Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 13128–13133, November 1997
Genetics

Genetically targeted cell disruption in *Caenorhabditis elegans*

(cell death/degenerin/ENaC superfamily/ion channels/cell ablation)

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Communicated by William B. Wood, III, University of Colorado, Boulder, CO, September 11, 1997 (received for review February 13, 1997)

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 [$p_{hsp\text{-}16}$*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 overnight. 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). pABL1 is a derivative of a $p_{mec\text{-}7}$GFP clone. A 769-bp fragment containing the GFP coding sequence was amplified from TU#61 (28) using the primers 5′-CGGGCTAGCTACCGTAGAAAAAATGAG-3′ (which introduced an *Nhe*I site) and 5′-GGGGTACCCCGCGGCCGCCCGAATGCTATTT-

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ATTTGT-3′ (which introduced *Not*I and *Kpn*I sites). This fragment was cloned into *Nhe*I + *Kpn*I-digested pPD52.102 (22) to generate $p_{mec\text{-}7}$GFP. A 3.8-kb fragment containing the toxic *mec-4*(*u231*) allele was PCR-amplified from genomic clone TU#14 (3) using the primers 5′-GCTGGCGGCCG-CATGTCATGGATGC-3′ and 5′-GGGGTACCGAATA-ATTTCCTACCC-3′, which included restriction sites for *Not*I and *Kpn*I, respectively. This fragment was cloned into *Not*I + *Kpn*I-digested $p_{mec\text{-}7}$GFP. Subsequently, a 1.7-kb fragment containing the *mec-7* promoter and the GFP gene was excised with *Sal*I and *Not*I and replaced with a 50-bp synthetic polylinker to obtain the basic promoterless vector pABL1. pABL2 and pABL3 were constructed by deleting one or two bases (respectively) from the *Bcl*I site of pABL1. $p_{unc\text{-}4}$*mec-4*(*d*) was constructed by amplifying a 3.4-kb fragment of the *unc-4* 5′ region from pNC4–21 (29) using the primers 5′-CCGTCGACCTGCAGCTCTGAAAATATATC-3′ and 5′-GACCCGGGTTTCACTTTTTGGAAGAAG-3′ (which included engineered *Sal*I and *Ava*I sites, respectively) and cloning this fragment into *Sal*I + *Ava*I-digested pABL1. $p_{unc\text{-}8}$*mec-4*(*d*) was constructed by amplifying nucleotides 7441–9451 from cosmid R13A1 (13) using 5′ primer 5′-GCTCTAGAGG-GAGCATTTCCGGTAGTTTG-3′ and 3′ primer 5′-CGCG-GATCCGATTTCCGGGATGACGTTCG-3′, which contained *Sph*I and *Bam*HI sites, respectively. The *unc-8* promoter region was cloned into vector pPD21.28 (24), and the *Xba*I-*Kpn*I *unc-8* promoter fragment from this construct was subsequently substituted for the *mec-7* promoter in the $p_{mec\text{-}7}$*mec-4*(*d*) gene fusion. $p_{mec\text{-}5}$*mec-4*(*d*) was constructed by amplifying a 1.3-kb fragment from the *mec-5* 5′ region using plasmid TU#234 (30) as template and primers 5′-CCAAGCTT-GATCGGCAAATTTTAAAATTTGCCGCTCACCCC-3′ and 5′-GGGGATCCTTTCTGAAAAGTTTGAAATAT-TATC-3′ (which included engineered *Hin*dIII and *Bam*HI sites, respectively) and cloning this fragment into *Hin*dIII + *Bam*HI-digested pABL1. To generate $p_{myo\text{-}2}$*mec-4*(*d*), we excised a 5.5-kb *Kpn*I–*Apa*I genomic *mec-4*(*d*) fragment from clone $p_{mec\text{-}7}$*Kpn*I066*mec-4*(*d*) [which is a $p_{mec\text{-}7}$*mec-4*(*d*) gene fusion that harbors a synthetic *Kpn*I site at position 1066 of the *mec-4* sequence reported by Driscoll and Chalfie (3)] and cloned this fragment into *Kpn*I–*Apa*I-digested pPD30.69 (22). The *mec-4* product synthesized from this construct lacks the N-terminal 20 amino acids (5) but appears as toxic as full length *mec-4*. To generate $p_{unc\text{-}54}$*mec-4*(*d*), we cloned the same 5.5-kb *Kpn*I–*Apa*I *mec-4*(*d*) fragment into *Kpn*I–*Apa*I-digested pPD30.38 (22). $p_{del\text{-}2}$*lacZ* is a translational fusion constructed by amplifying ≈2.2 kb of DNA containing putative promoter sequences and some N-terminal coding sequences of the predicted gene with N-terminal degenerin similarity on cosmid F58G6 (accession no. Z68217; nucleotides 2010–4241 in cosmid annotation) using primers 5′-ACGCGTCGACGATTGA-CATCTAGGAATTTTTAAGGGT-3′ and 5′-CGGGGTAC-CGGTACGCCGTGGATTGTTGCAGTCTCT-3′ (which contained *Sal*I and *Kpn*I sites, respectively) and cloning this fragment into *Sal*I–*Kpn*I-digested vector pPD21.28 (24). $p_{del\text{-}2}$-*mec-4*(*d*) was constructed by amplification of the same fragment from F58G6 using the alternate downstream primer 5′-CTGCGGCCGCGGTACGCCGTGGATTGTTGCAGT-CTCT-3′, which introduced a *Not*I site and cloning into *Sal*I + *Not*I-digested pABL1.

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 nongonadal cells (31–32). When transgenic animals harboring an integrated array of $p_{hsp\text{-}16}$*mec-4*(*d*) were heat shocked at 30°C for 2 or more h, we observed vacuolated cells throughout the body (Fig. 1*a*). 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. 1*b*). 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 $p_{hsp\text{-}16}$*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

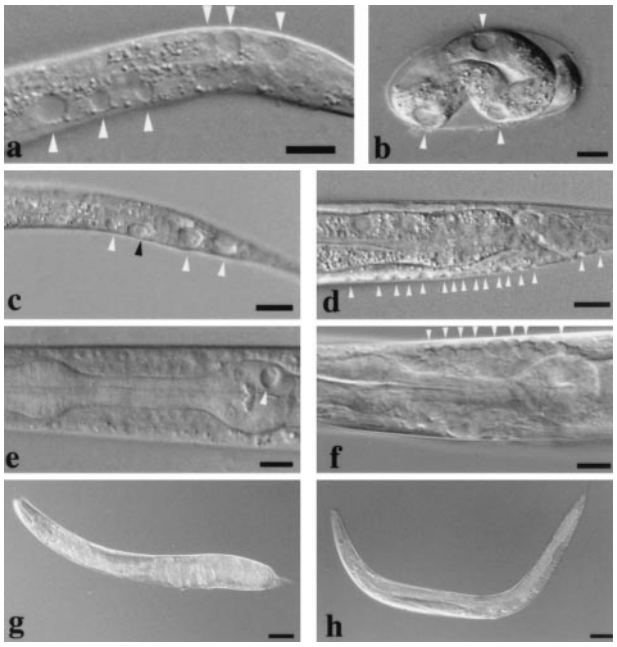

FIG. 1. Effects of ectopic expression of *mec-4*(*d*). Animals are oriented with anterior to the left and dorsal to the top, except for embryos. (*a–f*) Nomarski differential interference contrast microscopy images (Bar = 10 μm). (*g–h*) Bright-field images (Bar = 50 μm). Most prominent vacuoles, which typically appear as crater-like, swollen, membrane-bound units, are highlighted by arrows. Vacuoles may be evident in several different focal planes, and thus not all appear in sharp focus. In early stage degenerations, swollen nuclei can be observed within cells (for example, *c*, black arrow; see ref. 8). (*a*) Extensive vacuolation in a heat shocked L1 animal harboring $p_{hsp\text{-}16}$*mec-4*(*d*). (*b*) Heat shocked post-pretzel stage embryo harboring $p_{hsp\text{-}16}$*mec-4*(*d*). (*c*) Ventral cord neurons swelling in the posterior of an L1 stage animal harboring $p_{unc\text{-}4}$*mec-4*(*d*); black arrow indicates a cell with a clearly swollen nucleus inside. (*d*) Small vacuolated regions in the hypodermis of an L2 animal harboring $p_{mec\text{-}5}$*mec-4*(*d*). Note that hypodermal vacuoles are always small and nuclear inclusions within these vacuoles have not been observed. (*e*) Vacuolation of a pharyngeal muscle in an L2 stage animal harboring $p_{myo\text{-}2}$*mec-4*(*d*). (*f*) $p_{unc\text{-}54}$*mec-4*(*d*)-induced swelling of body wall muscle cells near the pharynx in an L3 stage animal. Indicated are several vacuoles along the dorsal side; the ventral side also exhibited small vacuoles in this animal that are not fully in focus. In body wall muscle, individual cells often appear to have multiple small vacuoles as shown. (*g*) The hypercontracted phenotype of an animal bearing the $p_{unc\text{-}54}$*mec-4*(*d*) transgene. (*h*) A *mec-6*(*u450*) mutant bearing the $p_{unc\text{-}54}$*mec-4*(*d*) transgene is not dramatically hypercontracted.

Table 1. Effects of ectopic expression of *mec-4*(*d*)

Construct	Expression	Phenotype	Suppression by *mec-6*(*u450*)
p_{hsp-16}*mec-4*(*d*)	Most nongonadal cells at 30°C	Paralysis at 30°C, death	+
p_{mec-7}*mec-4*(*d*)	Touch neurons, FLP, ALN, BDU, PVD	Touch-insensitive	+*
p_{unc-4}*mec-4*(*d*)	VA and VC ventral cord neurons	Uncoordinated, unable to back-up	+
p_{unc-8}*mec-4*(*d*)	Motor neurons, interneurons, ASH, FLP	Uncoordinated, nose touch insensitive	+
p_{mec-5}*mec-4*(*d*)	Hypodermal seam cells	Abnormal movement	+
p_{myo-2}*mec-4*(*d*)	Pharyngeal muscles	Starvation, arrested development	+
p_{unc-54}*mec-4*(*d*)	Body wall muscles	Hypercontraction, clearing, paralysis	+
p_{del-2}*mec-4*(*d*)	IL1, ASH, OLQ neurons	Nose touch defects	+

Phenotypes reported were observed in the majority of transgenic animals mosaic for the presence of the construct indicated although, as expected for mosaic animals, phenotypes typically occurred with a range of severity. Suppression in the *mec-6*(*u450*) background scored as + indicates that no transgenic animals in this background exhibited behavioral defects conferred by the *mec-4*(*d*) transgene (>100 animals scored for each).
**mec-6*(*u450*) animals are touch-insensitive, so phenotypic suppression is not expected and does not occur. However, whereas 100/100 transgenic animals harboring p_{mec-7}*mec-4*(*d*) in an otherwise wild type background exhibited degenerating neurons, only 6/100 transgenic animals had occasional vacuoles around the nose in the *mec-6*(*u450*) background, which is the background level we observed in nontransgenic wild-type animals.

neurons and at a lower level in the FLP, ALN, PVD, and BDU neurons (4, 33–34). In transgenic animals harboring p_{mec-7}*mec-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. 1*c*). 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 p_{unc-4}*mec-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 p_{unc-8}*mec-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 p_{unc-8}*mec-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 Nonneuronal 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 p_{mec-5}*mec-4*(*d*) displayed some vacuolation in hypodermal cells (Fig. 1*d*) 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 p_{myo-2}*mec-4*(*d*) construct had vacuoles localized within pharyngeal tissue (Fig. 1*e*). 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 p_{unc-54}*mec-4*(*d*) construct often had multiple small vacuoles in body wall muscles (Fig. 1*f*). 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. 1*g*), 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 p_{unc-54}*mec-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. 1 *g* 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 p_{hsp-16}*mec-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*(+) background.

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_{unc-8}*mec-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 *mec-4*(*d*)-induced degeneration 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 (*de*generin-*l*ike gene, designated as *del-2*). To determine the temporal and spatial expression pattern of the *del-2* promoter, we constructed a *lacZ* reporter fusion driven by upstream *del-2* regulatory sequences. Transgenic strains harboring the p_{del-2}*lacZ* fusion gene expressed β-galactosidase in a group of anterior neurons (Fig. 2*a*) that we tentatively identified as neurons of the ASH, OLQ, and IL1 classes after costaining transgenic animals with 4′,6-diamidino-2-phenylindole to visualize cell nuclei in the area. [This expression pattern is intriguing for a degenerin-related gene because candidate *del-2*-expressing neurons are mechanosensory; ASH and OLQ contribute to the response to head-on collision, and OLQ and IL 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

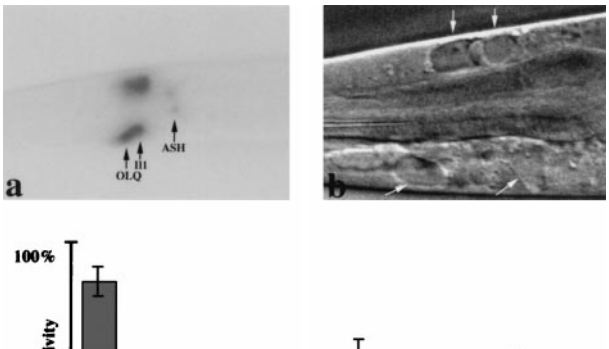

FIG. 2. Fusion of a promoter of interest to *mec-4*(*d*) verifies promoter activity profiles and creates behavioral defects. (*a*) β-galactosidase staining of candidate ASH, OLQ, and IL1 neurons in animals harboring a p_{del-2}*lacZ* gene fusion. (*b*) Nomarski differential interference contrast microscopy image of vacuolated cells (indicated by white arrows) in a transgenic L2 animal harboring p_{del-2}*mec-4*(*d*). (*c*) Assay of nose touch responsiveness in transgenic animals harboring p_{del-2}*mec-4*(*d*). Transgenic lines also include pRF4, which encodes dominant *rol-6*(*su1006*). Dark gray bars indicate assay in the wild type background; light gray bars indicate assay in the *mec-6*(*u450*) background. Scores are the average of 100 animals per line, 10 trials per animal, for two independently derived transgenic lines. Error bars indicate the SD.

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_{del-2}*mec-4*(*d*), induced degeneration of neurons in the vicinity of the IL1, OLQ, and ASH neurons (Fig. 2*b*), a phenotype suppressed in the *mec-6(u450)* background (Table 1; Fig. 2*c*). Transgenic animals exhibited a pronounced reduction in their ability to respond to mechanical stimuli delivered to the nose (Fig. 2*c*), supporting the hypothesis that *del-2*-expressing cells are the ASH, OLQ, 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 (pABL1). We introduced single or double nucleotide deletions within the *Bcl*I site of the pABL1 polylinker so that translational gene fusions could be made in all three reading frames (pABL2 and pABL3, 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 MEC-4(d) Channel. The working model for MEC-4 function postulates that MEC-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 MEC-4 channel is thought to depend on associations with additional proteins situated both inside and outside the touch neuron, and MEC-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 MEC-4(d) subunits, which might

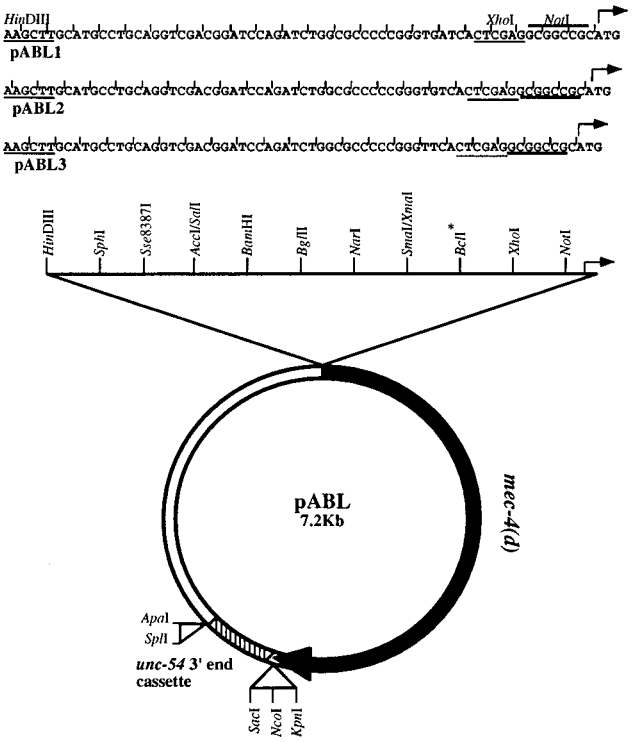

FIG. 3. *mec-4*(*d*) vectors for transcriptional and translational gene fusions. pABL1, pABL2, and pABL3 enable translational fusions in any reading frame to be constructed. They differ in the nucleotides corresponding to the *Bcl*I site in pABL1; the *Bcl*I site is present only in pABL1 (this site is 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 *Sse*8387I site in the polylinker enables fragments with *Pst*I ends to be inserted. In general, previously constructed *lacZ* and GFP fusion constructs made in standard *C. elegans* vectors (24, 28) can be readily converted to *mec-4*(*d*) fusion constructs. Swapping *Xba*I–*Apa*I or *Xba*I–*Spl*I reporter + 3′ end cassettes might be the most common conversion strategy; for constructs without the nuclear localization signal, substituting an *Xba*I–*Apa*I fragment from pABL2 into the *Age*I–*Apa*I region of the fusion construct should produce an in-frame *mec-4*(*d*) fusion equivalent to the original *lacZ* or GFP fusion.

assemble 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, *mec-4*(*d*) can still kill the touch receptor neurons in the absence of the activity of most *mec* genes that encode candidate channel-interacting proteins (10). Alternatively, the MEC-4(d) subunit might coassemble with other degenerin family members expressed in different cell types to form toxic heteromeric channels. Our data are consistent with the hypothesis that MEC-4(d) must interact in some way with MEC-6 for abnormal function.

Ectopic Toxicity Depends on *mec-6*. *mec-6* is needed for the deleterious activities of all *C. elegans* degenerins identified by mutation: *deg-1* (10), *mec-10* (11), *unc-8* (12), and *unc-105*. Our finding that *mec-6* is required for ectopic *mec-4*(*d*) toxicity in diverse cell types confirms its importance in degenerin channel activity and suggests that *mec-6* is expressed in a broad range of cells over most developmental stages. The requirement for *mec-6* renders *mec-4*(*d*) toxicity conditional such that lines harboring *mec-4*(*d*) constructs that are deleterious to nematode health or reproduction can be easily constructed and reared in the *mec-6*(*u450*) background. Degeneration can be induced in progeny of such a line by crossing in the *mec-6*(+) allele.

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*(*lf*) mutants; for *unc-8*, the uncoordinated phenotype of the p$_{unc\text{-}8}$*mec-4*(*d*) transgenic animals was similar to that of *unc-8*(*d*) mutations. 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 example, mutations that disrupt nonessential transcriptional activators 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 p$_{unc\text{-}8}$*mec-4*(*d*) indicate that nuclei do disappear, but more remain detectable than expected (N2, 51 ± 2 nuclei; *bzIs5* [p$_{unc\text{-}8}$*mec-4*(*d*)], 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 DD 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*(*lf*); *ced-3* or *ced-4 ced-9*(*lf*) double mutant backgrounds. 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* 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 *mec-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 substitution analogous to the 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.

We thank G. Kao and D. Xue for critical reading of the manuscript and D. Miller and M. Chalfie for plasmids. N.T. was supported by a fellowship from the Human Frontiers in Science Program Organization. M.D. was a fellow of the Alfred P. Sloan Foundation. Work was supported by grants from the National Institutes of Health (NS34435 to M.D. and GM37706 to A.F.) and the National Science Foundation (MCB-9205856 to M.D.).

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