Regulation of *Caenorhabditis elegans* degenerin proteins by a putative extracellular domain

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Background: Rare, dominant mutations in the degenerin genes of *Caenorhabditis elegans* (*deg-1*, *mec-4* and *mec-10*) cause neuronal degeneration. The extensive sequence similarity between degenerins and mammalian genes that encode subunits of the amiloride-sensitive sodium channel from kidney, colon and lung suggests that the *C. elegans* degenerins form ion channels. As *mec-4* and *mec-10* are needed for the reception of gentle touch stimuli, they may contribute to a mechanosensory ion channel. All the dominant degeneration-causing mutations in the *C. elegans* degenerin genes affect equivalent residues in a hydrophobic region that is structurally similar to the H5 domain of several ion channels, and so could form the channel lining. Increased channel activity may underlie the resulting degeneration, in which the affected cells vacuolate and swell.

Results: We now demonstrate that a missense change in a predicted extracellular region of the proteins encoded by *deg-1* and *mec-4* causes cell death similar to that caused by the dominant mutations. The missense mutation lies within a 22 amino-acid region found in all the *C. elegans* degenerins for which the sequences have been published, but not in the similar mammalian proteins. Deletion of nine amino acids surrounding the mutation site in *mec-4* also causes neuronal degeneration. The degeneration-causing mutations in either the predicted pore-lining or the predicted extracellular regions of *deg-1* are suppressed by additional, dominantly acting mutations that substitute larger for smaller residues within the channel lining.

Conclusions: Our data suggest that the putative extracellular domain negatively regulates degenerin activity, perhaps by gating the channel. As this region is only found in the *C. elegans* proteins, it may allow more rapid regulation of the nematode channels, which may be needed for them to function in mechanosensation. The suppressor mutations, by adding larger amino acids to the putative pore lining, could prevent degeneration by blocking the pore of a multisubunit channel.

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Background

Members of the degenerin gene family of the nematode *Caenorhabditis elegans* encode proteins that can be mutated to cause neuronal degeneration — cells become vacuolated, swell, and die (the Deg phenotype). This degeneration is caused by dominant gain-of-function mutations that result in the formation of abnormal products; loss-of-function mutations in any of the three degenerin genes — *deg-1* [1], *mec-4* [2] and *mec-10* [3] — do not affect cell viability. Two of the genes, *mec*-4 and *mec*-10, are needed for the function of a set of six touch receptor neurons [4,5]. The function of *deg-1* is unknown; loss of *deg-1* activity produces no mutant phenotype [1].

The *C. elegans* degenerin genes encode proteins that are similar to subunits of the amiloride-sensitive epithelial sodium channel (ENaC) of rats and humans [6–10]. Three degenerin-like proteins found in rats — the α, β and γ rENaCs — are thought to contribute to the rat channel [7]. The nematode and mammalian proteins have similar sizes (650–778 amino acids) and predicted structures. Each of the proteins has two hydrophobic stretches that are sufficiently long to be membrane-spanning and that surround an extensive region with several of the hallmarks of being extracellular domains (see below). Recent biochemical and antibody-based studies [11] support the identification of this region as extracellular. Because of the sequence similarity between the *C. elegans* and mammalian proteins, and the vacuolated appearance of the cells that die in the *C. elegans* mutants — a phenotype that suggests a disruption of membrane activity — we believe that the degenerins, like their mammalian counterparts, are subunits of ion channels. Furthermore, as dominant gain-of-function mutations, and not loss-of-function mutations, cause neuronal degeneration, cell death could result from an increased or altered ion flux through the channel (as hypothesized in [1]).

In this paper, we describe mutations in a predicted extracellular region of the proteins encoded by *deg-1* and *mec-4* that cause neuronal degeneration. This gain-of-function phenotype suggests that the region encompassing these mutations regulates degenerin activity. As this region is found in all *C. elegans* degenerins, but not in any of the similar mammalian proteins, this form of regulation may be restricted to the *C. elegans* proteins. These observations lead us to propose that the regulatory region results in degenerin and ENaC channels with very different activities.

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(a)

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MSNHHSKTKKTSMLGREDYIYSHDITNKNKKEKLNGASKNNDYNQDDDDETMKSKMMDFCDKTTAHGAKRVLIARNSFSKLMWGLIIFSF      90
LLMFAYQASKLIFKFSAHEKITDISLKFDDVEFPAITFCNLNPYKKSLVMMVPSIRDTMDVYDNAKTHSKSEGEKKKPKVSRKQHSDASQ     180
QMVRELFAKEIEEGMVELKKSNKTLQSQNKSGRRRSQRSIENRRYEAIEAHCKCVGNIGMECIRFESPPRDPSSKCICTYDRDMEVAWPC     270
FNISVWYDHECPLCHDDGYCESTLPSGTTSSDKWPCMCRNRGDTSERDDTPYCIGKAGVGKIEIRKLWLENNMTTTSTTTTTTTTPPPtt     360
tsttttttttppptttARPNQRAIVSNPETIKAMGFQGMTDGVAMLTRAKENLMFTMAALSDKQRIALSQSKHEFIEMCSFNGKECDIDE     450
DFRLHVDPEFGNCFTFNYDVNNNYTSSRAGFMYGIRVLLFVNTSDYMSTSESSGVRLAIHPPTEYPFPDTFGYSAPVGFASSFGIKKKVM     540
QRLPAPYGECVETKKVVDRNYIYAGYDYHPEGCHRSCFQNGLIDDCSCGDPRFPVPEGYRHCSAFNATARTCLEKNIGSVGDFHHITQKM     630
DKCVCKQSCEEIIHEVTFSCSKWPSGATDlgdcdgmteseceqyyrlnaamieVFYEQLNYELLQESEAYGLVNLIADFGGHLGLWLGFS     720
VITVMEVCVLLVDMISLFFKSRHEEKLLRQSTKRKDVPEDKRQITVGSGRKSDAFVSI                                    778
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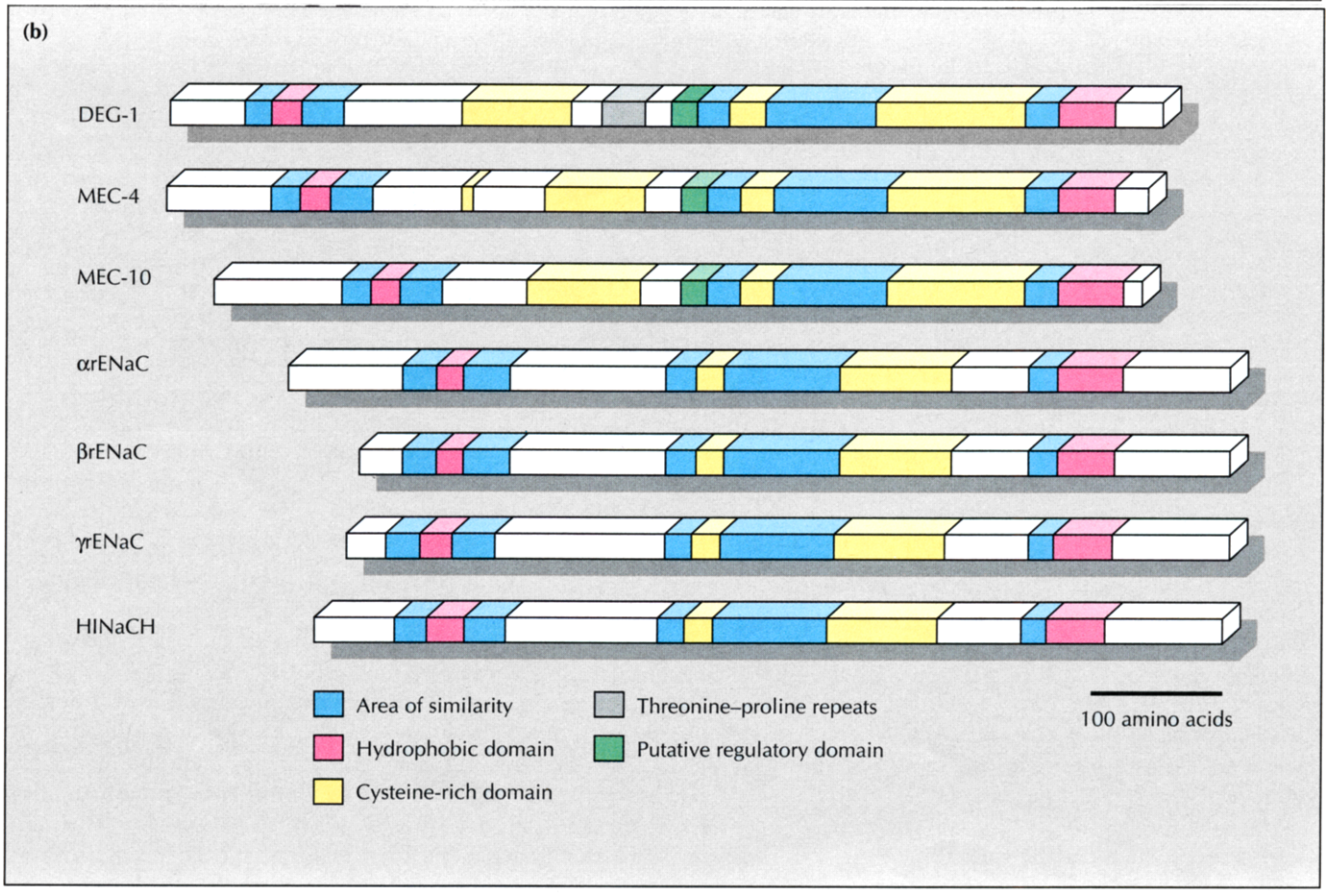
(b)



(c)

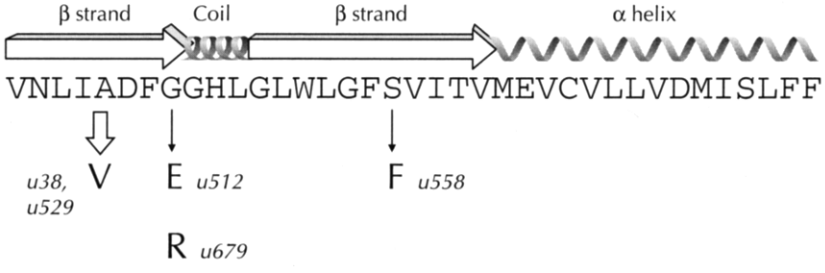


Fig. 1. The *deg-1* gene and the degenerin/ENaC superfamily. **(a)** Predicted protein sequence from *deg-1* cDNAs (GenBank Accession Number L34414). As is the case for the majority of *C. elegans* transcripts, the *deg-1* messages are *trans*-spliced to the SL1 leader sequence [45,48]. The longest predicted DEG-1 protein has 778 residues; two exons (lower case letters) have been found in only some cDNAs. Predicted *N*-glycosylation sites (open boxes), phosphorylation sites (underlined), and an RGD sequence (amino acids 311–313; red type) are indicated on the sequence. Predicted membrane-spanning (pink box) and cysteine-rich (yellow box) regions, and other regions of similarity (blue box) with other members of this superfamily are shown. Also indicated, with black lines above and below it (green in (b)), is the region that is found only in the *C. elegans* proteins and that contains the amino acid (residue 393) that is changed by the *deg-1(u506)* mutation. The grey box indicates the Thr–Pro repeats that have been seen only in DEG-1. **(b)** Comparison of the longest DEG-1 protein and homologous proteins. Sequences were obtained from [2,3,7,9] and M.Driscoll and M.C. (unpublished observations). The areas of similarity are indicated with the same colours as in (a). **(c)** Predicted secondary structure (analyzed by the method of Garnier [13]) of the second hydrophobic domain of DEG-1, and amino-acid substitutions caused by Deg (open arrow) and Sup (closed arrows) mutations in this domain.

Results

The *deg-1* gene and its products

We have determined the complete coding sequence for *deg-1*, and find cDNAs with two alternative exons . The longest predicted *deg-1*-encoded protein (DEG-1) contains 778 amino acids (Fig. 1a). DEG-1 is similar to other *C. elegans* degenerins and to the mammalian epithelial channel proteins (Fig. 1b). These proteins have two hydrophobic domains that are likely to be membrane-spanning (by the method of Rao and Argos [12]). In addition, analysis of the second hydrophobic domain by the method of Garnier [13] predicts that it should form a β strand, a coil, and a β strand (that could produce a β hairpin) followed by an α helix (Fig. 1c).

The bulk of the sequence lies between the two hydrophobic domains and contains several sequences characteristic of extracellular domains. In particular, all the proteins have potential *N*-linked glycosylation sites and cysteine-rich domains. The nematode and vertebrate proteins differ in this putative extracellular region, in that this region in the *C. elegans* proteins is larger and uniquely contains an additional cysteine-rich sequence and a 22 amino-acid sequence (near the middle of the region; Fig. 1b). In addition, DEG-1 has two motifs not found in the other proteins. The first contains two threonine- and proline-rich repeats. In muscins, similar domains are heavily *O*-glycosylated [14,15], form rigid, extended structures, and mediate cell adhesion by binding to selectins [16,17]. The second motif is an RGD (Arg–Gly–Asp) sequence (residues 311–313), a putative integrin-attachment site [18]. Immunoprecipitation and protease digestion studies of αrENaC also suggest that the region between the two hydrophobic domains is extracellular [11].

These considerations suggest that the amino and carboxyl termini of the proteins in the superfamily reside in the cytoplasm. Consistent with this proposed structure, the termini of the proteins have several predicted phosphorylation sites (see also [8,9]) that might be sites of regulation of the channels (see [19]).

deg-1 mutations that cause degeneration

The dominant Deg mutations in *deg*-1 (alleles *u38* and *u529*) result in a substitution at amino acid 707, from alanine to valine ([20] and see Fig. 1c). This amino acid, which is equivalent to the alanine substituted in the *mec-4* and *mec-10* dominant Deg mutations, is found in the second hydrophobic domain in the predicted β hairpin.

The recessive, gain-of-function *deg-1(u506)* mutation causes a similar, if not identical, pattern of neuronal degeneration to that seen in the dominant *deg-1(u38)* mutation (Fig. 2a). The *u506* mutation was identified by looking for mutants that had vacuolated cells. Because of the degeneration of the PVC neurons, the mutant animals are 'touch abnormal' (the 'Tab' phenotype) — they do not respond to a harsh touch stimulus at the tail [1]. Although *u506* is recessive, it is not a null mutation; null mutations do not have a detectable mutant phenotype [1], and *u506*, which has a Deg and Tab phenotype, is dominant to null alleles (data not shown).

Unlike the dominant, degeneration-causing mutations of *deg-1* and *mec-4*, which alter an amino acid in the predicted β-hairpin region, the *deg-1(u506)* mutation substitutes threonine for alanine at residue 393 in the 22 amino-acid region that is found only in the *C. elegans* proteins (Figs 1a and 1b). Degeneration-causing mutations at this position are not restricted to *deg-1* — the equivalent mutation at codon 404 of *mec-4* results in the death of the touch receptor neurons (Fig. 2b).

Table 1. Touch cell deaths induced by *mec-4* mutations in the region of amino acid 404.

Genotype	Amino acid 404 (codon)	No. animals	Degeneration (%)
mec-4(u253); uEx164	Ala^{WT} (GCT)	80	0
mec-4(u253); uEx150	Thr (ACT)	28	39
mec-4(u253); uEx152	Thr (ACT)	30	37
mec-4(u253); uEx160	Lys (AAG)	49	18
mec-4(u253); uEx153	Leu (CTT)	46	26
mec-4(u253); uEx158	Cys (TGT)	59	0
mec-4(u253); uEx159	Gly (GGT)	110	1
mec-4(u253); uEx161	Df399–407	71	34

Animals with *uEx158, uEx159,* and *uEx164,* although they did not have the Deg phenotype, were touch-insensitive (Mec phenotype), presumably because multiple copies of *mec-4* cause a Mec phenotype (M. Huang and M.C., unpublished observations) The *uEx161* array has a deletion of the 27 base-pairs that encode amino acids 399–407.

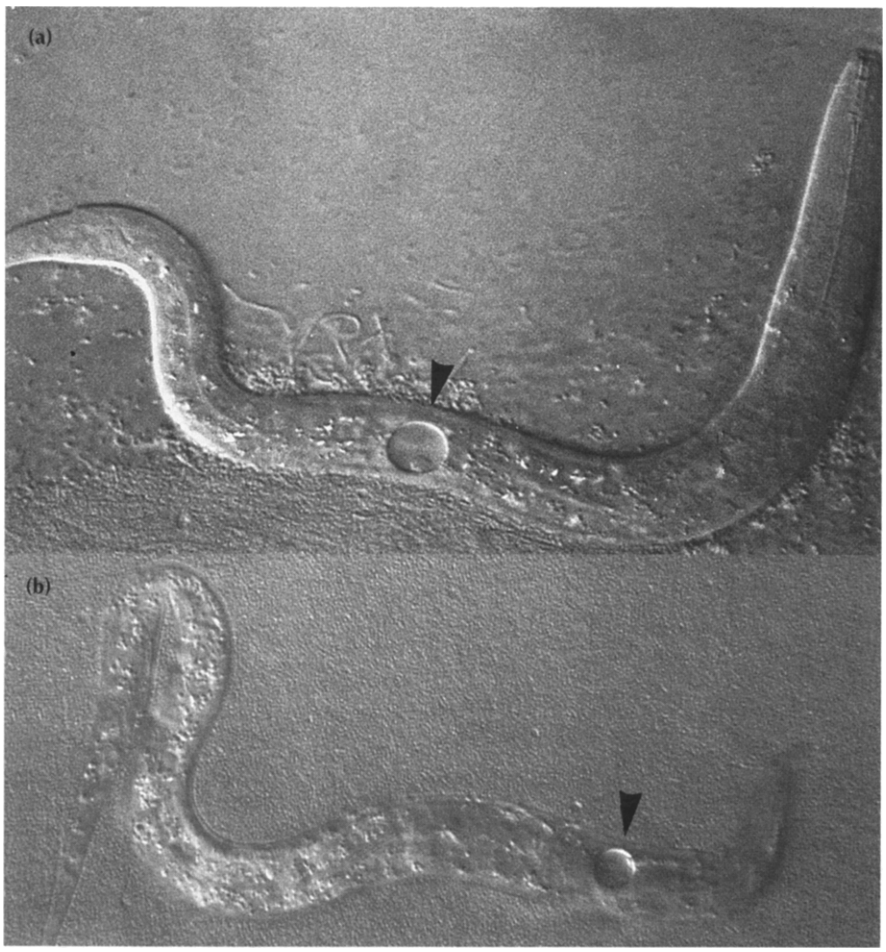


Fig. 2. Effects of degeneration mutations in *deg-1* and *mec-4*. **(a)** Degeneration of IL1 and AVG neurons (arrohwead) in a newly hatched *deg-1(u506)* larvae. **(b)** ALM degeneration (arrohwead) in a newly hatched larva containing an extra-chromosomal array (*uEx152*) of *mec*-4 DNA mutated to give the equivalent change (residue 404 Ala→Thr) to that in *deg-1(u506)*. Although *deg-1(u506)* is recessive to wild type, this array, *uEx152,* is dominant to the wild type, presumably because it contains multiple copies of the mutant *mec-4* gene.

Furthermore, touch cell death also resulted from the substitution of large-side-chain amino acids (threonine, leucine or lysine), but not small-side-chain amino acids (glycine or cysteine), for Ala 404 in *mec-4* (Table 1). These results suggest that steric constraints at this site are important for the function of the region. Deletion of DNA encoding the nine residues surrounding the amino acid (codons 399–407) in *mec-4* also produces the Deg phenotype (Table 1).

deg-1 mutations that prevent degeneration

We have identified 66 intragenic mutations that suppress degeneration caused by the *deg*-1(*u506*) mutation (J.G-A. and M.C., unpublished observations). Three of these mutations — *u512*, *u558* and *u679* — dominantly suppress both the *u506*-induced and *u38*-induced degenerations (Fig. 3); all of these mutations substitute larger (and in two cases, charged) amino acids at sites in the predicted β-hairpin region of DEG-1 (Fig. 1c). The *u512* mutation substitutes glutamine for glycine at residue 710; the *u679* mutation substitutes arginine for glycine at residue 710; and the *u558* mutation substitutes phenylalanine for serine at residue 720. E. Wolinsky [20] has also found that a Gly 710→Arg substitution in *u38* homozygotes suppressed *u38 in trans*. The equivalent mutations in *mec-4* [21] and *mec-10* [3] also suppress dominant degeneration-causing mutations of these genes *in trans*.

Discussion

Do the *C. elegans* degenerin proteins form channels?

Several lines of evidence suggest that the *C. elegans* degenerins form ion channels. First, and most suggestive, is their extensive sequence similarity with the mammalian subunits of the epithelial amiloride-sensitive sodium channel. Each of these proteins has features that predict they will all have similar topologies as membrane proteins. Of particular note are the 22 amino acids at the beginning of the second hydrophobic region in each of these proteins which could form a β hairpin (Fig. 1c). The β hairpins from several subunits could form a β barrel that would serve as a pore lining, as has been proposed for the H5 region of several voltage-gated channels [22–25]. Jan and Jan [25] have made similar suggestions about the *C. elegans* degenerins. Secondly, Hong and Driscoll [21] have shown that most of the second hydrophobic domain from αrENaC, which is predicted to form the pore lining of the channel, can be substituted for the equivalent region in MEC-4, to give a product that is functional in *C. elegans*. Thirdly, the degeneration-causing mutations in the *C. elegans* genes produce vacuolation, swelling and eventual cell lysis ([1–4]; this paper; D. Hall, G. Gu, M. Driscoll and M.C., unpublished observations). This gain-of-function phenotype could result from channel hyperactivation — by increased conductance, open time or opening frequency, or by

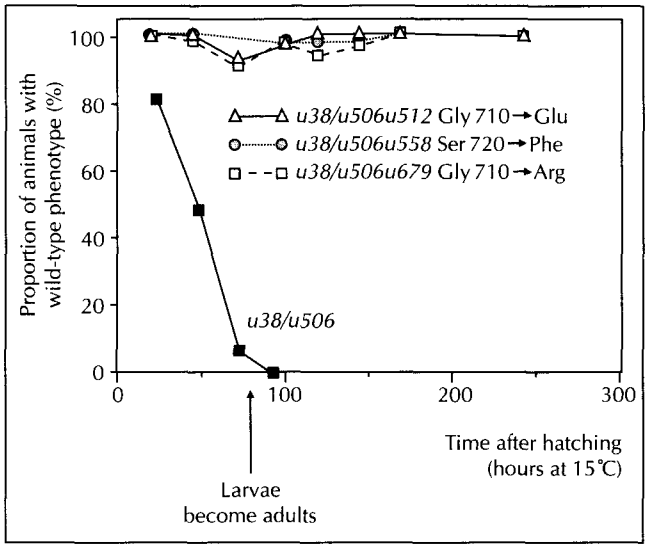


Fig. 3. Onset of the Tab phenotype in *u38/u506* animals, and dominant suppression of the dominant Tab phenotype by mutations affecting the predicted β-hairpin, pore-lining (H5-like) domain. Complete suppression of the Tab phenotype is seen at 15 °C. At 25°C, *u38/u506u512, u38/u506u558,* and *u38/u506u679* animals become Tab later than *u38/u506* animals (data not shown). In addition, *u506/u506u512, u506/u506u558,* and *u506/u506u679* animals are not Tab at either temperature (data not shown).

altered ion specificity. Identical-appearing cell deaths occur in a *C. elegans* mutant in which the pore lining of an acetylcholine receptor subunit is mutated [26]. Hyperactivation is implicated in these deaths, as growth of the mutants in the presence of cholinergic antagonists prevents cell death.

Dominant Deg and Sup mutations disrupt predicted pore-domain residues

Alterations of H5 domains affect the the ion selectivity, conductance and inactivation properties of several different channels [23,24,27–29]. As all of the dominant, degeneration-causing mutations of the *C. elegans* degenerins change an alanine residue situated in the predicted β hairpin (H5-like) region, these mutations may hyperactivate the channel by affecting pore properties (modeled in Fig. 4).

We have identified three *trans*-dominant suppressor mutations that prevent degeneration caused by both the dominant degeneration-causing mutations and mutations in the extracellular region. All three suppressor mutations introduce larger residues in the predicted β-hairpin pore lining which may obstruct or distort the pore, preventing or reducing currents that would otherwise lead to cell degeneration (see Fig. 4). This *trans*-dominant suppression suggests that *deg-1* products interact, perhaps as subunits of a multimeric protein. A similar suggestion has been made for *deg-1* [20], *mec-4* [21] and *mec-10* [3]. Alternatively, degenerins with the suppressor defects may compete for other factors needed for channel function or formation.

Predicted extracellular sequences regulate degenerin activity

Alterations in equivalent amino acids of the predicted extracellular portion of DEG-1 (residue 393) and MEC-4 (residue 404) cause vacuolated cell death. A similar phenotype is seen when the nine amino acids surrounding residue 404 in MEC-4 are deleted, so this region may negatively regulate degenerin function. Because large, but not small, residues substituted at position 404 of MEC-4 also cause degeneration, this amino acid appears to be sterically constrained. This inhibitory region of the protein may regulate channel activity by being part of a gating domain that occludes the channel (either as a plug or as a hinge attached to a plug), a binding site for another protein that actually gates the channel, or a ligand-binding site. This last possibility seems less likely for the *mec-4*- and *mec-10*-encoded degenerins, as these proteins are needed for touch sensitivity and may be touch-activated. Thus, an attractive hypothesis is that the regulatory region is (or is part of) a structure that 'gates' the pore (see Fig. 4). Previously identified gating structures, such as the 'ball-and-chain' that inactivates Na^+ and K^+ voltage-sensitive channels [30,31] and control elements for Cl^- and other K^+ channels (reviewed in [25]), are located intracellularly. The putative gating domain that we have identified in the *C. elegans* degenerins is unusual because it is located extracellularly.

We think that the extracellular inhibitory region could be important for the suspected mechanosensory role of the *C. elegans* degenerins in the touch receptor neurons. Mechanical manipulation of an extracellular regulatory domain may gate such a channel. This hypothesis is similar

Fig. 4. Model of degenerin mutant activity. Each panel shows a cross-sectional representation of two subunits of the putative degenerin channel in the 'closed' configuration. Because the wild-type channel is hypothesized to be closed by either of at least two gating domains, homozygous (*u506/u506*), but not heterozygous (*u506/+*) mutations affecting the gating domain will lead to an 'open' channel. In contrast, a single mutation affecting pore structure (*u38/+*) can lead to an 'open' state. Both types of defect can be prevented by a single further mutation (*u512*) of the pore structure.

to the gating-spring model for hair cell regulation [32], which postulates that physical manipulation of a gate activates mechanosensory channels, most probably by the action of the extracellularly located tip links (reviewed in [33,34]).

A model for a recessive gain-of-function mutation

The *trans*-dominant suppression of the dominant and recessive degeneration-causing mutations suggests that *deg-1* products interact such that each channel contains more than one copy of the DEG-1 protein. Multiple copies of DEG-1 in the channel may explain why mutations in the putative pore domain are dominant, but *u506* is recessive. A defect in the pore domain of one subunit could disrupt the pore as a whole, either forcing it open (as in the dominant Deg mutations), or keeping it blocked (as in the *trans*-dominant suppressors); removal of only one of several regulatory domains (as in *u506*) will not prevent channel closure by the remaining domains. The *Shaker* potassium channel shows such redundancy, as it can be inactivated by any one of its four identical subunits [35]. Thus, the *u506* mutation is gain-of-function, because it produces a phenotype not caused by loss-of-function alleles, but recessive, because it disrupts a redundant regulatory domain. Recessive gain-of-function mutations are rare (see [36], for example). Perhaps these mutations, too, are in genes whose products contribute several subunits to a multimeric protein.

Different mutations within the same gene can produce dominant and recessive forms of some human diseases. The recessive forms of some of these diseases (caused, for example, by rhodopsin mutation in retinitis pigmentosa [37] and by disruption of the skeletal muscle chloride channel in myotonia [38]) are thought to be due to null mutations. Other inherited neuronal degeneration disorders, such as several forms of spinocerebellar degeneration, appear in both dominant and recessive forms [39,40], but the genetic defects underlying them are unknown. Here, we have shown that gain-of-function mutations in different domains of the same gene lead to recessive and dominant forms of neurodegeneration.

Differences between the *C. elegans* and mammalian proteins

The most obvious structural difference between the *C. elegans* and mammalian proteins in this superfamily is that the *C. elegans* proteins all contain additional sequences at the amino-terminal part of the putative extracellular region. Specifically, only the nematode proteins have an additional cysteine-rich domain and a 22 amino-acid sequence (Fig. 1b). The 22 amino-acid region is where the *u506* alteration occurs (five amino acids of this region are absent in the deletion that causes degeneration in *mec*-4).

The occurrence of the 22 amino-acid sequence in the *C. elegans* proteins, but not in the mammalian proteins, may explain functional differences in the properties of the resulting channels. The rat channel, comprised of α, β and γ rENaCs, appears to be one that is constitutively active, with long open times [7]; its regulation involves protein synthesis and is very slow [41]. These kinetic properties are in keeping with the function of the kidney and colon epithelia in sodium reabsorption. In contrast, the *C. elegans* proteins, at least those expressed in the touch receptor neurons and required for their function (MEC-4 and MEC-10), are likely to be subunits of mechanosensitive ion channels, and as such would have to be gated rapidly. The 22 amino-acid sequence may make this rapid gating possible. These two types of channel may provide an example of how proteins could evolve to produce channels with significantly different gating properties. We think it likely that genes encoding degenerin-like proteins with inactivation domains may be found in mammals, and these, like their counterparts in *C. elegans*, may underlie mechanosensory signal transduction. Similarly, degenerin-like proteins without the inhibitory domain may be found in *C. elegans*.

Materials and methods

Isolation and characterization of deg-1 *mutants*

All strains of *C. elegans* var. Bristol were grown as described previously [42]. The *u506* and *u529* mutations were identified after ethyl-methanesulfonate (EMS) mutagenesis [42] of *mec-4(e1611)* because they produced additional cell deaths. These mutations were identified as *deg-1* mutations by their map position, their failure to complement a deletion of *deg-1* (for *u506*), and their sequence (see below). Intragenic suppressors were obtained by reverting the cold-sensitive embryonic lethality that is associated with the *u506* mutation (J.G-A. and M.C., unpublished observations) by EMS mutagenesis.

To characterize the dominant suppression of *deg-1(u38)* by intragenic *deg-1* mutations, we generated heterozygous animals by mating *lon-2(e678) deg-1(u506)*, *deg-1(u506u512)*, *deg-1(u506u558)*, and *deg-1(u506u679)* males and *dpy-20(e2017); deg-1(u38)* hermaphrodites, and synchronized the progeny at hatching at 25 °C [1]. The newly hatched larvae were either maintained at 25 °C or shifted to 15 °C, and the non-Dpy animals (the cross progeny) were tested for the Tab (Touch abnormal) phenotype by touching them with a platinum wire on the tail [1]. The vacuolated cell deaths in *deg-1(u506)* animals were examined at 25 °C by differential interference contrast microscopy [1].

Sequencing of wild-type and mutant deg-1 *genes*

Genomic fragments (6.7, 2.4 and 3.7 kb) containing *deg-1* coding sequences were identified by Southern blotting [43] *Eco*RI-digested DNA from cosmid R02A8, using the *mec-4* genomic clone TU#12 [2] as a probe. The fragments were subcloned into SK^+ bluescript (Stratagene). The 6.7 kb clone, which did not hybridize to the partial *deg-1* cDNA clone representing the 3′ half of the transcript (starting at codon 485 of the complete coding sequence) [1], was sequenced. The 76 base-pair fragment that joins the 6.7 and 2.4 kb *Eco*RI fragments was sequenced using R02A8 as template. The cDNA sequence between codons 315 and 494 was obtained by reverse-transcription (RT) and inverse polymerase chain reaction (PCR) [44] from poly(A)$^+$ mRNA using two primers from known portions of the transcript (GATGTGTTTAC-GAATAGAAGGACACG and TTGCCAGCACCATATG-GAGAATGT). The sequence of the mRNAs from their 5′ ends to codon 325 was determined from cDNA using

RT–PCR [44], using an anti-sense primer (CCAATG-CAATAGGGAGTGTCAT) and an SL1-specific primer (GGTTTAATTACCCAAGTTTCAG) [45]. In each case, single sequences matched the genomic sequence. RT–PCR using primers bracketing the middle of the sequence (codons 326–483) gave two products, one of which lacked the sequence from the exon that encodes the second threonine- and proline-rich repeat. Introns interrupting the previously available partial cDNA (which starts at codon 485) were detected by sequencing the 2.4 and 3.7 kb genomic fragments with primers homologous to the cDNA sequence. Sequences were analyzed by PCgene (release 6.26; Intelligenetics).

Coding sequences from genomic DNA obtained from *deg-1* mutants were PCR amplified [44], cloned into pCR-Script SK^+ (Stratagene), and sequenced (this procedure was repeated to confirm mutant sequences). The primers used for PCR amplification are available upon request. All *deg-1* exons were analyzed for *u506* and *u506u512*. Only part of the *deg-1* coding sequence was analyzed for *u506u558*, *u506u679*, *u38* and *u529*. The changed codons in the mutants are: 393 GCT→ACT (*u506*), 707 GCC→GTC (*u38* and *u529*), 710 GGA→GAA (*u512*), 710 GGA→AGA (*u679*) and 720 TCC→TTC (*u558*).

Mutations induced in mec-4

To produce the *u506*-like mutation in *mec-4*, the *mec-4* genomic clone TU#12 was mutagenized (Muta-Gene *in vitro* mutagenesis kit, Biorad) to make the changes in MEC-4 indicated in Table 1. The oligonucleotides used for the mutagenesis *in vitro* were homologous to *mec-4* genomic sequences for 10 or 11 nucleotides on each side of the mismatching nucleotides of the missense mutations, and for 15 nucleotides on each side of the 27 base-pair deletion. The mutated construct was co-injected with pRF4 into *mec-4(u253)* animals (*u253* is a null mutation; [5]) to produce lines carrying stable extrachromosomal arrays (each with a different *uEx* number) of these DNAs [46,47].

Degenerations in *mec-4* mutants were examined as above, in newly hatched animals. At this time only the ALM and PLM touch receptor neurons are present. For the *mec-4*-transgenic mutants, only the progeny of Roller parents (which carry the transgenic extrachromosomal arrays, were examined (the newly hatched animals do not yet express the marker Roller phenotype). As the extrachromosomal arrays are unstable and are inherited by only some of the progeny, not all of the progeny we examined would be transgenic, and not all of the cells of a transgenic animal would carry the extrachromosomal array.

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