NEUROBIOLOGY

Death Gives Birth to the Nervous System. But How?

In the nervous system, as in other parts of the body, mortality is an important aspect of vitality. Early on in the development of the embryo, many more neurons are made than the nervous system ultimately requires, and as a result huge numbers of surplus neurons die off as part of the process that sculpts brains and nerves into final form. Researchers have long puzzled over what directs this mass destruction. And since the nervous system is unique in many ways, they have generally assumed the answer would be peculiar to the nervous system. Not so.

In the past 5 years, researchers have discovered that neurons share mechanisms of programmed cell death, or "apoptosis," with many other cellular systems (see story on page 760). And that has brought new life to the field of neuronal cell death, as researchers seek—and, increasingly, find—clues to what is driving the process in such divergent quarters as the mammalian immune system and nematode worms. Among the very newest of this cascade of findings is an immune-system gene that can protect neurons from death—a discovery that could lead to treatments for stroke or neurodegenerative disease. Those benefits, though, won't come until much more is learned about how neuronal cell death actually occurs.

Why the mass die-off of neurons occurs during embryonic development is easier to explain than how it takes place. A few deaths kill off neurons that have wound up in the wrong places or have served a transient purpose and are no longer needed; but the majority of the carnage seems to be "size-matching"—the final step in a process in which the animal first makes a large excess of nerve cells, then weeds many of them out until the right number remain. That may sound wasteful—indeed, in many parts of the nervous system, more than 50% of the neurons die before embryonic development is complete—but it is one way of solving a problem that arises in complex organisms. The problem results from the fact that genes don't dictate exactly how many cells of a particular type will be made. The final size of a muscle, for example, is a bit uncertain, and one way to be sure there will be enough neurons to activate the muscle is to make extras and let them compete for survival.

The precise nature of this fatal competition, however, remains a mystery. "The evidence would suggest that it is survival of the fittest," says Eugene Johnson, who studies neuronal death at Washington University in St. Louis. "But nobody really understands what distinguishes the winners from the losers." Hints have come from one of the systems in which neuronal cell death is best understood: the sympathetic nervous system, which controls unconscious visceral processes. There, developing neurons growing toward internal organs appear to compete for a protein called nerve growth factor (NGF), which is made by the target tissues. Some neurons get enough of this elixir and surive; other neurons either don't get enough, or don't respond strongly enough to NGF, and die.

Precisely how those neurons go about dying, though, was a question neuroscientists tended to overlook as they concentrated on the external factors that control competition between winners and losers. "There was a real separation between the people who were interested in cell death in other systems and people who studied cell death in the nervous system," says Johnson. "We [neuroscientists] were focusing on what factors control the decision of the cell to live or die, not the question of what the molecular mechanism is by which the cell dies, once that decision has been made." While immunologists and others explored those mechanisms in their systems, Johnson says, "I think the general feeling in the [neuroscience] field was that the cell loses its [growth-factor] support and just kind of peters out."

In 1987, however, Johnson and his graduate student David Martin did an experiment that turned such thinking around. They found that drugs that inhibit protein synthesis can block the death of cultured sympathetic neurons that ordinarily die when deprived of NGF. That finding was "crucial," says developmental biologist Martin Raff of University College, London—because it suggested the neurons were not just petering out, but were undergoing a specific "death program." "That was the first real evidence that when you take away NGF, the cells are committing suicide," Raff says. And that put neuronal cell death smack in the cell-death research mainstream, equating it with apoptosis in other systems, such as the immune system, where the process aids in the removal of self-reactive T cells and executes the death order delivered by killer cells.

In addition to bringing neuronal cell death under the larger umbrella of apoptosis research, Martin and Johnson's finding stimulated the search for the "death genes" whose products carry out apoptosis in neurons. In that search, the new alliance with immunologists wasn't initially very helpful, since no one in that field had been successful in finding a death gene. Even today, says Johnson, "we really cannot identify any gene yet whose expression is critical for the [neuron] to die."

Yet if the search for neuronal death genes has been frustrating for those working on higher organisms, researchers working with simpler creatures have had considerable success—and their results are pointing the way forward. The model that has provided the

Cashing In on Cell Death

Growing excitement among researchers about programmed cell death and its possible role in neuronal and immunological diseases and cancer hasn't escaped Wall Street's attention. Indeed there are signs that a young crop of apoptosis-oriented startup companies is springing to life, with plans to seek drugs that would block cell death caused by disease, or turn the death program on as a way of killing cancer cells.

Not only are ideas abundant, but the research has progressed to the point that companies now have an idea of what genes or proteins to work with, says Kevin Gorman of Avalon Ventures Inc., a San Diego venture capital firm. Avalon, with the help of Robert Horvitz of MIT, Martin Raff of University College, London, and Stanley Korsmeyer of Washington University, all major players in cell-death research, is launching Idun Pharmaceuticals, which will conduct research on cell death in cancer, the immune system, and the nervous system.

Cambridge, Massachusetts-based ImmunoGen, in partnership with the Dana-Farber Cancer Institute, has thrown its hat in the cell-death ring with a new company called Apoptosis Technology Inc., or ATI. ATI will focus initially on TIA-1, a protein discovered by Paul Anderson and colleagues at Dana-Farber that killer T cells apparently use to induce apoptosis in their targets. ATI plans to expand its cell-death research through collaborations with academic labs.

Says Tim Wilson, biotech analyst with Hambrecht and Quist securities, "Cell death is very hot now in the scientific community." How hot it stays in the financial community could depend on the early fortunes of these two pioneers.

–M.B.

key results is the roundworm *Caenorhabditis elegans*, a simple, extremely well-characterized organism. Every one of the 1090 cells that appear during the worm's embryonic development has been identified; 131 of them are known to die in a programmed way as the worm matures. In the early 1980s, Robert Horvitz and his colleagues at the Massachusetts Institute of Technology (MIT) found two *C. elegans* genes, *ced-3* and *ced-4*, whose protein products seemed to trigger programmed cell death, and later they found another gene, *ced-9*, which had the opposite effect—saving the cells that express it from programmed extinction.

Given the frustration neuroscientists were experiencing with vertebrate systems in the search for death genes, some hoped that studies of such genes in *C. elegans* would provide clues to how the death process worked in higher animals. But on the face of it, cell death in the tiny roundworm is very different from the vertebrate variety. Neither growth factors nor competition—both key elements in vertebrate cell death—seems to play a role in *C. elegans*; instead, certain cells are fated to die from the moment they are created. That apparently fundamental difference left some researchers worrying that *C. elegans* may not provide the answers they sought.

As it turned out, those fears were unfounded. In a wave of advances that swept through the field in the past year, work on one gene has managed to stitch together cell-death studies in worms, immune-system cells, and vertebrate neurons. That gene is *bcl-2*, an oncogene first identified in a B cell leukemia that was recently found to prevent lymphocytes from undergoing programmed cell death. That finding triggered a flurry of experiments in which researchers tested the effect of *bcl-2* in various cell-death systems.

For those studying the nervous system, the results were particularly revivifying. Jean-Claude Martinou of the Centre Medical Universitaire in Geneva, Switzerland, and his co-workers reported last October that they had expressed the *bcl-2* gene in cultured neurons, and it kept them from dying in the absence of NGF. In December, Stuart Kim and his colleagues at Stanford showed that when the mammalian *bcl-2* gene was inserted into *C. elegans*, it worked its magic there, too.

In fact, *C. elegans* has a gene of its own that is homologous to *bcl-2*. Horvitz announced at several conferences last fall that *ced-9*, which saves *C. elegans* cells from death, is 23% identical in DNA sequence to the *bcl-2* gene. Together, those findings imply that whatever *bcl-2* does is "not just a lymphoid thing or a mammalian thing," says David Vaux, a postdoc with Irving Weissman at Stanford who worked on the project with Kim. "It's common to many cell types, and goes back as far as *C. elegans*." And that, adds Raff, suggests that "programmed cell death is as fundamental as cell proliferation, or any other basic process in animal cells."

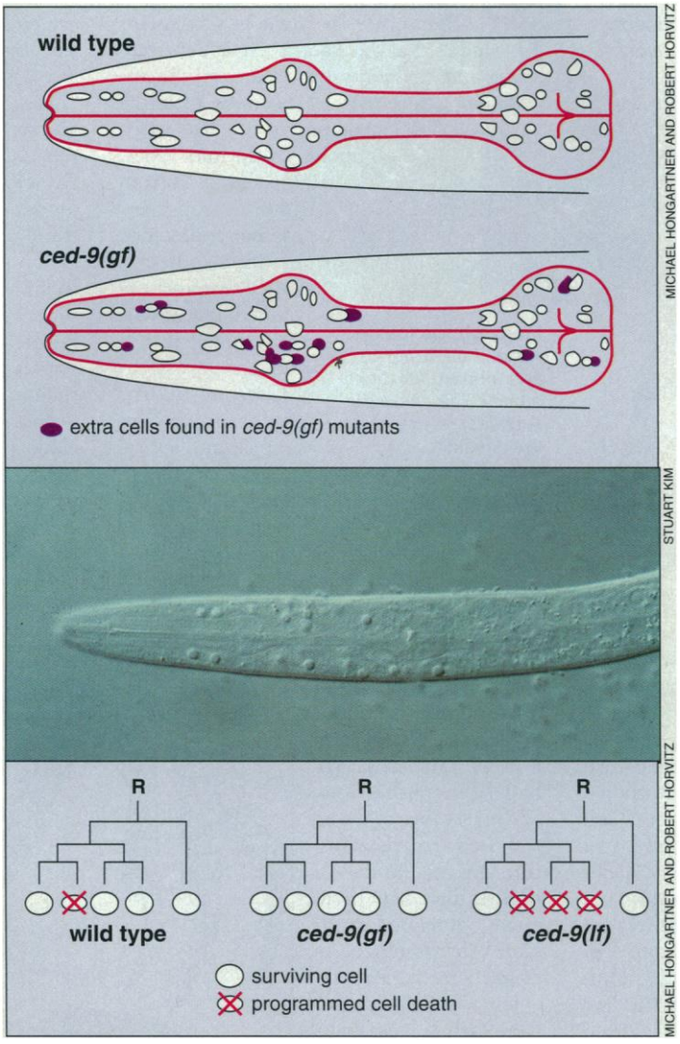
The reason neuroscientists are so excited about these findings is that if, as Vaux says, the mechanisms of programmed cell death are similar from organism to organism and from organ to organ, that should quicken the pace of advances in understanding how the nervous system picks survivors during embryonic development. Yet neuroscientists are cautious about jumping to the conclusion that *bcl-2* itself is a player in neuronal death. For that to be the case, says neuroscientist Lloyd Greene, who studies cell death at Columbia University, there must be evidence that "*bcl-2* is present and functioning in the nervous system."

Evidence of its presence is, in fact, on the way. Diane Merry, a postdoc with Stanley Korsmeyer at Washington University, has found *bcl-2* expressed in neurons throughout the developing brains and nervous systems of embryonic and young mice, often just at the time neurons are deciding whether to live or die. The level of *bcl-2* expression could be helping to decide the neurons' fate, says Merry, but that remains an untested hypothesis, she adds.

Even if *bcl-2* itself eventually turns out not to be involved in the normal life-and-death decisions of neurons, the findings so far have prompted researchers to intensify the search for other cell-death genes that may be similar in worms and man. "We know the cell death pathway in worms involves the killer genes *ced-3* and *ced-4*," says MIT's Horvitz. "Maybe there are comparable killer genes in mammals."

There is also the tantalizing possibility that *bcl-2* could be useful in fighting neuronal death resulting from strokes and neurodegenerative diseases such as Parkinson's or Alzheimer's disease. Neuron death following a stroke doesn't seem to be classic apoptosis, but *bcl-2* may still be able to help. The neurons seem to die as the result of a calcium-ion influx, and Martinou, now at the Swiss pharmaceutical company Glaxo, recently found that *bcl-2* blocks calcium-triggered death in cultured neurons. Using transgenic mice whose neurons overexpress *bcl-2*, he plans to test whether the gene protects against stroke damage. That's all quite speculative for the moment, and the case of neurodegenerative diseases is even more so, since little is known about how neurons die in those diseases. But the payoff will be large if therapy for even one major neurodegenerative condition comes out of apoptosis research. For that reason, in addition to the sheer intellectual interest of the quest, cell death in the nervous system is sure to remain a lively research area for years to come.

–Marcia Barinaga



Lifesaver. In *C. elegans* embryos, over-expression of a gene called *ced-9* causes the survival of cells that would normally die *(dark spots in top panel)*. In specific lineages of *C. elegans* cells *(bottom)*, extra *ced-9* (gf) saves a cell that would normally die; loss of *ced-9* function (lf) causes abnormal death of two cells. *C. elegans* cells undergoing programmed death have a button-like appearance *(middle)*.