# The *unc-8* and *sup-40* Genes Regulate Ion Channel Function in *Caenorhabditis elegans* Motorneurons

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ABSTRACT

Two *Caenorhabditis elegans* genes, *unc-8* and *sup-40*, have been newly identified, by genetic criteria, as regulating ion channel function in motorneurons. Two dominant *unc-8* alleles cause motorneuron swelling similar to that of other neuronal types in dominant mutants of the *deg-1* gene family, which is homologous to a mammalian gene family encoding amiloride-sensitive sodium channel subunits. As for previously identified *deg-1* family members, *unc-8* dominant mutations are recessively suppressed by mutations in the *mec-6* gene, which probably encodes a second type of channel component. An unusual dominant mutation, *sup-41(lb125)*, also co-suppresses *unc-8* and *deg-1*, suggesting the existence of yet another common component of ion channels containing *unc-8* or *deg-1* subunits. Dominant, *trans*acting, intragenic suppressor mutations have been isolated for both *unc-8* and *deg-1*, consistent with the idea that, like their mammalian homologues, the two gene products function as multimers. The *sup-40(lb130)* mutation dominantly suppresses *unc-8* motorneuron swelling and produces a novel swelling phenotype in hypodermal nuclei. *sup-40* may encode an ion channel component or regulator that can correct the osmotic defect caused by abnormal *unc-8* channels.

MAINTENANCE of osmotic balance is a vital cell function. Cell volume and membrane potential both depend on proper ion distribution across the plasma membrane. Ubiquitous ion transport proteins such as the $Na^+/K^+$-ATPase, $Na^+/H^+$-anti-porter and $Na^+/Ca^{2+}$ exchanger subserve this housekeeping function in virtually all cell types. Specialized functions of some cell types, such as neuronal signaling, and transepithelial transport by intestine, lung and kidney, are also critically dependent on electrochemical gradients and require cell-type-specific ion channels responsive to external solutes, neurotransmitters, hormones or membrane voltage. *In vivo*, ion channel malfunctions, such as those produced by mutations at the human cystic fibrosis locus, can cause serious disease (WELSH and SMITH 1993). In the laboratory, mutational analysis can be used to identify new ion channel genes and to examine the relationship between ion channel structure and function.

Here we describe two *Caenorhabditis elegans* genes that play a role in cellular osmoregulation: *unc-8* and its suppressor locus, *sup-40*, which together influence ion channel function in motorneurons. The *sup-40(lb130)* mutation also causes apparent osmotic abnormalities in the hypodermis and in developing oocytes, suggesting that its role in ion transport is not limited to neurons. Both *unc-8* and *sup-40* can mutate to confer increased survival after exposure to a toxic level of nordihydroguairetic acid (NDG), a nonspecific lipoxygenase inhibitor that blocks opening of Aplysia S-$K^+$ channels (BELARDETTI *et al.* 1989; BRAHA *et al.* 1993; SCHACHER *et al.* 1993). The mechanism of NDG toxicity in *C. elegans* is not yet known; however, its usefulness in isolation of ion channel mutants suggests an effect on cell membrane permeability.

Genetic evidence strongly suggests that *unc-8* is a member of the previously characterized *deg-1* gene family encoding homologues of mammalian amiloride-sensitive sodium channel subunits (DRISCOLL and CHALFIE 1991; CANESSA *et al.* 1993; CHALFIE *et al.* 1993; LINGUEGLIA *et al.* 1993). Electrophysiological measurements of Xenopus oocytes expressing cloned mammalian cDNAs encoding different members of the gene family showed that channel conductance properties vary with subunit composition (CANESSA *et al.* 1994; LINGUEGLIA *et al.* 1994). $K^+$ channel subunits, encoded by a large gene family distinct from the *deg-1* homologues, also function as heteromultimeric complexes whose electrophysiological properties depend on subunit composition (RUPPERSBERG *et al.* 1990; SHENG *et al.* 1993). Both *deg-1* and *unc-8* exhibit interallelic interactions that suggest significant subunit interaction within the channel complex: *trans*acting suppressor mutations have been isolated in both genes, and, in the case of *unc-8*, a *trans*-acting enhancer mutation as well. As for previously identified *deg-1* family members, *unc-8* dominant mutations are recessively suppressed by *mec-6* mutations. *mec-6* seems to encode a ubiquitous channel component or

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**TABLE 1**

**Strains**

| Genotype | Isolation |
|---|---|
| *deg-1(u38)* | CHALFIE and WOLINSKY (1990) |
| *deg-1(u38u424)* | CHALFIE and WOLINSKY (1990) |
| *mec-6(e1342)* | CHALFIE and SULSTON (1981) |
| *mec-6(lb126)* | *mut-2(r459);unc-8(e15)* revertant |
| *mec-6(lb127)* | *mut-2(r459);unc-8(e15)* revertant |
| *mec-6(lb128)* | EMS reversion of *unc-8(e15)* |
| *mec-6(lb81)* | EMS reversion of *unc-8(n491)* |
| *mec-6(lb83)* | EMS reversion of *unc-8(n491)* |
| *mec-6(lb84)* | EMS reversion of *unc-8(n491)* |
| *mec-6(lb89)* | EMS reversion of *unc-8(n491)* |
| *mut-2(r459)* | COLLINS *et al.* (1987) |
| *ndg-4(lb108)* | NDG selection of EMS-treated N2 |
| *sup-40(lb130)* | EMS reversion of *unc-8(e15)* |
| *sup-41(lb125)* | EMS reversion of *unc-8(n491)* |
| *unc-8(e49)* | BRENNER (1974) |
| *unc-8(e15)* | BRENNER (1974) |
| *unc-8(n491)* | PARK and HORVITZ (1986) |
| *unc-8(lb109)* | NDG selection of EMS-treated N2 |
| *unc-8(e15lb54)* | EMS reversion of *unc-8(e15)* |
| *unc-8(e15lb55)* | *mut-2(r459);unc-8(e15)* revertant |
| *unc-8(e15lb56)* | *mut-2(r459);unc-8(e15)* revertant |
| *unc-8(e15lb57)* | *mut-2(r459);unc-8(e15)* revertant |
| *unc-8(e15lb129)* | EMS reversion of *unc-8(e15)* |

auxiliary protein required for activity of all known *deg-1* family members. *sup-40(lb130)*, however, does not cross-suppress *deg-1* mutations, nor do *mec-6* or *unc-8* putative null mutations suppress the hypodermal nuclear swelling phenotype of *sup-40(lb130)*. Thus, *sup-40* may encode a distinct ion channel component or regulator that can indirectly counteract the effect of abnormal *unc-8* channels by permitting a compensatory ion flux.

## MATERIALS AND METHODS

**Nematode strains:** Worm stocks were maintained as described (Brenner 1974) at 20°, unless otherwise stated. Mutations used in this work were isolated as shown in Table 1. The short body shape of *dpy-13(e184)* homozygotes is abbreviated as Dpy and the intermediate dumpy body size of *dpy-13(e184)/dpy-13(+)* heterozygotes as semi-Dpy. The coiling, backing-deficient phenotype of *unc-8(e15)* and *unc-8(n491)* homozygotes is abbreviated as Unc and the weaker coiling, backing proficient phenotype of *unc-8(e15)/unc-8(+)* and *unc-8(n491)/unc-8(+)* heterozygotes and *unc-8(e49)* and *unc-8(lb109)* homozygotes as semi-Unc.

**NDG lethality assay:** To measure NDG resistance, 100–200 adults were placed in 0.5 ml of NDG solution in a 1.5 ml Eppendorf tube and incubated 12–16 hr at 20° on a Nutator rocker platform. The NDG solution consisted of M9 buffer supplemented with a 2–3× suspension of bacteria (*Escherichia coli* strain 0P50/1 from a saturated overnight grown in Luria broth), to provide food. NDG (Sigma or Aldrich) was diluted to the desired concentrations from stock solutions of 30 or 50 mg NDG/ml dissolved in dimethylsulfoxide (DMSO) and stored at −20°. DMSO was added to a final concentration of 2%. The next morning, the contents of each Eppendorf tube were transferred using a Pipetman 1000 to fresh NGM agar plates spread with bacteria, and the number of motile and immobile worms was scored using a dissecting microscope after a 1–2-hr recovery period, during which the excess liquid was absorbed by the agar plate. The percentage of survivors was calculated as 100 times the number of motile animals divided by the total number scored.

**Selection for NDG resistance:** The NDG lethality assay described above was scaled up to isolate resistant mutants. F2 progeny of N2 treated with EMS were washed off 100-mm culture dishes with M9 buffer and resuspended in 0.5 mg NDG/ml, using 0.5 ml of NDG solution for each plate, and incubated 12–16 hr at 20° in 15- or 50-ml disposable plastic conical centrifuge tubes on a Nutator rocker platform. The NDG-treated animals were allowed to settle on ice, excess liquid was aspirated and the worm pellet was transferred by pasteur pipet to fresh culture plates, which were then visually screened using a dissecting microscope for healthy, motile animals. Candidates were picked singly, and their progeny retested in a small scale NDG survival test. Strains with reproducible drug resistance were outcrossed with *deg-1(u38)* males and resulting F1 cross-progeny expressing the dominant *deg-1* tail-touch-insensitive phenotype were picked singly. The resulting F2 were then rescreened with NDG to recover outcrossed resistance mutations. Outcrossing was repeated at least three times. Seven recessive NDG resistance mutations, including *ndg-4(lb108)* and *unc-8(lb109)*, have been isolated at a frequency of approximately 1 per 5000 haploid genomes.

The *ndg-4(lb108)* mutation confers the strongest level of NDG resistance so far observed (Figure 6). It also confers a recessive visible phenotype of abnormally pale egg color and is tightly linked to *dpy-17(e164)* on chromosome *III*.

The *lb109* mutation confers moderate NDG resistance (Figure 6). Its linkage to *dpy-13(e184)* on chromosome *IV* and semi-dominant coiler phenotype suggested allelism with *unc-8*. *Trans* heterozygotes were obtained by mating *lb109* males with the marked putative *unc-8* null mutant *dpy-13(e184)unc-8(e15lb55)*. *lb55* is a possible transposon insertion obtained by *mut-2(r459)* reversion of *e15*. The coiler phenotype of *dpy-13(e184)unc-8(e15lb55)/dpy-13(+)unc-8(lb109)* animals is as strong as that of *dpy-13(e184)unc-8(lb109)/dpy-13(+)unc-8(lb109)* and nearly as strong as that of *dpy-13(e184)unc-8(e49)/dpy-13(+)unc-8(e49)* homozygotes, whereas that of *dpy-13(e184)unc-8(lb109)/dpy-13(+)unc-8(+)* and *dpy-13(e184)unc-8(e49)/dpy-13(+)-unc-8(+)* is considerably weaker. *unc-8(e15lb55)* homozygotes move normally, so *e15lb55* enhancement of the coiler phenotype in *trans* with *lb109* and *e49* implies allelism of all three mutations. Similar results were obtained using a second putative null allele obtained from EMS mutagenesis, *e15lb54*. *dpy-13(e184)unc-8(e15)/dpy-13(+)unc-8(e49)* and *dpy-13(e184)-unc-8(e15)/dpy-13(+)unc-8(lb109)* also express similar uncoordinated phenotypes, consistent with the idea that *lb109* is a new *unc-8* mutation. *lb109/e15*, *lb109/n491* and *lb109/e49* are more strongly Unc than *e15/+*, *n491/+* or *e49/+* animals, respectively. Furthermore, no wild-type recombinants have been obtained from *lb109/e15*, *lb109/n491* or *lb109/e49* heterozygotes. Yet another piece of evidence for allelism of *lb109* with *unc-8* mutations is its suppression by *mec-6(e1342)*, *mec-6(lb127)* and *sup-40(lb130)*, which also suppress the previously isolated mutations *unc-8(e15)*, *unc-8(n491)* and *unc-8(e49)*.

**Construction of *mec-6;unc-8* double mutants:** N2 males were mated with *dpy-13(e184)unc-8(e15)* hermaphrodites. Resulting semi-Dpy coiler males, *dpy-13(e184)unc-8(e15)/dpy-13(+)unc-8(+)*, were mated with *mec-6(e1342)* hermaphrodites. Semi-Dpy coiler cross-progeny were picked singly, as

were their Dpy Unc self-progeny. Dpy non-Unc were picked singly from broods segregating both Dpy Unc and Dpy non-Unc as putative *mec-6(e1342);dpy-13(e184)unc-8(e15)*. These strains were outcrossed with wild-type males to obtain semi-Dpy coiler cross-progeny, confirming the presence of the *e15* mutation and the recessive nature of *e1342* suppression. *unc-8(n491)/unc-8(+)* males do not mate, therefore *dpy-5(e61)mec-6(e1342);unc-8(n491)* was constructed by mating *dpy-5(e61)mec-6(e1342)/dpy-5(+)mec-6(+)* heterozygous males, obtained by mating wild-type males with *dpy-5(e61)mec-6(e1342)*, with *unc-8(n491)*. Coiler cross-progeny F1 were picked singly, and non-Dpy backing-deficient Unc F2, homozygous for *n491*, were picked singly. From broods segregating both non-Dpy Unc and Dpy non-Unc F3, Dpy non-Unc were picked singly as putative *dpy-5(e61)mec-6(e1342);unc-8(n491)*. Outcrossing with wild-type males to obtain non-Dpy coiler cross-progeny confirmed the presence of the *n491* mutation. Full suppression of *unc-8* mutations is observed in four independently constructed strains: *mec-6(e1342);unc-8(e15)*, *mec-6(e1342);unc-8(n491)*, *mec-6(e1342);dpy-13(e184)unc-8(e15)* and *mec-6(e1342);dpy-13(e184)unc-8(n491)*.

**Isolation of *unc-8* revertants:** *unc-8(e15)*, *unc-8(n491)* or *unc-8(n491);deg-1(u38)* animals were treated with EMS as decribed (SULSTON and HODGKIN 1988) and their progeny (approximately 600,000 haploid genomes) visually screened for backing proficient animals in either the F1 generation, to obtain dominant suppressor mutations, or in the F2 generation, to obtain recessive mutations suppressing the Unc phenotype. A strain containing active transposons, *mut-2(r459);unc-8(e15)*, was also screened to obtain candidate insertional mutants. Fifteen wild-type revertants were recovered from *mut-2(r459);unc-8(e15)*, 14 from EMS-mutagenesis of *unc-8(n491)* or *unc-8(n491);deg-1(u38)* and 13 from EMS-treated *unc-8(e15)*. Nine revertant strains contained dominant *unc-8* mutations recovered after outcrossing to wild-type males and were thus considered to be extragenic. Seven extragenic mutations, *lb81*, *lb83*, *lb84*, *lb89*, *lb126*, *lb127* and *lb128*, fail to complement *mec-6(e1342)* in suppression of both *unc-8* and *deg-1* dominant mutations. Two other extragenic mutations, *sup-40(lb130)* and *sup-41(lb125)*, dominantly suppress *unc-8(15)* and *unc-8(n491)*. Mapping of *sup-40(lb130)* is described below. Heterozygotes obtained by mating *unc-8(n491)sup-41(lb125)* males with *dpy-13(e184)unc-8(n491)* hermaphrodites produce <1% Dpy non-Unc progeny, indicating linkage to *dpy-13* and *unc-8*. *unc-8(n491)* is, however, recovered, at very low frequency: <0.5% Unc, among the progeny of *dpy-13(e184)unc-8(+)sup-41(+)/dpy-13(+)unc-8(n491)sup-41(lb125)* (slow growth of Unc recombinants probably reduces their observed frequency).

**Construction of *mec-6 sup-40* double mutants:** *dpy-5(e61)-sup-40(+)mec-6(lb127)/dpy-5(+)sup-40(lb130)mec-6(+);unc-8-(e15);deg-1(u38)* were constructed as follows. Wild-type males were mated with *sup-40(lb130)/sup-40(+);dpy-13(e184)unc-8(e15);deg-1(u38)* hermaphrodites and the semi-Dpy non-Unc male progeny [due to dominant suppression of *unc-8-(e15)/unc-8(+)* by *sup-40(lb130)/sup-40(+)*] were crossed with *dpy-5(e61)mec-6(lb127);unc-8(e15);lon-2(e678)deg-1(u38)* hermaphrodites. Non-Unc, semi-Dumpy cross progeny were picked singly, of the genotype *dpy-5(e61)sup-40(+)mec-6-(lb127)/dpy-5(+)sup-40(lb130)mec-6(+);dpy-13(+)unc-8(e15)/dpy-13(e184)unc-8(e15);lon-2(e678)deg-1(u38)/lon-2(+)deg-1(u38)*. From their progeny, fertile non-Unc, tail-touch-insensitive animals with normal body shape were picked singly as *dpy-5(e61)sup-40(+)mec-6(lb127)/dpy-5(+)sup-40(lb130)mec-6-(+);unc-8(e15);deg-1(u38)*. Nine hundred progeny of these heterozygotes were examined to obtain exceptional Dpy, tail-touch-insensitive [loss of *mec-6(lb127)* from the *dpy-5(e61)* chromosome] and non-Dpy, tail-touch-sensitive recombinants (gain of *mec-6(lb127)* mutation by the *sup-40(lb130)* chromosome). Two recombinants of the first type and three of the latter type were found, indicating that *sup-40* is located approximately halfway between *dpy-5* and *mec-6*. Similar results were obtained in parallel experiments with heterozygotes carrying the *mec-6-(lb128)* mutation. *sup-40(lb130)mec-6(lb127 or lb128);unc-8(e15);deg-1(u38)* adults are sterile, tail-touch sensitive and exhibit swollen hypodermal nuclei as observed using Nomarski microscopy.

**Sequence of *deg-1* missense mutations:** *deg-1(u38u424)* was isolated as a touch-sensitive revertant of *deg-1(u38)* after EMS mutagenesis as described previously (CHALFIE and WOLINSKY 1990). A 35-kb cosmid clone of *deg-1(u38)* genomic DNA (designated TU#3) capable of conferring the tail-touch-insensitive and PVC cell degeneration phenotypes on transgenic animals, and a *deg-1* cDNA sequence were obtained previously (CHALFIE and WOLINSKY 1990). Identical methods were used to clone the equivalent fragment from *deg-1(u38u424)* genomic DNA. Sequences encoding amino acids 197 to 248 were amplified from each cosmid clone using the following primers by use of the polymerase chain reaction: 5′ ATGATCGAGGTATTCTACGAACAA 3′ (sense) and 5′ AAAGAAAAGGGAAATCATATCAAC 3′ (antisense). The amplified fragments were subcloned into the pKS$^-$ vector (Stratagene) and sequenced on both strands using the same primers and "Sequenase" DNA polymerase (United States Biochemical) according to manufacturer's instructions. The *u38* mutant clone contains a C→T mutation at base 668 of the coding strand. The *u38u424* suppressor allele retains the *u38* mutation and also contains a G→A change at base 677.

**Egg-laying assays:** Egg-laying assays were performed as decribed previously (TRENT *et al.* 1983). SKF525a (Research Biochemicals, Inc.) stock solutions were prepared fresh before use in M9. NDG stock solutions in DMSO were prepared fresh or were stored at −20° and were diluted in M9 supplemented with DMSO to a final concentration of 2%. Basal egg laying was determined for each strain in each experiment using M9 only, for comparison with SKF525a treatment, or with M9 supplemented with 2% DMSO, for comparison with NDG treatment.

## RESULTS

**The *unc-8* motorneuron swelling phenotype:** Dominant mutations of the *unc-8* gene cause a locomotion defect (BRENNER 1974; PARK and HORVITZ 1986), whose anatomical focus and biochemical basis are described here. Strong alleles such as *e15* and *n491* produce a fully Unc (uncoordinated) phenotype including coiling and inability to move backwards. Heterozygotes of either strong allele express a semi-Unc phenotype: they coil less severely and are able to back up in response to a tap on the head. Weak alleles such as *e49* and a new mutation, *lb109*, isolated in an NDG resistance screen (see MATERIALS AND METHODS) also confer the semi-Unc phenotype in homozygotes. The two strong alleles cause swelling of embryonically born motorneurons (Figure 1), as well as swelling of some neurons in head and tail ganglia. The clear-cut difference in backing ability between strong and weak *unc-8* mutations and between homozygotes and heterozygotes of the strong mutations also correlates with the presence

FIGURE 1.—*unc-8* Swollen motorneuron phenotype. Nomarski photomicrographs of late L1 *unc-8(e15)* (top) and *unc-8(n491)* (bottom). Ten swollen cells are visible in this plane of focus in the ventral nerve cord of each animal. Examples of individual swollen cells are marked with arrows.

of motorneuron swelling (Table 2), suggesting that defective motorneuron osmoregulation underlies the Unc phenotype.

Motorneuron swelling is absent at hatching and peaks in severity during late L1 and early L2 stages of larval development. No ventral nerve cord swelling is seen in older animals. *n491* is a stronger mutation than *e15*: homozygotes are more severely Unc, and unlike *e15*/+ males, *n491*/+ males fail to mate. The anatomical pattern of neuronal swelling is similar for *e15* and *n491*, but occurs more rapidly in *n491* larvae. Although the exact pattern of swollen cells differs from animal to animal, even at the same age, examples of swelling of each of the 15 midbody embryonically born motorneurons (DB3 to DA7) have been observed, including cases in which 15 swollen cells are seen simultaneously. We conclude that most or all motorneurons eventually swell in the majority of animals, although the time of onset varies for some cells. The swelling is similar in appearance to that of cells degenerating under the influence of *deg-1*, *mec-4* or *mec-10* dominant mutations (CHALFIE and WOLINSKY 1990; HUANG and CHALFIE 1994). *unc-8* mutant motorneurons, however, do not swell to as large a size as the PVC interneurons of *deg-1* mutants. It is possible that the *unc-8* induced swelling is transient, rather than lethal to the cells.

**TABLE 2**

**Correlation of motorneuron swelling and backing-defective phenotypes**

| Parental genotype | Swollen L1 motorneurons[a] | Adult backing[b] |
|---|---|---|
| N2 (wild-type) | 0 (20) | + |
| *unc-8(e15)* | 8.3 ± 1.8 (18) | − |
| *unc-8(n491)* | 12 ± 2.5 (19) | − |
| *unc-8(e49)* | 0 (30) | + |
| *unc-8(lb109)* | 0 (30) | + |
| *dpy-13(e184)unc-8(e15)/ dpy-13(+)unc-8(+)*[c] | 0[d] (10) | + |
| *dpy-13(e184)unc-8(n491)/ dpy-13(+)unc-8(+)* | 0[e] (11) | + |

[a] Synchronized late L1 progeny were obtained by allowing parents to lay eggs for 2 hr on plates subsequently incubated at 20°. Swollen cells with visible nuclei within the ventral nerve cord between the pharynx and anus were scored using Nomarski microscopy at 20–22 hr after egg laying. The number of larvae scored is in parentheses.

[b] Adults from nonsynchronized cultures were tapped on the head with a thin platinum wire to stimulate backing. Fully penetrant inability to back is indicated as −. Fully penetrant ability to back is indicated as +.

[c] Seventy-five percent of progeny of heterozygous parents are expected to exhibit swollen motorneurons if this phenotype is dominantly transmitted, 25% if the trait is recessive. The observed transmission is 16 and 21% for *e15*/+ and *n491*/+, respectively, consistent with recessivity. *dpy-13(e184)* is a semidominant mutation causing dumpy body size, three map units to the left of *unc-8* on chromosome *IV*, used in these experiments as a genetic marker to distinguish self- from cross-progeny of mated *dpy-13unc-8* double mutant hermaphrodites.

[d] Ten of 12 animals examined displayed no swollen cells; six ventral cord swellings were observed in each of the remaining two.

[e] Eleven of 14 animals examined displayed no swollen cells; the remaining three contained 8, 12 and 15 swollen cells, respectively.

Ventral cord nuclei in 4,6-diamidino-2-phenylindole (DAPI)-stained *n491* or *e15* L3 animals do not differ noticeably from wild-type in pattern or number (data not shown), suggesting that the nuclei of embryonically born motorneurons that had swollen earlier are still present. In addition, regression of swelling of individual neurons has been observed in L1 animals over an 8–10-hr period (Figure 2). Thus, at least some neurons appear to recover from *unc-8*-induced swelling.

**Interaction between *unc-8* and *mec-6*:** *deg-1*, *mec-4* and *mec-10* are members of a gene family with significant sequence (CANESSA *et al.* 1993; CHALFIE *et al.* 1993; HUANG and CHALFIE 1994) and functional (HONG and DRISCOLL 1994) homologies to the amiloride-sensitive epithelial sodium channel of mammals. Recessive *mec-6* mutations suppress the dominant cell-swelling phenotypes caused by *deg-1*, *mec-4* and *mec-10* mutations (CHALFIE and WOLINSKY 1990; HUANG and CHALFIE

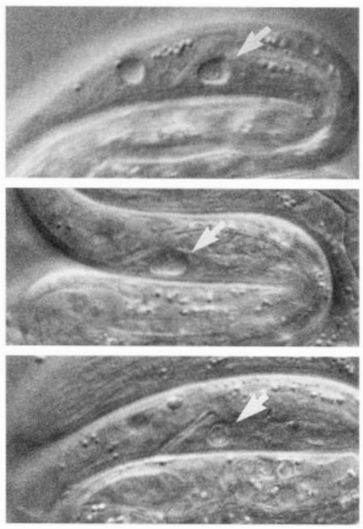

FIGURE 2.—Regression of neuron swelling. A single swollen cell (arrows) of the pre-anal ganglion was photographed using Nomarski microscopy at 22 hr (top), 24 hr (middle) and 26 hr (bottom) after the egg from which this *unc-8(n491)* L1 hatched was laid. The cell increases, then decreases, in size. The animal was reared and photographed at 20°.

1994). Thus, mec-6 is thought to encode a common protein partner of each of the homologous ion channel subunits encoded by the three genes. To examine the possible relationship between *unc-8* and *deg-1* family members, we constructed *mec-6(e1324);unc-8(n491)* and *mec-6(e1324);unc-8(e15)* double mutants (see MATERIALS AND METHODS). Uncoordinated locomotion, motor neuron swelling and NDG resistance (Figure 6) are fully suppressed in these double mutants. Furthermore, seven recessive *unc-8* extragenic suppressor mutations identified in *unc-8(e15)* and *n491* reversion screens were found to be new *mec-6* alleles (MATERIALS AND METHODS). Two of these are candidate transposon insertion alleles obtained from the mutator strain *mut-2(r459);unc-8(e15)*.

**Intragenic *unc-8* reversion events:** Two further genetic parallels between *deg-1* and *unc-8* support the idea that they are functional homologues. First, putative null mutations in *unc-8* confer a wild-type phenotype, suggesting that *unc-8* (PARK and HORVITZ 1986), like *deg-1* (CHALFIE and WOLINSKY 1990), is a dispensable gene, as expected for a redundant gene family member. We have obtained at least 42 wild-type revertants of dominant mutations following chemical and transposon mutagenesis (see MATERIALS AND METHODS). These are likely to be reduction-of-function alleles consisting of a second intragenic mutation modifying the coding capacity or level of expression of the original dominant allele. Two putative null alleles tested (*e15lb54* and *e15lb55*, from EMS mutagenesis and transposon mutagenesis, respectively) confer a stronger Unc phenotype in *trans* with any of the four *unc-8* dominant mutations

**TABLE 3**

***deg-1(u38u424)* is a *trans*-acting suppressor**

| Genotype | Temperature | % touch sensitive |
|---|---|---|
| *lon-2(e678)deg-1(u38)* / *lon-2(+) deg-1(+)* | 15° | 14 (79) |
| | 20° | 4 (56) |
| | 25° | 0 (126) |
| *lon-2(e678)deg-1(u38u424)* / *lon-2(+) deg-1(u38 +)* | 15° | 95 (80) |
| | 20° | 58 (73) |
| | 25° | 7 (134) |

Adult cross-progeny of wild-type males crossed with *lon-2(e678)deg-1(u38)* or of *deg-1(u38)* males crossed with *lon-2(e678)deg-1(u38u424)* hermaphrodites were identified by their non-Lon phenotype (*lon-2(e678)* is a recessive mutation conferring long body shape). Forward movement in response to touch on the tail with a thin platinum wire was scored as described previously (CHALFIE and WOLINSKY 1990). The number of animals scored is in parentheses.

(*n491*, *e15*, *e49* or *lb109*) than does the wild-type allele, as expected for loss-of-function mutations.

**Interallelic suppression:** Second, *trans*acting intragenic suppressor mutations of both *deg-1* and *unc-8* have been found. The *unc-8* allele *e15lb129* fully suppresses *e15* and *n491 in trans*, while conferring a wild-type phenotype in homozygotes. A second unusual revertant allele, *unc-8(n491lb82)*, is a *trans*acting enhancer: *n491lb82* homozygotes move normally, but *n491lb82/+* heterozygotes, remarkably, are backing defective and markedly more coiled than *n491/+* animals, which can back up. This suggests that the double mutant protein encoded by *n491lb82* interacts with wild-type *unc-8* subunits differently than does the original *n491* mutant protein. Unlike the putative null intragenic revertant mutations, which lack *trans*suppressing or -enhancing activity, *e15lb129* and *n491lb82* most likely contain second-site missense mutations that allow a functional, albeit abnormal, gene product to be made.

**Amino acid changes in *deg-1* interallelic suppressor mutations:** An analogous intragenic *trans*acting suppressor mutation of *deg-1*, *u38u424*, also acts homozygously as a wild-type revertant (Table 3). *deg-1(u38)/deg-1(+)* adults express a fully penetrant tail-touch-insensitive phenotype at 25°, due to the degeneration of the PVC interneurons that receive input from the posterior mechanosensory PLM cells and synapse onto motorneurons mediating forward movement (CHALFIE and SULSTON 1981; CHALFIE and WOLINSKY 1990). Seven percent of *deg-1(u38+)/deg-1(u38u424)* heterozygotes at this temperature, however, move forward when touched on the tail, indicating the presence of functioning PVC neurons, while *deg-1(u38u424)* homozygotes are all wild-type. The penetrance of the tail touch insensitive phenotype of *deg-1(u38)/deg-1(+)*

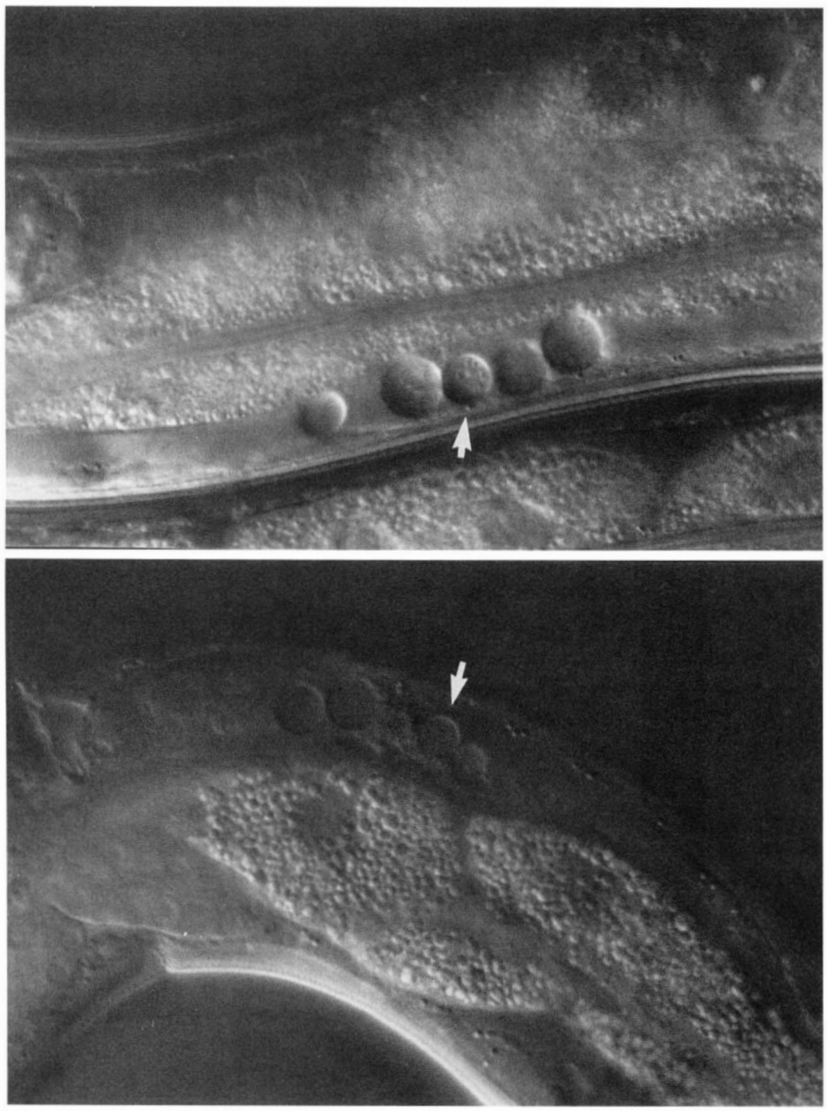

FIGURE 3.—Hypodermal phenotype conferred by *sup-40(lb130)*. Five (top) and four (bottom) unanchored swollen hypodermal nuclei in *sup-40(lb130);unc-8(e15lb54)* adults (arrows).

animals is heat sensitive, and the *trans* suppression conferred by *deg-1(u38)/ deg-1(u38u424)* is cold sensitive. The idea of conformationally sensitive interactions between *deg-1* proteins suggested by this result is supported by sequence data (see MATERIALS AND METHODS). *deg-1(u38)* is a missense mutation changing $Ala^{223}$ (numbered as in CHALFIE and WOLINSKY 1990) to Val. This change is homologous to that reported for *mec-4(e1611)*, a dominant mutation inducing mechanosensory cell degeneration (DRISCOLL and CHALFIE 1991) and for an *in vitro*-engineered *mec-10* degeneration allele (HUANG and CHALFIE 1994). The *trans* suppressor allele *deg-1(u38u424)* contains a second missense mutation, changing $Gly^{226}$ to Arg, only two codons downstream of the first. Each of these missense mutations has, evidently, opposite effects on how *deg-1* proteins interact with each other or with other components of a functional ion channel complex. The existence of *trans*suppressing alleles of both *unc-8* and *deg-1* suggest their gene products function as multimers, consistent with the known ability of the homologous mammalian $Na^+$ channel subunits to form functional complexes (CANESSA *et al.* 1994).

**Novel *unc-8* suppressor loci:** *unc-8* reversion screens also yielded two novel extragenic suppressor loci, each identified by a dominant mutation. *sup-40*, defined by the *lb130* mutation, maps within the *dpy-5*-to-*mec-6* interval of chromosome *I* (see MATERIALS AND METHODS). *sup-41(lb125)* is tightly linked to, but separable from, *unc-8*. *unc-8(n491)sup-41(lb125);deg-1(u38)* triple mutants move normally, and, when reared at 20°, 5% of adults respond to tail touch ($n = 224$). At 25° no tail-touch-sensitive animals are found ($n = 103$), while at 15°, 30% respond to tail touch ($n = 105$). The tail-touch-insensitive phenotype of *deg-1(u38)* and *unc-8(n491);deg-1(u38)* homozygotes is fully penetrant at all three temperatures (at least 100 adults of both genotypes tested at each temperature). Since backing profi-

ciency is restored at all three temperatures, the cold-sensitive suppression of tail-touch insensitivity may result from the heat sensitivity of *deg-1(u38)*-induced PVC degeneration (CHALFIE and WOLINSKY 1990) rather than cold sensitivity of *sup-41(lb125)* itself. The cross-suppression of *unc-8* and *deg-1* by *sup-41*, in addition to *mec-6*, strengthens the idea that *unc-8* and *deg-1* proteins are biochemically similar.

*sup-40(lb130)* dominantly suppresses all phenotypes associated with the *unc-8* dominant mutations *e15*, *n491*, *e49* or *lb109*: coiling, defective backing, motor neuron swelling and NDG resistance. However, *sup-40(lb130)* homozygotes also express four unselected recessive phenotypes: slow growth, sterility (Figure 4), swelling of adult hypodermal nuclei (Figure 3) and strong NDG resistance (Figure 6). These four phenotypes are expressed whether the *unc-8* genotype is wild type, dominant or null, suggesting that the *unc-8* gene product does not directly contribute to them. *sup-40(lb130)* does not cross-suppress *deg-1(u38)*, nor do *mec-6* mutations visibly suppress any *sup-40(lb130)* phenotypes. Lack of suppression of *sup-40(lb130)* by *mec-6* mutations suggests the two gene products do not interact, either because *sup-40* encodes a protein dissimilar in function to *deg-1* gene family members, *i.e.*, is not a channel subunit, or because *mec-6* does not act in oocytes and hypodermis. Similarly, *sup-40(lb130)* does not suppress PVC cell death in double mutants with *deg-1(u38)*. The lack of genetic interaction between *sup-40* and *deg-1* also suggests a lack of protein interactions or restriction of expression to different cell types.

The recessive hypodermal nuclear swelling phenotype conferred by *sup-40(lb130)* is novel, affecting 80% of *sup-40(lb130);unc-8(e15)* adults, as scored using Nomarski microscopy. A few swollen nuclei are seen in young adults; more accumulate with age. In many cases, as shown in Figure 3, the swollen nuclei become detached from their anchored positions within the hypodermal syncytium and float freely within the hypodermal cytoplasm. Such nuclei shift back and forth contrary to the direction of body movement, or become entrained by the rhythm of pharyngeal pumping, if located behind the head. Similar mobility of hypodermal nuclei, but without swelling, was described as a phenotype of *anc-1* mutations (HEDGECOCK and THOMSON 1982) and interpreted as a defect in cytoskeletal elements that normally fix nuclei at specific positions within the hypodermal syncytium. In the case of *sup-40(lb130)*, nuclear detachment is likely a secondary effect of swelling, since swollen fixed nuclei are also seen. Nuclear swelling could result from osmotic or pH changes (OBERLEITHNER *et al.* 1993; WUNSCH *et al.* 1993) within the hypodermis, consistent with the idea of an ion channel malfunction caused by the *sup-40(lb130)* mutation.

The sterility of *sup-40(lb130)* homozygotes is due to failure to produce normal oocytes. The somatic gonad of these mutants appears normal, as observed with Nomarski optics. DAPI staining (data not shown) reveals the presence of dividing germline precursors, as well as appropriately localized mature sperm. However, mature oocytes are rarely seen. The abnormal oocytes (Figure 4) are distorted in shape and larger than nor-

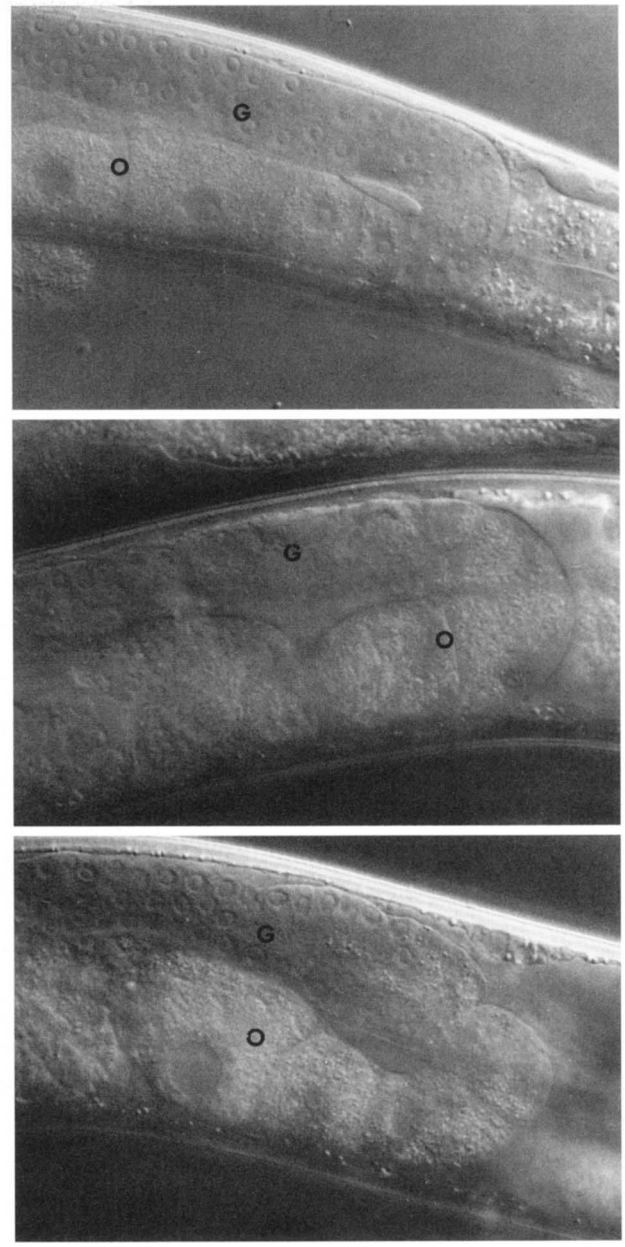


FIGURE 4.—Sterile phenotype conferred by *sup-40(lb130)*. (Top) One arm of a wild-type adult gonad, containing a normal germ line proliferation zone (G) and four maturing unfertilized oocytes (O). (Middle) One arm of a *sup-40(lb130);unc-8(e15lb54)* adult gonad, which contains a germ line (G) proliferation zone and two highly abnormal oocytes (O). (Bottom) One arm of a *sup-40(lb130);unc-8(+)* adult gonad, containing a germline proliferation zone (G) and three highly abnormal oocytes (O).

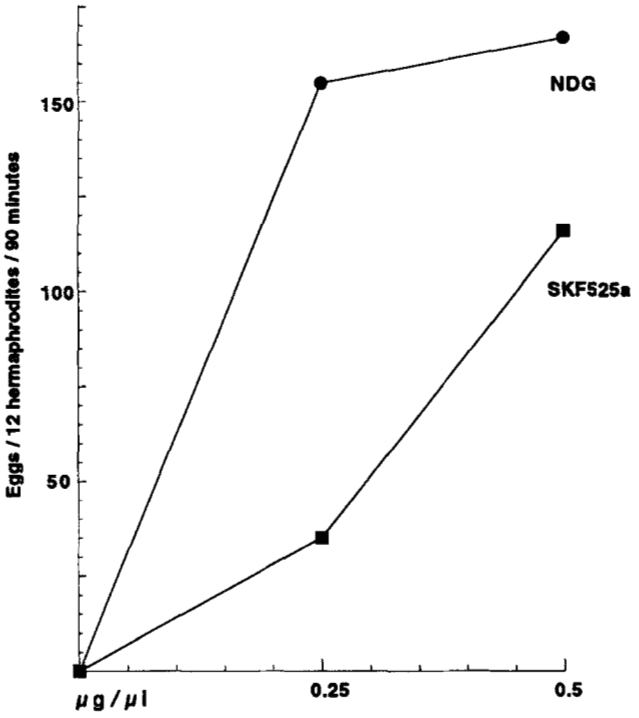


FIGURE 5.—Induction of egg laying by NDG and SKF525a. Horizontal axis, drug dose in $\mu g/\mu l$; vertical axis, the total number of eggs laid by 12 hermaphrodites during a 90-min incubation in NDG (●) or SKF525a (■).

mal. They appear to be fragile, often bursting during normal body flexion. A second mutation conferring strong NDG resistance (Figure 6) without visible behavioral defects, *ndg-4(lb108)* on linkage group *III* (see MATERIALS AND METHODS), also produces abnormal oocytes, unusually pale in color as if yolk deficient and yielding only 20% as many progeny per brood as wild type.

**NDG and egg-laying behavior:** NDG has been used in Aplysia to interrupt an intracellular signaling pathway leading to S-$K^+$ channel opening in response to the neuropeptide FMRFamide (BELARDETTI *et al.* 1989; SCHACHER *et al.* 1993). In this system, the channel is thought to respond directly to an eicosanoid second messenger molecule, hepoxilin, formed by lipoxygenase-mediated conversion of arachidonic acid to the precursor HPETE and a subsequent heme-dependent P450 oxidation step. These enzymes are inhibited, respectively, by NDG and a second drug, SKF525a, which block the effect of FMRFamide on the channel. FMRFamide is also present within several neuronal types in *C. elegans*, including six ventral cord motorneurons, the VC cells, which synapse onto the egg-laying muscles (SCHINKMAN and LI 1992; WHITE *et al.* 1986). Both NDG and SKF525a induce egg laying by wild-type animals (Figure 5), suggesting a neuroactive effect of these compounds in *C. elegans*.

Serotonin, the endogenous transmitter responsible for activation of the egg-laying muscles, exerts its maximal effect at a dose of 5 mg/ml (TRENT *et al.* 1983). NDG optimally induces about half the egg-laying response of serotonin, but does so at 10-fold lower concentration. Up to 2.5 $\mu g$/ml of NDG does not, however, elicit egg laying by *egl-1(n487)* mutants (data not shown), which lack the serotonergic motorneurons, the HSN cells, that drive the egg-laying muscles (DESAI *et al.* 1988). NDG either acts directly on the HSN cells or modulates egg-laying muscle response to the HSN cells.

**TABLE 4**

**Elevated basal egg laying by *unc-8* mutants**

| Genotype | Eggs/ 12 animals/ 90 min |
|---|---|
| Experiment 1 | |
| N2 (wild-type) | 2 |
| *unc-8(lb109)* | 98 |
| *unc-8(e15)* | 72 |
| *dpy-13(e184)unc-8(e15)/dpy-13(+)unc-8(+)* | 33 |
| *sup-40(lb130)/sup-40(+);unc-8(e15)* | 7 |
| *unc-8(n491)* | 86 |
| *dpy-13(e184)unc-8(n491)/dpy-13(+)unc-8(+)* | 59 |
| *sup-40(lb130)/sup-40(+);unc-8(n491)* | 13 |
| Experiment 2 | |
| N2 (wild-type) | 8 |
| *dpy-13(e184)/dpy-13(+)* | 11 |

Egg laying was assayed as described (TRENT *et al.* 1983). *dpy-13* is used in these experiments as a genetic marker to distinguish self- from cross-progeny of mated *dpy-13unc-8* double-mutant hermaphrodites.

**Constitutive egg-laying by *unc-8* mutants:** *unc-8* dominant mutants display significantly elevated basal egg laying compared with wild-type (Table 4). This phenotype is also *sup-40*-suppressible. Thus, *unc-8* ion channel subunits and their *sup-40* partner are components of egg laying as well as locomotory circuitry. Two percent DMSO, the solvent used for NDG in these experiments, abolishes spontaneous egg laying by *unc-8* mutants. Although this effect is almost certainly not specific, it is noteworthy, because it implies that the mechanisms of NDG-induced egglaying by wild type and elevated basal egg laying by *unc-8* mutants are distinct.

## DISCUSSION

Five genetic properties shared with the *deg-1* gene suggest that *unc-8* encodes a related ion channel subunit: (1) two dominant *unc-8* mutations cause neuronal swelling; (2) *mec-6* is a recessive suppressor of *unc-8* dominant mutations; (3) *unc-8* appears to be a dispensible gene, since the null phenotype is wild type; (4) *unc-8* can mutate to *trans*suppress or enhance its own product, implying interaction or competition between *unc-8* proteins and (5) the *sup-41(n491lb125)* mutation

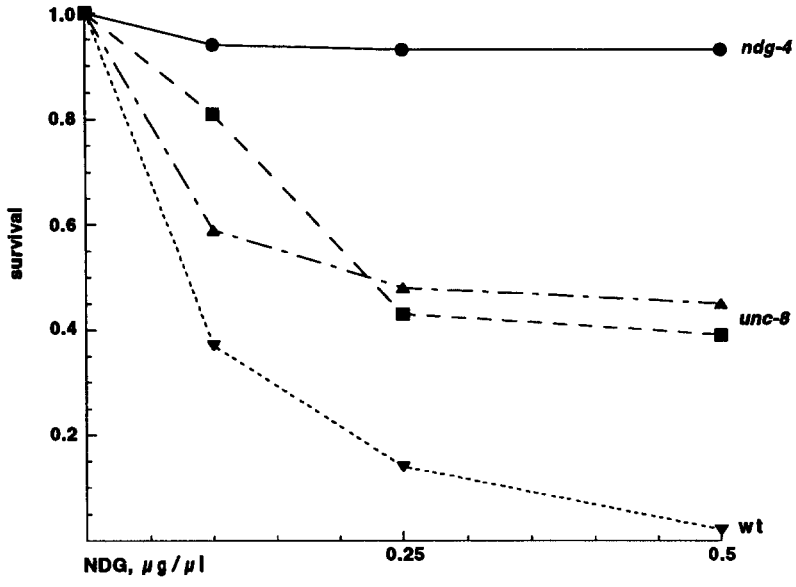


FIGURE 6.—NDG-resistance of mutant strains. Wild type is classified as nonresistant (wt, ---◀---, 0–15% survival). *ndg-4(lb108)* is classified as strongly resistant (——●——, +++, 70–95% survival). *unc-8(e15)* (– – –■– – –) and *unc-8(lb108)* (– – –▲– – –) mutants are classified as moderately resistant (+, 30–60% survival). Resistance of each strain was ranked after at least three independent tests comparing survival with that of wild type. The survival tests were performed and percentage survival was calculated, as described in MATERIALS AND METHODS.

| STRAIN | NDG RESISTANCE |
|---|---|
| N2 (wild type) | – |
| *unc-8(e15), (e49), (n491), (lb109)* | + |
| *unc-8(lb54), (lb55)* (putative null alleles) | – |
| *mec-6(lb128); unc-8(e15)* | – |
| *sup-40(lb130/+); unc-8(e15)* | – |
| *sup-40(lb130); unc-8(e15)* | +++ |
| *sup-40(lb130); unc-8(+)* | +++ |
| *sup-40(lb130); unc-8(lb54)* | +++ |
| *ndg-4(lb108)* | +++ |

suppresses both *unc-8* and *deg-1* dominant mutations. Future work to clone and sequence the *unc-8* gene is required to confirm its membership in the *deg-1* ion channel family. The *sup-40(lb130)* mutation dominantly suppresses *unc-8* locomotion defects and confers two recessive phenotypes: hypodermal nuclear swelling and sterility due to enlarged, fragile oocytes. The influence of *sup-40* on cell membrane permeability thus extends beyond motorneurons to at least two quite different cell types, suggesting a broader physiological role. Recognition of the role of *unc-8* and *sup-40* in ion channel function in our laboratory originated in drug-resistance screens aimed at identifying genes involved in second messenger signaling by eicosanoids. The enzyme inhibitors NDG and SKF525a, which block FMRFamide-induced $K^+$ channel opening in Aplysia, induce egg laying in *C. elegans*, suggesting that endogenous eicosanoids may play an inhibitory role in modulating this behavior. It has also proved feasible to isolate mutants resistant to the lethal effects of longer-term NDG exposure, among which was found a new allele of the *unc-8* gene, *lb109*. The NDG resistance of pre-existing *unc-8* mutants was therefore also tested, at which time the swollen motorneuron phenotype of *n491* and *e15* larvae, previously unnoted, was observed. The mechanism by which *unc-8* ion channel mutations confer moderate NDG resistance is currently unknown and is probably distinct from that of the strong NDG resistance phenotype of *ndg-4(lb108)* mutants, which appear behaviorally normal. Remarkably, strong NDG resistance is conferred as an unselected recessive phenotype by the *sup-40(lb130)* mutation. This raises the possibility that NDG, a hydrophobic compound, may interact with some types of ion channels directly, or indirectly, like a local anesthetic dissolved in cell membranes (FRANKS and LIEB 1994). NDG may prove to be a useful reagent for identifying additional loci affecting neurotransmission and ion channel function.

The *C. elegans* $Na^+$ channel sequence homologues, *deg-1*, *mec-4* and *mec-10*, were first identifed through mutations causing defective sensitivity to touch (CHALFIE and SULSTON 1981; CHALFIE and Au 1989). These genes encode proteins with two predicted membrane spanning domains and a large variable extracellular do-

main (CHALFIE and WOLINSKY 1990; DRISCOLL and CHALFIE 1991; CHALFIE *et al.* 1993; HUANG and CHALFIE 1994; E. WOLINSKY, unpublished results). Homologous alanine-to-valine missense mutations near the second hydrophobic domain of each gene cause dominant neuronal degeneration, *mec-4* and *mec-10* affecting mechanosensory neurons and *deg-1* affecting interneurons, including the PVC cells that relay posterior-touch-receptor input to motor neurons. The swelling and death of these cells probably results from excessive ion influx through the abnormal channels. The *unc-8* dominant mutations (in order from most to least severe) *n491*, *e15*, *e49* and *lb109*, each confer a distinct locomotion phenotype. It is likely that each will prove to consist of a different missense mutation resulting in increased channel activation or conductance. The *trans*acting suppressor and enhancer mutations *lb129* and *lb82*, respectively, are also likely to be novel and distinct missense mutations. The elaboration of these structure-function relationships at the molecular level will require cloning the *unc-8* gene.

The isolation of the *trans*acting *unc-8(e15lb129)* and *unc-8(n491lb82)* mutations, as well as extragenic suppressor mutations in *mec-6* and *sup-41* support the idea that, like their mammalian homologues (CANESSA *et al.* 1994), the nematode channel proteins function in heteromeric complexes. The product of the *mec-6* gene positively regulates *deg-1*, *mec-4* and *mec-10* channel complexes, since reduction-of-function *mec-6* mutations suppress the dominant degeneration-producing mutations. Furthermore, *deg-1* and *unc-8* each display interallelic suppression, suggesting that ion channel complexes contain multiples of these gene products. Finally, one unusual *unc-8*-suppressing mutation, *sup-41(lb125)*, also partially suppresses the PVC neuron-degeneration phenotype of *deg-1(u38)*, suggesting that the two types of channel subunit both interact with the *sup-41* as well as *mec-6* gene product. These genetic interactions suggest that functional motorneuron channel complexes contain more than one molecule of either *unc-8* protein, as well as at least one molecule of *mec-6* and *sup-41* proteins. Both homotypic and heterotypic protein interactions are probably important in channel function.

The recessive phenotypes of hypodermal nuclear swelling, sterility and NDG resistance conferred by *sup-40(lb130)* suggest that it may be a loss-of-function mutation. In this case, its dominant suppression of *unc-8* dominant mutations would arise from haplo-insufficiency. Alternatively, *lb130* may be a gain-of-function mutation whose gonadal and hypodermal effects are antagonized by the wild-type gene product in heterozygotes. Construction of deficiency and duplication strains, isolation of additional *sup-40* mutations and *sup-40(lb130)* reversion screens will address this question.

The *C. elegans* hypodermis is a polarized epithelium. It secretes cuticle on the exterior of the worm and extracellular matrix on the interior; it is exposed to the external environment on one side and to the fluid compartment of the pseudocoelom on the other. Polarized epithelia, such as lung, kidney and intestine, contain amiloride-sensitive $Na^+$ channels on the apical surface, and $Na^+$-extruding exchangers on the basal side, to control transepithelial ion and water flow (SMITH and BENOS 1991; TURNHEIM 1991). Motorneurons are not polarized in this way. This difference may explain how the *sup-40(lb130)* mutation suppresses swelling in motorneurons, yet causes it in hypodermis. *unc-8* protein in hypodermis may be expected to be expressed on the apical surface, exposed to the compartment beneath the cuticle, while *unc-8* protein in motorneurons is exposed to a different ionic environment in the interior of the animal. Other types of ion channels expressed on the basal surface of the hypodermis, whose activity might compensate for abnormal *unc-8* function, even if coexpressed in motorneurons, would not be properly oriented to allow similar compensation in motorneurons.

Another possibility is that *unc-8* is not expressed in hypodermis, since the recessive *sup-40(lb130)* phenotypes are expressed even in *unc-8* null mutants. In that case, another, as yet unidentified, member of the *deg-1* gene family may take its place. It is also possible that *sup-40* encodes yet another *deg-1* family homologue that forms a heteromeric channel including *unc-8* protein. Gene cloning is the most direct way to determine whether *unc-8* and *sup-40* encode homologous proteins. Associations between different coexpressed $K^+$-channel homologues are limited to certain combinations (COVARRUBIAS *et al.* 1991). This raises the possiblity even if *unc-8* and *sup-40* do encode homologous products coexpressed within the hypodermis, they might not interact with each other.

Our working hypothesis is that *sup-40(lb130)* produces an ion flux opposing that of dominant mutant *unc-8* channels in motorneurons. If mutant *unc-8* channels allow excessive $Na^+$ influx, as suggested by the *deg-1* gene family homology to mammalian amiloride-sensitive $Na^+$ channels, then *sup-40* may encode or regulate a $Na^+$-extruding channel, such as a $Na^+$/cation-exchanger. Gain-of-function mutations activating two different ion channels could thus compensate for each other. One approach to cystic fibrosis treatment is to compensate for abnormal lung chloride transport by lowering apical $Na^+$ channel activity with amiloride (JIANG *et al.* 1994; SMITH *et al.* 1994). The human lung amiloride sensitive $Na^+$ channel is also a member of the *deg-1* family (VOILLEY *et al.* 1994), with overall 50% conserved or identical amino acids when compared to either *deg-1* (CHALFIE and WOLINSKY 1990; E. WOLINSKY, unpublished results) or *mec-4* (DRISCOLL and CHALFIE 1991). Identification of *unc-8* suppressor loci may thus suggest other therapeutic

targets, as well as revealing basic mechanisms underlying cellular osmoregulation.

A major question remains elucidation of the basis of $Na^+$ channel diversity. What is the spectrum of cell-type-specific expression of channel components? What governs the protein-protein associations of active channel complex formation? What determines the susceptibility of particular types of channel complexes to different regulators, such as hormones and second messengers (LIGHT *et al.* 1990; LINGUEGLIA *et al.* 1994)? These question hinge on knowing the identities of proteins interacting with known ion channel subunits. The relatively subtle mechanosensory defects of *deg-1*, *mec-4*, *mec-10* and *mec-6* mutants, scored by touching individual animals, makes screening for interacting suppressor or enhancer mutations extremely difficult. We have recognized a likely new member of the $Na^+$ channel family, *unc-8*, which can mutate to cause motorneuron swelling and visibly abnormal locomotion. The ease of scoring the *unc-8* mutant phenotype allows the large-scale revertant screening that is required to detect rare suppressor and enhancer mutations. One *unc-8* suppressor locus that we have identified, *sup-40*, affects hypodermis and oocytes as well as neurons, suggesting sharing of some osmoregulatory proteins between these diverse cell types. Thus, we expect that genetic and molecular analysis of *unc-8* and interacting genes will lead to clearer understanding of how $Na^+$ channel complexes are assembled and their activity controlled in a variety of cell types.

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