DEVELOPMENTAL BIOLOGY

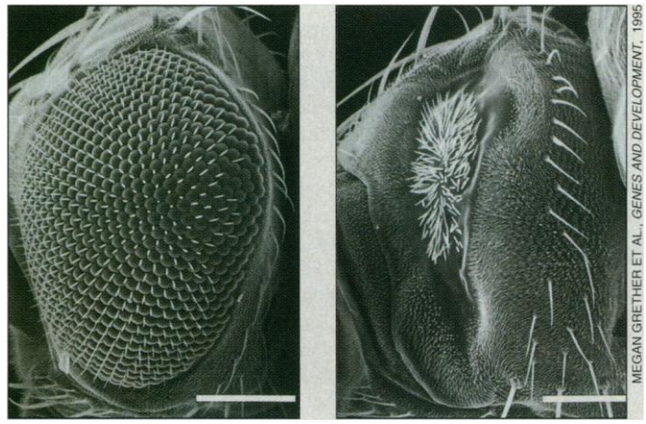
In the Fruit Fly, Cell Death Genes May Come in Pairs

While the thought of suicide can offer "calm passage across many a bad night," as Nietzsche wrote, few people go so far as to keep both a loaded gun and a lethal dose of pills at their bedside. Yet cells in embryos of the fruit fly *Drosophila melanogaster* may employ just such a fail-safe strategy to ensure that they carry out the self-destruction that is a necessary part of normal embryonic development.

Last year, a research team led by developmental neurobiologist Hermann Steller at the Howard Hughes Medical Institute (HHMI) and the Massachusetts Institute of Technology (MIT) discovered the first fruit fly gene needed for "apoptosis," the sequence of programmed biochemical changes inside cells that ends with their shutdown, fragmentation, and ultimate digestion by surrounding cells (*Science*, 29 April 1994, pp. 668 and 677). The researchers found that this gene, which they grimly called *reaper*, initiates the series of events culminating in apoptosis. Now, in work described in the 15 July issue of *Genes and Development*, the same team has located a second death gene, called *hid* (for *head-involution defective*), which lies close to *reaper* in the fruit fly genome and appears to act independently in triggering apoptosis.

The identification of the second gene solves a long-standing puzzle in developmental genetics: why no apoptosis-defective mutants were known in *Drosophila*. Although researchers had found several apoptosis-defective mutants in the nematode *Caenorhabditis elegans*, their searches for similar mutants in the fruit fly, normally a species highly amenable to such studies, long proved fruitless. Now, the MIT group has shown that the apoptosis machinery is crippled only in flies missing the entire chromosomal region containing both *reaper* and *hid*. This suggests that some of the genes regulating cell death in *Drosophila* and higher organisms may be redundant, with one sometimes compensating for the loss of the other, thereby making single gene mutations hard to find. Indeed, only the fortuitous pairing of *reaper* and *hid* allowed Steller's group to find the two genes. If *hid* were hiding in an entirely different genomic neighborhood from *reaper*, Steller says, "we would never have found a cell-death-defective mutant, and our original paper on *reaper* would never have appeared."

But luck prevailed, and the two discoveries together will now enable researchers to use the powerful genetic tools that exist for the study of *Drosophila* to search for more genes and proteins involved in apoptosis. "Anomalously, until 2 years ago, *Drosophila* had made contributions to every other area of development except cell death," says Gerald Rubin, a developmental geneticist at HHMI and the University of California, Berkeley. "Now the first of its contributions there are coming to light."



MEGAN GRETHER ET AL., *GENES AND DEVELOPMENT*, 1995

Eye for an eye. Targeting expression of the *hid* gene to the cells of the developing eye of *Drosophila melanogaster* results in a missing eye *(right)*. Scale bar equals 100 micrometers.

Ever since *reaper*'s discovery 2 years ago, Steller and his MIT colleagues, Megan Grether, John Abrams, Kristin White, Lynn Young, Kim Farrell, and Julie Agapite, had suspected that it wasn't the only cell-death gene at work in its region of the *Drosophila* genome. One reason was that the many point mutations (single basepair changes) they induced in the region around the *reaper* gene failed to produce significant changes in the pattern of embryonic cell death. Since a point mutation can inactivate only one gene at a time, one possible explanation for this failure was the existence of a second, functionally redundant cell death gene lying in the same chromosomal region as *reaper*.

The MIT workers' suspicions fell on *hid*, a gene that developmental biologists Judy Lengyel and Michael Abbott at the University of California, Los Angeles, had already located on that section of the *Drosophila* genome. Mutations in the gene produce embryos with misshapen heads, a defect that occurs because a region of the embryo known as the dorsal fold, which lies near the developing head, fails to migrate to the anterior as it should. As a result, the head is unable to retract properly into place. The Steller team guessed that the fold's migration might be blocked by the persistence of cells around the head that should be eliminated by apoptosis. If true, this could indicate that the gene inactivated in *hid* mutants is actually a cell-death initiator.

To test their hypothesis, the MIT researchers cloned and sequenced the *hid* gene. When they introduced it into mutant flies whose embryos can't carry out apoptosis, cell death occurred throughout the embryo. The team also found that the gene, when expressed at higher than normal levels, can kill cells that would normally live. When they placed *hid* under the control of a gene regulator involved in the development of *Drosophila*'s compound eye, for example, the resulting adult flies had shriveled, scar-like eyes.

At present, the roles of *hid* and *reaper* are poorly understood, since neither is related to any of the three genes known to control apoptosis in *C. elegans*, or to other known genes that might provide clues to their function. The work so far suggests, however, that they act early in the apoptosis pathway. *Reaper* and *hid*'s messenger expression comes on 1 to 2 hours before apoptosis begins, indicating that the genes may be passing on the suicide signal, but are not involved in the actual machinery of fragmentation.

And the two genes seem to be acting to trigger the same pathway. The MIT researchers found that the cell-killing abilities of both can be completely blocked by the addition of the baculovirus *p35* gene, which normally inactivates cell death mechanisms in the viruses' host cells as a way of prolonging viral replication. Steller thus believes *reaper* and *hid* to be "integrators" that link different signaling pathways with other genes acting "downstream" in the apoptosis pathway. Scientists would now like to use the *reaper* and *hid* genes as an entree to finding some of these downstream genes.

Indeed, the effort to uncover the full apoptosis pathway may proceed twice as quickly now that *Drosophila* geneticists are joining those studying *C. elegans*, the only other organism that can be easily subjected to gene mutation studies. Steller's laboratory at MIT, says Michael Hengartner, a geneticist at Cold Spring Harbor Laboratory on Long Island, has "almost single-handedly raised *Drosophila* to a level where it is a very serious contender" in the study of dying cells. If biologists can continue to decipher the cryptic notes passed along by these cells' genes, they may also come to understand why, for some cells, suicide is the only way out.

–Wade Roush