Genetically targeted cell disruption in Caenorhabditis elegans
(cell death/degenerin/ENaC superfamily/ion channels/cell ablation)

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ABSTRACT The elimination of identified cells is a powerful tool for investigating development and system function. Here we report on genetically mediated cell disruption effected by the toxic Caenorhabditis elegans mec-4(d) allele. We found that ectopic expression of mec-4(d) in the nematode causes dysfunction of a wide range of nerve, muscle, and hypodermal cells. mec-4(d)-mediated toxicity is dependent on the activity of a second gene, mec-6, rendering cell disruption conditionally dependent on genetic background. We describe a set of mec-4(d) vectors that facilitate construction of cell-specific disruption reagents and note that genetic cell disruption can be used for functional analyses of specific neurons or neuronal classes, for confirmation of neuronal circuitry, for generation of nematode populations lacking defined classes of functional cells, and for genetic screens. We suggest that mec-4(d) and/or related genes may be effective general tools for cell inactivation that could be used toward similar purposes in higher organisms.

Targeted removal of specific cells can yield significant insights into biological processes. Cells can be physically removed through surgical manipulations, for example, as in the classic experiments that established target dependence for motor neuron survival (1) or in higher resolution laser ablation procedures (2). Cells also can be eliminated via genetic approaches in which a cell-specific promoter drives expression of a cytotoxic gene. Genetic ablations offer the advantage that multiple, physically dispersed cells can be simultaneously removed without extensive manipulation. Here we report on the utilization of the toxic Caenorhabditis elegans mec-4(d) as a genetic cell disruption reagent.

mec-4 encodes a subunit of a candidate mechanotransducing channel expressed in the six C. elegans body touch receptor neurons (3–5). Dominant mec-4 alleles [mec-4(d)] induce swelling and degeneration of these six neurons (6). Death-inducing amino acid substitutions are believed to cause elevated ion influx through the MEC-4 channel (3, 7), initiating a degenerative process that involves formation of internal membraneous whorls and large vacuoles, chromatin clumping, and degradation of intracellular contents (8) that is distinct from programmed cell death (reviewed in ref. 9). Several other C. elegans genes related in sequence to mec-4 can mutate to induce neurodegeneration (10–13), and therefore the C. elegans gene family has been named the “degenerin family.” The neurotoxicity of mutant mec-4, mec-10, deg-1, and unc-8 degenerin genes depends on the activity of another gene of unknown function, mec-6 (10–13). Mammalian genes similar to degenerins encode subunits of the epithelial amiloride-sensitive Na⁺ channel (the ENaC family; refs. 14–15) and related channels, at least some of which are assembled in neurons (16–18).

We have found that ectopic expression of mec-4(d) is an efficient means of disrupting C. elegans cell function. mec-4(d) induces detectable vacuolation of a broad range of cells, including nerve, muscle, and hypodermal cell types. Degeneration is dependent on the activity of the mec-6 gene in every case tested, enabling cell function to be rendered conditional depending on the genetic background. mec-4(d)-induced cell dysfunction can facilitate cell identification, assignment of neuronal function, and design of genetic screens. A mutant form of the related mammalian MDEG gene can induce degeneration of oocytes and fibroblasts (17). The broad spectrum effects of mec-4(d) in C. elegans suggest that a mec-4(d)-based (or possibly MDEG-based) vector may be generally useful for genetically mediated cell ablation in diverse organisms.

MATERIALS AND METHODS

C. elegans Manipulations. Unless otherwise noted, C. elegans strains were constructed and maintained at 20°C as described (19). Transformation was done according to Mello et al. (20): plasmid constructs and the cotransformation marker pRF4 [which harbors the dominant transformation marker rol-6 (su1006) (21)] were introduced at 50 µg/ml each. Lines assayed harbored introduced DNA as extrachromosomal arrays except for strain ZB15. ZB15 harbors an integrated array of plasmid pPD62.39 [popal-mec-4(d)], pPD38.61 (hlh-1 genomic clone), and pPD37.48 (hlh-1::lacZ) and was constructed by irradiating an extrachromosomal array as described (22). ZB15 was maintained at 15°C and heat shocked at 30°C for 2 h to overcome Cell degeneration was assayed by scoring for vacuolated cells under Nomarski differential interference contrast microscopy optics (23). β-galactosidase assays were performed as described (24). Behavioral assays were conducted on a sample size of at least 100 animals from each of at least two independent lines. Nose touch responsiveness was tested by modification of the assay described by Kaplan and Horvitz (25) in which an eyelash hair was positioned in the space between the head and tail of a transgenic roller that adopted a semicircular posture. Transgenic rollers reversed direction in 90% of encounters when they bumped into the eyelash hair; insensitive animals did not reverse direction in 10/10 trials per animal.

Molecular Techniques. Basic molecular techniques were as described (26), as was long fragment PCR amplification (27). pABL is a derivative of a punc-7::GFP clone. A 769-bp fragment containing the GFP coding sequence was amplified from TU#61 (28) using the primers 5’-GGGGTACCCGCGGCCGCCCAGATGCTATTGTT-3’ to which reprint requests should be addressed. e-mail: driscoll@mbcl.rutgers.edu.

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RESULTS

mec-4(d)-Induced Degeneration Can Occur in Multiple Cell Types. To determine whether mec-4(d) could induce degeneration of cells other than the six touch receptor neurons, we expressed mec-4(d) ectopically under the control of the heat-inducible hsp-16–2 promoter, which is active in most nonnonglial cells (31–32). When transgenic animals harboring an integrated array of punc-mec-4(d) were heat shocked at 30°C for 2 or more h, we observed vacuolated cells throughout the body (Fig. 1a). Behavioral effects of induced mec-4(d) expression in these lines are dramatic; animals become paralyzed within 2 to 3 h and die by the following day (Table 1). Embryonic cells also were susceptible to the deleterious effects of ectopic expression of mec-4(d) (Fig. 1b). These observations establish that mec-4(d) can be toxic when expressed ectopically and further suggest that mec-4(d)-induced degeneration can occur in a variety of cell types.

Sensory Neurons, Interneurons, and Motor Neurons Can Undergo mec-4(d)-Induced Degeneration. Although our initial ectopic expression experiments suggested that a large number of cells are susceptible to mec-4(d)-induced toxicity, it was difficult to determine whether all cell types are affected by punc-mec-4(d). To investigate toxicity in individual cell types, we fused mec-4(d) to various cell type-specific promoters and assayed vacuolation and behavioral defects in transgenic lines. As a first test, we assayed susceptibility of sensory neurons using the mec-7 promoter to drive expression of mec-4(d). mec-7 is expressed at high levels in the six touch sensory neurons.
neurons and at a lower level in the FLP, ALN, PVD, and BDU neurons (4, 33–34). In transgenic animals harboring puncmec-4(d), we observed degeneration of the FLP and ALN neurons as well as degeneration of the touch receptor neurons, establishing that different types of sensory neurons are vulnerable to the toxic effects of mec-4(d) (BDU and PVD are situated relatively close to the anterior touch neurons and PVM, respectively; their positions are somewhat skewed in transgenic rollers, and not all cells die simultaneously, making definitive cell assignments for these neurons difficult; data not shown). We then expressed mec-4(d) under the control of the unc-4 promoter, which functions in VA, DA, and VC motor neurons (ref. 29 and D. Miller and C. Li, personal communication). In late embryonic and early larval stages, we observed swollen neurons periodically positioned within the ventral nerve cord in these transgenic lines (Fig. 1c). That vacuolated cells could be detected before hatching is consistent with toxic degenerin effects shortly after the transgene is first expressed (29). Cells were clearly dysfunctional because many transgenic animals harboring puncmec-4(d) were uncoordinated and unable to back-up when touched on the head (Table 1), similar to the phenotype of unc-4 mutants, which lack differentiated VA motor neurons (35).

We further tested the spectrum of susceptible neurons by constructing and analyzing lines in which mec-4(d) was expressed under control of the unc-8 promoter. In puncmec-4(d) transgenic lines, many interneurons and ventral cord motor neurons degenerate, consistent with the characterized expression profile of unc-8 (13). Transgenic animals harboring the puncmec-4(d) fusion gene adopt the coiled phenotype of unc-8(d) mutants (Table 1). mec-4(d) also causes cell degeneration and behavioral abnormalities when expressed under the control of the glr-1 promoter in a group of neurons that includes interneurons (36). Thus, it appears that many neuronal classes, if not all, are susceptible to mec-4(d)-induced degeneration. In all cases that we tested, affected neurons exhibited a characteristic vacuolar morphology. We noted that the size and numbers of apparent vacuoles vary, probably because of the mosaic presence of the transgene, promoter strength, gene dosage, temporal regulation of expression, cell type, and body position. We did not observe degeneration of cells in which the promoters assayed are inactive.

Ectopic mec-4(d) Expression Also Causes Dysfunction of Non-neuronal Cells. To determine whether ectopic expression of mec-4(d) could affect hypodermal cells, we expressed this degenerin under the control of the hypodermal cell-specific mec-5 promoter (30). Transgenic animals expressing puncmec-4(d) displayed some vacuolation in hypodermal cells (Fig. 1d) and moved irregularly (Table 1). We also expressed mec-4(d) in muscle cells under the control of regulatory sequences of the myo-2 and unc-54 promoters. The myo-2 gene encodes a pharyngeal-specific myosin (37–38). Some transgenic animals bearing the puncmec-4(d) construct had vacuoles localized within pharyngeal tissue (Fig. 1e). Most severely affected animals exhibited very slow growth and appeared starved, most likely because of defective pharyngeal pumping caused by dysfunction of damaged pharyngeal muscle (Table 1). unc-54 encodes a myosin expressed in all body wall muscles, vulval and uterine muscles, intestinal muscle, and the anal depressor muscle (39).

Animals mosaic for the puncmec-4(d) construct often had multiple small vacuoles in body wall muscles (Fig. 1f). Some transgenic animals had bodies that appeared more transparent than wild type, possibly the consequence of muscle degeneration. Mosaic animals from these transgenic lines exhibited a range of behavioral defects including uncoordinated locomotion and hypercontraction (Table 1; Fig. 1g), suggesting that mec-4(d) can disrupt muscle function. Semi-dominant alleles of the muscle degenerin unc-105 (40) cause similar hypercontraction (41), suggesting that puncmec-4(d) and the unc-105(sd) allele may affect muscle cells in similar ways. Taken together, results of our ectopic expression experiments establish that multiple cell types are susceptible to mec-4(d)-mediated toxicity.

Ectopic Toxicity Induced by mec-4(d) Is Dependent on mec-6 Activity. Genetic experiments suggest that mec-6 influences degenerin channel complexes of different subunit composition in different cell types. mec-6 mutations suppress mec-4(d)-induced death of the six touch receptor neurons (10), swelling and vacuolation of sensory neurons, interneurons, and motor neurons in unc-8(d) mutants (12), degeneration of sensory neurons and interneurons in deg-1(d) mutants (10), and partially suppress hypercontraction of unc-105(sd) mutants (N.T. and M.D., unpublished results). Thus, mec-6 is inferred to be widely expressed.

mec-4(d) toxicity depended on mec-6 activity in all ectopic expression paradigms we tested (Table 1; Fig. 1g and h). The presence of the mec-6(u450) mutation in various transgenic backgrounds fully ameliorated behavioral defects and reduced detectable vacuoles to the rare level seen in nontransformed animals. Even when mec-4(d) was expressed from a strong heat shock promoter, suppression was strikingly efficient. In the wild-type background, a 2-h heat shock followed by incubation at 20°C was lethal to puncmec-4(d) transgenic animals that became populated by vacuolated cells; by contrast, in the mec-6(u450) background, similarly treated transgenic animals and embryos remained viable and contained at most an occasional vacuole. The fact that mec-6(u450) can effectively suppress degeneration allows the construction of lines bearing integrated mec-4(d) fusion constructs that would be toxic to the animal in the mec-6(+). The existence of a temperature-sensitive mec-6 allele (u247; ref. 6), raised the possibility that neurodegeneration could be rendered temperature-conditional in the u247 background. To
test this, we constructed the double mutant mec-6(u247); bzIs5 [p_{mec-6mec-4(d)}] [bzIs5 is an integrated array that directs synthesis of mec-4(d) in unc-8-expressing neurons (K. Xu and M.D., unpublished work)] and compared behavioral defects in animals reared at permissive (15°C) and nonpermissive (25°C) temperatures. We found that animals remained uncoordinated even when raised for several generations at the nonpermissive temperature, suggesting that the MEC-6 mutant protein encoded by allele u247 is partially functional at 25°C and confers enough degenerin channel activity to allow toxicity when the mec-4(d) copy number is high.

Exploiting mec-4(d) for Characterization of Genes and Behavior. We anticipated that genetic cell ablation could have useful applications for the analysis of C. elegans genes and behavior. For example, cell-specific expression of mec-4(d) in mec-4(d)-expressing neurons (K. Xu and M.D., unpublished work) could be used to establish gene expression patterns. In a database search, we identified a gene on cosmID F58G6 that exhibits sequence similarity with the amino termini of C. elegans degenerins (degenerin-like gene, designated as del-2).

To determine the temporal and spatial expression pattern of del-2 promoter, we constructed a lacZ reporter fusion driven by upstream del-2 regulatory sequences. Transgenic strains harboring the p\text{del-2lacZ} fusion gene expressed β-galactosidase in a group of anterior neurons (Fig. 2a) that we tentatively identified as neurons of the ASH, OLO, and IL1 classes after coantaining transgenic animals with 4',6-diamidino-2-phenylindole to visualize cell nuclei in the larval head (25). This expression pattern is intriguing for a degenerin-related gene because candidate del-2-expressing neurons are mechanosensory; ASH and OLO contribute to the response to head-on collision, and OLO and IL1 neurons contribute to the head withdrawal reflex in response to light touch on the side of the nose (25, 42). The absence of staining of the neuronal processes and proximity of other nuclei in the region made the cell assignments ambiguous in this case. To gain experimental support for our cell identifications, we fused del-2 promoter sequences to the mec-4(d) coding region and assayed transgenic lines for defects in mechanosensitivity expected for disruption of the nose touch sensory neurons. p\text{del-smec-4(d)}, induced degeneration of neurons in the vicinity of the IL1, OLO, and ASH neurons (Fig. 2b), a phenotype suppressed in the mec-6(u450) background (Table 1; Fig. 2c). Transgenic animals exhibited a pronounced reduction in their ability to respond to mechanical stimuli delivered to the nose (Fig. 2c), supporting the hypothesis that del-2-expressing cells are the ASH, OLO, and IL1 neurons and illustrating how mec-4(d) can be used to characterize promoter expression patterns.

In every case in which we expressed mec-4(d) from a characterized neuronal-specific promoter, we found that predicted behavioral phenotypes were observed (see Table 1). This correlation suggests that targeted mec-4(d)-mediated cell disruption can be used (somewhat analogously to laser ablation) to determine neuronal function in instances in which the activity of a neuron or neuronal class is not known. Use of ectopic mec-4(d) expression for such a purpose has been applied in analysis of glutamate receptor function (36).

Given the potential utility of mec-4(d)-mediated cell disruption, we constructed a set of vectors that would facilitate construction of gene fusions (Fig. 3). We positioned a polylinker 5' to the mec-4(d) initiation codon so that promoter sequences could be cloned using several different restriction sites (pAB1). We introduced single or double nucleotide deletions within the BglII site of the pAB1 polylinker so that translational gene fusions could be made in all three reading frames (pAB2 and pAB3, respectively). In tests of various mec-4(d) translational fusion genes, we found that toxicity is unaffected by either the addition of up to 100 foreign amino acids to the MEC-4 amino terminus or by the deletion of the first 20 MEC-4 amino acids (data not shown).

**Discussion**

Cell-specific expression of toxins such as ricin A and diphtheria toxin-A-chain has been used for elucidation of cell functions in mice and flies (43–46). Unfortunately, these proteins are extremely potent (for example, the presence of a single diphtheria toxin transcript is sufficient to kill a cell (47)), and most promoters are inherently leaky, limiting the facile use of these reagents for cell-specific genetic ablation. Diphtheria toxin-A-chain also appears to be highly toxic in C. elegans (DT-A coding sequences expressed from a heat shock promoter can kill C. elegans even without heat shock; S.Q.X. and A.F., unpublished observations). In contrast, ectopic expression of mec-4(d) induces degeneration of a wide variety of cell types and appears well restricted to the cells in which a given promoter is active. Moreover, deleterious effects of mec-4(d) can be effectively eliminated in a mec-6 mutant background, rendering toxicity conditional. Our studies demonstrate that mec-4(d)-mediated cell disruption can be added to the arsenal of tools for analyses of genes and behavior in C. elegans.

**Biology of the MECP-4(d) Channel.** The working model for MECP-4 function postulates that MECP-4 is a subunit of a multimeric mechanosensitive ion channel assembled in the six C. elegans touch receptor neurons (reviewed in refs. 48–49). The normal gating activity of the MECP-4 channel is thought to depend on associations with additional proteins situated both inside and outside the touch neuron, and MECP-4(d)-mediated toxicity is proposed to depend on increased channel opening (3, 7). Thus, our finding that mec-4(d) is toxic in a wide range of C. elegans cells when expressed ectopically was unexpected. How is it that mec-4(d) is deleterious to such a broad spectrum of cells? We suggest that ectopic expression results in a high cellular concentration of MECP-4(d) subunits, which might

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**Fig. 2.** Fusion of a promoter of interest to mec-4(d) verifies promoter activity profiles and creates behavioral defects. (a) β-galactosidase staining of candidate ASH, OLO, and IL1 neurons in animals harboring a p\text{del-2lacZ} gene fusion. (b) Nomarski differential interference contrast microscopy image of vacuolated cells (indicated by white arrows) in a transgenic L2 animal harboring p\text{del-smec-4(d)}-lacZ. Transgenic lines also include pRF4, which encodes the normal gating activity of the MEC-4 channel is thought to depend on associations with additional proteins situated both inside and outside the touch neuron, and MECP-4(d)-mediated toxicity is proposed to depend on increased channel opening (3, 7). Thus, our finding that mec-4(d) is toxic in a wide range of C. elegans cells when expressed ectopically was unexpected. How is it that mec-4(d) is deleterious to such a broad spectrum of cells? We suggest that ectopic expression results in a high cellular concentration of MECP-4(d) subunits, which might
assembled to form a homo-multimeric channel locked into an ion-permeable conformation. Most normal contacts with specialized cytoskeletal proteins or extracellular matrix could be dispensable in this situation. Consistent with this hypothesis, the mec-4(d) subunit might coassemble with other degenerin channel-interacting proteins (10). Alternatively, the mec-6 harboring an integrated array of pABL1, pABL2, and pABL3 enable translational fusions in any reading frame to be constructed. They differ in the nucleotides corresponding to the BcII site in pABL1; the BcII site is present only in pABL1 (this site is blocked by dam methylase and can only be used when DNA is prepared from a dam- host). Unique restriction sites that facilitate cloning are indicated. The mec-4(d) genomic sequence includes a conserved poly(A) addition site. The unc-54 3' end cassette (24) is positioned after this site. Note that the unique ScaI site in the polylinker enables fragments with PstI ends to be inserted. In general, previously constructed lacZ and GFP fusion constructs made in standard C. elegans vectors (2A, 2B) can be readily converted to mec-4(d) fusion constructs. Swapping XbaI-Apal or XbaI-Spal reporter + 3' end cassettes might be the most common conversion strategy; for constructs without the nuclear localization signal, substituting an XbaI-Apal fragment from pABL2 into the AgeI-Apal region of the fusion construct should produce an in-frame mec-4(d) fusion equivalent to the original lacZ or GFP fusion.

**Fig. 3. mec-4(d) vectors for transcriptional and translational gene fusions.** pABL1, pABL2, and pABL3 enable translational fusions in any reading frame to be constructed. They differ in the nucleotides corresponding to the BcII site in pABL1; the BcII site is present only in pABL1 (this site is blocked by dam methylase and can only be used when DNA is prepared from a dam- host). Unique restriction sites that facilitate cloning are indicated. The mec-4(d) genomic sequence includes a conserved poly(A) addition site. The unc-54 3' end cassette (24) is positioned after this site. Note that the unique ScaI site in the polylinker enables fragments with PstI ends to be inserted. In general, previously constructed lacZ and GFP fusion constructs made in standard C. elegans vectors (2A, 2B) can be readily converted to mec-4(d) fusion constructs. Swapping XbaI-Apal or XbaI-Spal reporter + 3' end cassettes might be the most common conversion strategy; for constructs without the nuclear localization signal, substituting an XbaI-Apal fragment from pABL2 into the AgeI-Apal region of the fusion construct should produce an in-frame mec-4(d) fusion equivalent to the original lacZ or GFP fusion.

**Potential Applications of mec-4(d)-Mediated Cell Disruption.** We have illustrated how mec-4(d)-mediated cell disruption can be used in conjunction with reporter gene expression to confirm gene expression patterns. In addition, our ectopic expression studies suggest that genetic cell disruption may be particularly useful for the analysis of the C. elegans nervous system. Most, if not all, neuronal classes appear susceptible to the deleterious effects of mec-4(d), and induced behavioral phenotypes reflect those predicted for the elimination of characterized neurons. Thus, genetic cell disruption may be exploited to probe the function of specific neurons or neuronal classes, to confirm predicted neuronal circuitry (50), and to generate populations of animals lacking defined classes of functional neurons for genetic and behavioral studies. These approaches should be most useful when promoters expressed in restricted cell types are used. Given a characterized promoter, the genetic cell disruption approach can be applied with an objective similar to that for laser ablation, conferring the advantages that many neurons can be simultaneously rendered inactive in large numbers of animals and that special equipment and training in cell recognition are not required for the execution of the experiment.

We have noted that, in some cases in which we inactivated neurons by expressing mec-4(d) from various promoters, transgenic animals exhibited phenotypes similar to those conferred by mutations in the corresponding gene. For unc-4, genetic ablations prevented animals from backing up normally, which is a phenotype of unc-4(k0) mutants; for unc-8, the uncoordinated phenotype of the punc-mec-4(d) transgenic animals was similar to that of unc-8(k0) mutants. Although phenocopying can only occur when a defect in the gene corresponds to the defect conferred by cell loss (clearly not always the case), mec-4(d)-induced cell inactivation may still be useful as an initial indication for reverse genetic investigations of genes with highly limited expression patterns. For example, if candidate 5' regulatory sequences of a gene identified by the C. elegans Genome Sequencing Consortium are fused to mec-4(d), the phenotype of the transgenic animal harboring this construct might facilitate correlation of the gene in question with an available genetic mutant.

Additionally, we suggest that transgene toxicity might be exploited for analyses of gene expression mechanisms. For examples, mutations that disrupt nonessential transcriptional activities could be selected by their ability to block cell lethality conferred by ectopic mec-4(d) expression.

**Caveats to Consider: Cell Death vs. Dysfunction.** Although mec-4(d)-expressing cells are clearly dysfunctional, it appears that not all die or completely disappear. Counts of 4',6-diamidino-2-phenylindole-stained ventral cord nuclei in adults harboring an integrated array of punc-mec-4(d) indicate that nuclei do disappear, but more remain detectable than expected (N2, 51 ± 2 nuclei; bzIs5 [punc-mec-4(d)l], 36 ± 10 nuclei; 20 worms counted). Death may be slow because of inefficient channel activity in a given cell type. Also, because in the touch neurons the rate of degeneration is correlated with mec-4(d) gene dosage (8), it seems plausible that weak promoters or transgene mosaicism might induce slow or incomplete killing. Conceivably, the transgene might not be expressed uniformly in all cells of a given type. Finally, it is possible that affected cells die, but the removal of their remains [which involves engulfment and probable degradative activities of neighboring cells for the degenerating touch cells (ref. 8; S. Chung and M.D., unpublished work)] may be ineffective in certain regions of the body or during certain developmental stages.

In mec-4(d) mutants, touch receptor cell swelling and degeneration occurs shortly after onset of mec-4 expression (8), but degenerating cells can persist for hours. Thus, temporal aspects need to be taken into account for experimental design.
Slow cell disintegration in transgenic lines expressing mec-4(d) suggests that genetic cell disruption may not be optimal for applications dependent on the complete elimination of cell contents such as preparation of cDNA libraries for subtractive hybridization or differential display. Cell persistence and the relatively slow time course of degeneration also limits the utility of mec-4(d) in studies designed to investigate cell–cell influences at precise times during development. The persistence of gene products produced in cells that die late might complicate analysis of nonautonomously acting genes.

Comparison with Alternatives for C. elegans Cell Disruption. Shaham and Horvitz (51) found that ectopic expression of C. elegans ced-3 and ced-4 in the touch receptor neurons or the Dv motor neurons induces programmed cell death of these cells and suggested the use of ced-3 and ced-4 for genetic cell ablations. Use of the programmed cell death executors for this purpose carries the advantage that cells ectopically expressing ced-3 and ced-4 clearly die and corpses are engulfed. Disadvantages of ced-3- or ced-4-mediated killing are that ectopic deaths reported thus far are quite sensitive to the dosage of the toxic gene and that deaths are induced efficiently only in the absence of ced-9 activity (51) [ced-9 is a negative regulator of programmed cell death (52)]. Thus, the authors recommend that ced-3- or ced-4-mediated killing be conducted in the ced-9(fu); ced-3 or ced-4 ced-9(fu) double mutant background. The advantages of mec-4(d)-mediated cell disruption are that: (i) degenerating cells persist for some time so that their swollen morphologies can be easily recognized [in contrast, programmed cell deaths can be easily visualized only in the background of mutant engulfment ced-4 genes (53–54)]; (ii) mec-4(d) does not require alteration of genetic background for potentiation of toxicity, and (iii) mec-4(d)-induced killing can be efficiently rendered conditional in the ced-6 mutant background. Effective application of genetic toxicity strategies will require consideration of these advantages/disadvantages.

mec-4-Related Genes as Vectors for Mammalian Cell Disruption. Although here we describe in detail a system for cell disruption in C. elegans, we note that a mammalian member of the DEG/ENaC channel superfamily engineered to encode a toxic MEC-4 A713V can induce degeneration of Xenopus oocytes and embryonic hamster kidney cells (17). This raises the exciting possibility that mec-4(d) or its mammalian counterparts could constitute the basis of genetic ablation vectors for higher organisms.

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