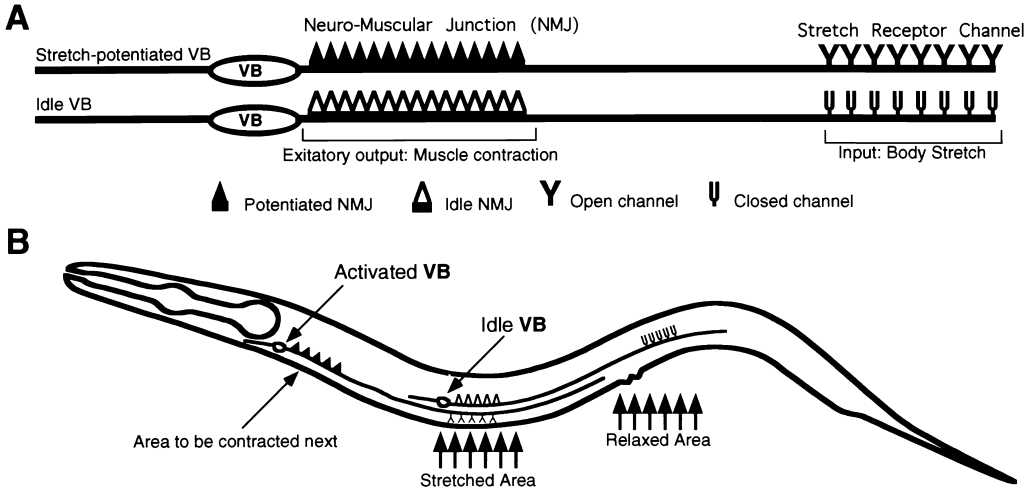


Figure 6. A Model for UNC-8 Involvement in Stretch-Regulated Control of Locomotion

(A) Schematic diagram of potentiated and inactive VB class motor neurons. Neuromuscular junctions (signified by triangles) are made near the cell body (White et al., 1986). Mechanically activated channels postulated to include UNC-8 (and, possibly in VB motor neurons, DEL-1) subunits (signified by Y figures) are hypothesized to be concentrated at the synapse-free, undifferentiated ends of the VB neuron.

(B) Mechanically gated channels could potentiate local excitation of muscle. Body stretch is postulated to activate mechanically gated channels, which potentiate the motor neuron signal that excites a specific muscle field. A strong muscle contraction results in a sustained body turn. In *unc-8(lf)* mutants, VB motor neurons lack the stretch-sensitive component that potentiates their signaling and consequently elicits a muscle contraction that is shortened in intensity or duration so that the body turns less deeply. Note that although we depict VB as an example of one motor neuron class that affects locomotion, other motor neuron classes must also be involved in the modification of locomotion in response to body stretch.

in the absence of *unc-8* activity. Our understanding of neuronal circuitry (Chalfie et al., 1985) and characterized behavioral mutants argues that if these neurons were not functional, *unc-8* null mutants would exhibit severely defective locomotion. Given that *unc-8* null mutants move in a manner only marginally different from wild-type animals, the case that the UNC-8 channel maintains an osmotic milieu required for the function of other neuronal channels is weakened. One caveat to this discussion is that we cannot rule out the possibility that a functionally redundant and as yet unidentified degenerin family member might be coexpressed with *unc-8* and could nearly compensate for its absence.

Mechanical Gating as a Common Property of DEG/ENaC Channels?

A key question that remains to be resolved is how broadly DEG/ENaC superfamily members will prove to be involved in mechanotransduction. Analyses of the mammalian ENaC channel in lipid bilayers suggest that its gating can be influenced by membrane stretch (Awayda et al., 1995), although interpretation of these studies requires attention to experimental caveats (Hamill and McBride, 1996). One recently identified mammalian family member, MDEG, behaves analogously to *C. elegans* degenerins in that it can be similarly altered to create a toxic form that induces cell degeneration (Waldmann et al., 1996), but MDEG is fairly distantly related to the *C. elegans* degenerins, and the gating mechanism of the MDEG channel is unknown. Not all family members are expected to be exclusively mechanically gated. For example, one distant member of the DEG/ENaC superfamily, *helix* FaNaC, is gated by FMRFamide (Lingueglia et al., 1995). Nonetheless, given the implication of multiple *C. elegans* degenerin family members in stretch-sensitive behaviors (work discussed here and by Driscoll and Chalfie, 1991; Huang and Chalfie, 1994; Liu et al., 1996), we speculate that closely related genes may encode the elusive mammalian mechanically gated channels.

Experimental Procedures

Computer Analyses

The amino acid sequences of MEC-4 (Lai et al., 1996), MEC-10 (Huang and Chalfie, 1994), DEG-1 (García-Añoveros et al., 1995), and UNC-105 (Liu et al., 1996) were used to search databases including GenBank, SwissProt, PIR, the Brook Haven Protein Data Bank, the Genome Sequencing Center at Washington University School of Medicine, St. Louis, and the Sanger Center, Cambridge, UK. BLAST searches were performed with National Center for Biotechnology Information BLAST network service (Altschul et al., 1990). Multiple sequence alignments and other computational analyses were performed using the Genetics Computer Group software package (1994).

C. elegans Strains

C. elegans strains were grown at 20°C as described by Brenner (1974). *unc-8* alleles utilized in this study were *gf*: *e15*, *e49* (Brenner, 1974), and *n491* (Park and Horvitz, 1986a); *lf*: *n491n1192*, *n491n1193*, *n491n1194*, *n491n1195*, *n491n1196* (Park and Horvitz, 1986a), *e15lb55*, *e15lb56*, *e15lb57*, *e15lb143*, *e15lb144*, *e15lb145*, *e15lb146*, and *e15lb147* (Shreffler et al., 1995). *mec-6* alleles utilized were: *e1432* (Chalfie and Sulston, 1981), *u450* (Chalfie and Au, 1989), *lb126* (Shreffler et al., 1995), *bz20*, *bz22*, and *bz23* (isolated as trimethylpsoralen-induced *unc-8[n491]* suppressors). *mec-6; unc-8(gf)* double mutant strains assayed included all pairwise combinations of *mec-6* alleles *u450*, *bz20*, *bz22*, and *bz23* with *unc-8* alleles *e15* and *n491*. *mec-6; unc-8(lf)* strain was *mec-6(u450)*; *unc-8(e15lb145)* (*e15lb145* harbors a substantial deletion of *unc-8* coding sequence). *mec-4(u253)* is described by Chalfie and Au (1989). Genotypes of transgenic lines were ZB166: *bzEx4* [$p_{R13A1-4}$*lacZ* pRF4], ZB167: *bzIs4*

[p_{R13A1_4}*lacZ* pRF4], ZB170: *bzIs6* [$p_{del\text{-}1}$*lacZ* pRF4], ZB171: *bzIs7* [$p_{del\text{-}1}$*mec-4(u231)* pRF4].

Recombinant DNA

Cosmids R13A1 and E02H4 were obtained from the Sanger Centre Network site, maintained by the *C. elegans* Genome Sequencing Consortium (Sulston et al., 1992). Plasmid pRF4, which harbors the dominant *rol-6(su1006)* allele, is described by Kramer et al. (1990). Plasmid constructions employed standard molecular biology methods (Sambrook et al., 1989). *LacZ* reporter plasmids were translational fusions constructed by PCR amplification of DNA fragments of approximately 3 kb that contained putative promoter sequences and some N-terminal coding sequences of the predicted degenerin genes. Primers used to amplify sequences of the R13A1_4 promoter (nucleotides 7441–9451) were 5′GCTCTAGAGGGAGCATTTCCGGT AGTTTG3′ and 5′CGCGGATCCGATTTCCGGGATGACGTTCG3′ and contained SphI and BamHI sites to facilitate subsequent cloning into vector pPD21.28 (Fire et al., 1990). A similar strategy was used to construct $p_{del\text{-}1}$*lacZ* using primers 5′ACATGCATGCTCAAGTCC CACCTCAACCC3′ and 5′CGCGGATCCCTCCAAAATTCCACATC TAG3′ (nucleotides 19201–21269; E02H4 accession number: Z68003). To construct $p_{del\text{-}1}$*mec-4(u231)*, plasmid ZB#54, which harbors a $p_{mec\text{-}7}$*mec-4(u231)* gene fusion, was digested with XbaI and KpnI to remove the *mec-7* promoter, and XbaI-KpnI degenerin promoter fragments from the $p_{del\text{-}1}$*lacZ* reporter constructs were introduced.

PCR and DNA Sequence Analysis

Coding sequences of R13A1_4 and E02H4_1 were confirmed by sequencing DNA fragments obtained by RT–PCR with degenerin-specific primers corresponding to predicted protein coding regions. DNA sequence analysis was performed using the Cycle-Sequencing method (GIBCO/BRL) or using an LI-COR automated sequencer. For PCR amplification of the entire *unc-8* genomic region for gene rescue experiments, we generated 6 kb DNA fragments from wild type, *unc-8(e49)*, and *unc-8(n491)* animals by long PCR as described (Barnes, 1994) using approximately 1 μg of *C. elegans* genomic DNA as a template and a 1:10 ratio of Pfu and Taq polymerases; PCR primers were 5′GCTCTAGAGGGAGCATTTCCGGTAGTTTG3′ and 5′GTTTCTGCTATTCAAATGTCAGTC3′, which amplified sequences between 7441 and 12865 in the R13A1 cosmid (accession number U40798). Three sets of primers were used for PCR-mediated detection of Tc1 insertions within R13A1_4: 5′GCTCTAGAGGGAGCATT TCCGGTAGTTTG3′ and 5′CGCGGATCCGATTTCCGGGATGACGT TCG3′, 5′ACGAAGTTGCCAAGGTTTTCAG3′, 5′CGTATTCAGGAT CAACGTGAAG3′, 5′CGTTTAATTCGAAGGATTGTAGTATG3′, and 5′GTTTCTGCTATTCAAATGTCAGTC3′. These primers span three overlapping DNA fragments (7441–9451, 9353–10980, and 10873–12865) that cover the entire *unc-8* gene.

C. elegans Transformation

Transgenic lines were constructed by injecting plasmids at a concentration of 50 μg/ml and cosmid at 10 μg/ml (Mello et al., 1992; Driscoll, 1995). Most constructs tested were coinjected with pRF4, which carries *rol-6(su1006)* and confers a roller phenotype on transformants (Kramer et al., 1990). For construction of transgenic lines using PCR-amplified DNA, 6 kb PCR fragments containing the wild-type *unc-8* gene or semidominant *unc-8(n491)* and *unc-8(e49)* were purified using a Quiagen column and injected at a final concentration of 100 μg/ml together with pRF4 DNA at 50 μg/ml. Since transgenic animals are mosaic for the presence of transforming DNA, three independently derived lines that exhibited a high frequency of segregation of the introduced DNA array were always selected for assays of phenotype. To alleviate instability problems in the inheritance and mitotic segregation of arrays carrying expression reporter genes and ablation fusions, several arrays were integrated by γ irradiation from a ^{137}Cs source (Kari et al., 1995).

LacZ Staining and Cell Identifications

Staining for β-galactosidase activity in transgenic animals was performed as described (Fire et al., 1990) except that 10 ng/ml DAPI (final concentration) was routinely added to fixed animals so that all nuclei would be visualized. For p_{R13A1_4}*lacZ*, transgenic animals were incubated for 5–8 hr; for $p_{del\text{-}1}$*lacZ*, transgenic animals were incubated for 10–15 hr. Cell identifications were made by examining the position of stained cells relative to positions of other nuclei and by observing the morphologies of neuronal processes of individual stained cells (White et al., 1986) in mosaic animals that harbored the reporter genes as extrachromosomal arrays. Observation of degenerating neurons was assayed by scoring swollen–vacuolated cells under Nomarski differential interference illumination as described (Driscoll, 1995).

Locomotion Assays

Phenotypes were scored by comparing age-matched animals grown at 20°C on NGM plates seeded with E. coli strain OP50 (Brenner, 1974). Individual animals were transferred to fresh plates and allowed to cut tracks for 10–20 min before paths were measured. Tracks were measured from photographs or on a video monitor. The locomotion defect was quantitated by measuring the amplitude of the path and the distance between successive peaks in the path (the wavelength) as shown in Figure 5B. For each strain, 10 trials were conducted and 50 measurements were recorded per trial. Values were normalized to a value of 10 for wild type and tested for statistical significance using Excel P-test software. *unc-8* phenotypes were detectable from L2 through adult stages but were best photographed for L4 animals.

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