A Screen For Mutants Defective in the Specification of M4 Sister Cell Death

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In *C. elegans*, 131 somatic cells undergo programmed cell death during wild-type hermaphrodite development. The process of programmed cell death includes four distinct steps: cell-death specification, execution, engulfment and degradation. While many genes involved in cell-death execution, engulfment and degradation have been identified, the process of cell-death specification is poorly understood.

The M4 motor neuron regulates muscle contraction in the pharynx, while the M4 sister undergoes programmed cell death in embryonic development. The reporter *Pceh-28::gfp* (kindly provided by Brian Harfe and Andrew Fire) is expressed in the M4 of wild-type animals and in both the M4 and the M4 sister of mutants defective in programmed cell death. We constructed strains carrying a *Pceh-28::4XNLSgfp* reporter. These strains have fluorescence tightly localized in the nucleus and allowed us to efficiently screen for mutants defective in M4 sister cell death by using a dissecting microscope equipped with fluorescence optics.

Screens of 71,820 mutagenized haploid genomes yielded at least 75 independent mutations that cause extra gfp-expressing cells. By complementation tests, we identified at least 34 alleles of *ced-3*, 11 alleles of *ced-4* and four alleles of *egl-1*. Five other isolates defining at least three genes had an extra gfp-expressing cell inside the pharynx. We do not know if the extra gfp-expressing pharyngeal cell in each of these mutants is the M4 sister cell. We are currently mapping the unidentified mutations in which one extra gfp-expressing cell is inside the pharynx. One, *n4796*, has been located to a 180 kbp interval on LG V. Rescue experiments are currently in progress.

We also mapped a mutant in which one or two extra gfp-expressing cells are outside the pharynx. *n4784* is located within a 86 kbp interval on LG X, and the mutant phenotype could be rescued by two overlapping cosmids. In the overlapping region, there is a single predicted gene, *F49E10.5*, which is most similar to adenovirus E1A C-terminal binding protein, a transcriptional corepressor. We have identified mutations in *F49E10.5* in *n4784* animals and in five other isolates that showed a similar phenotype. Further phenotypic characterization is in progress.

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