Cell Suicide: By ICE, Not Fire

A cascade of new research findings is giving researchers insights into the genes that control programmed cell death

Like people, cells die in different ways: accident, murder, old age, even suicide. In fact, cellular suicide isn't just a curiosity, it's necessary for the health of the organism. During embryonic development, for example, it helps weed out superfluous nerve cells, as well as immune cells that might attack and damage the body's own tissues. Like a spy-plane pilot who carries a little vial of poison under his seat in case he's captured, cells carry in their nuclei a genetic program for suicide that can be set in motion, should the cell receive orders to self-destruct.

Now, after years of eluding researchers, the genes that carry out the suicide program are coming into the light. "Clearly it is an exciting time in cell death, unlike 3 or 4 years ago, when [the field] was very quiet," says neuroscientist Eugene Johnson, who studies cell death at Washington University in St. Louis. "The pieces are coming in fast and furious."

One particularly hot area of the cell-death field concerns several genes that seem to direct and execute the death program inside cells. Over the past 6 months, an outpouring of papers in *Cell* and *Science* has provided insights into the identities and modus operandi of these genes. The latest discovery comes from a team led by Junying Yuan of Harvard Medical School, which reports their work on page 826. Yuan's group finds that the gene for an enzyme known as "ICE" that plays a key role in triggering inflammation may do double duty as a suicide gene in neurons that receive the command to die.

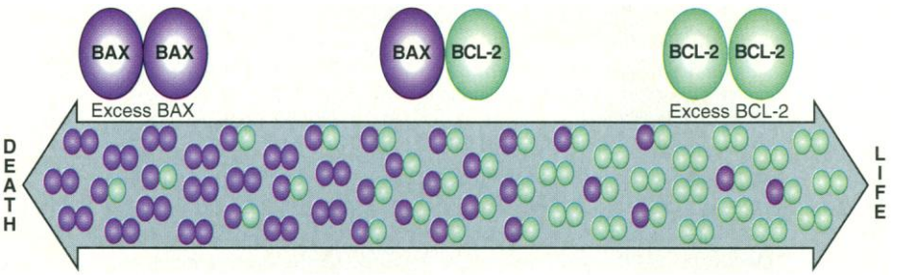
It's still not clear how all the rapidly accumulating pieces fit together in the overall picture of programmed cell death. Indeed, many parts of the picture are still missing. But, like the first pieces of a jigsaw puzzle falling in place, this recent flurry of findings has infused researchers with energy, spurring them to push harder yet to find the other pieces that will bring the complete image into view. Their hope is that the full pattern will provide answers not only about "healthy" cell death, but also about situations in which programmed cell death goes wrong, causing disease. Failure of cells to die when they should may, for example, contribute to the abnormal growth of tumors, and inappropriate cell death in the nervous system may contribute to neurodegenerative diseases such as Alzheimer's or Parkinson's (*Science*, 5 February 1993, p. 760 and 762).

If those answers are found, they will be an extension of a long line of work on programmed cell death and the role it plays during both the development and the later life of an organism. Studies dating back more than 20 years showed that cells that are chosen to die—either because they are superfluous, diseased, or have served their useful purpose—don't just fall apart and expire; they go through a predictable, well-choreographed series of events. The cells round up, their outer membranes form bulges called blebs, nuclear membranes and some internal structures break down, the nuclear DNA is fragmented by enzymes, and finally the cell breaks into pieces that are scarfed up by still-vital neighboring cells.

This distinctive pattern was dubbed apoptosis, after a Greek word describing a plant shedding its leaves. The early studies of apoptosis provided more than a name; they showed that drugs that block protein synthesis prevent apoptosis, suggesting that this programmed form of death requires specific proteins. That spurred researchers to find the genes for those proteins.

A key aid in finding the suicide genes was the emergence in the 1980s of the tiny, transparent roundworm *Caenorhabditis elegans* as a valuable resource for identifying genes active in embryonic development. Because this microscopic worm has only 1090 cells, developmental biologists were able to trace the lineage of each cell as the worm matures, and found that 131 of the embryonic cells undergo programmed cell death. That finding opened the way for Robert Horvitz and his colleagues at the Massachusetts Institute of Technology to search for mutations that influence apoptosis in C. *elegans* embryos. Among the genes identified by those genetic studies were the death genes *ced-3* and *ced-4*, as well as *ced-9*, an "anti-death" gene that protects cells from apoptosis.

Meanwhile, researchers searching for suicide genes in less tractable mammalian cells found it slow going. Their first breakthrough came only in the late 1980s, with the discovery that the gene *bcl-2*, which had been identified as a cancer-causing oncogene, protects immune cells called lymphocytes from suicide; later work showed that it protects neurons as well. But researchers had few other clues to the genes regulating death in mammalian cells, and they hoped that work on C. *elegans* would give their own research a boost.



To live or to die. The ratio of two proteins called Bcl-2 and Bax, which bind either to themselves or to each other, determines whether cultured lymphocytes will heed or ignore an order to die.

ILLUSTRATION: K. SUTLIFF

But if they were to rely on C. *elegans*, apoptosis in the worm would have to resemble the process in mammalian cells. "It really was a question of whether the [cell death] pathway of C. *elegans* was going to be evolutionarily conserved in the vertebrates," says Yuan, who studied cell death as a postdoc in Horvitz's lab before moving to Harvard 3 years ago. "And that was not really clear."

The first bit of clarity came in the fall of 1992, when Horvitz announced that his group had cloned and sequenced the worm suicide-protection gene, *ced-9*, and it was 23% identical to the mammalian protection gene, *bcl-2*. The resemblance between the two genes went beyond sequence similarities to function. Work reported a few months later by Stuart Kim and his colleagues at Stanford University School of Medicine showed that the mammalian *bcl-2* gene could substitute for *ced-9* in C. *elegans*, rescuing *ced-9* mutants from runaway cell death. Those findings gave researchers working on mammalian systems the sought-after boost, raising the possibility that, like the protection genes, the suicide genes would be conserved all the way from worms to humans.

As a matter of fact, evidence for just that kind of conservation was already being gath-

ered when the connection between *bcl-2* and *ced-9* was made. After Yuan moved to Harvard in early 1991, she and her colleagues continued looking for vertebrate counterparts of the suicide genes *ced-3* and *ced-4*, by screening the gene banks for similar sequences. That search proved fruitless, though, until a day in the spring of 1992, when Horvitz graduate student Shai Shaham gave it another ounce of persistence. Checking the data bank one more time, he hit pay dirt: a match between *ced-3* and a new gene sequence that had just been deposited.

The new gene, cloned by teams at Immunex Corp. in Seattle and Merck Research Laboratories in Rahway, New Jersey, coded for a protein called "ICE," for interleukin-1 β-converting enzyme. ICE is a protease (a protein-splitting enzyme) that activates interleukin-1 β, an important mediator of inflammation, by cutting an inactive precursor protein. "Immediately we got very excited," says Yuan, because the similarity suggested that ICE might well be a human cell-suicide gene. Overall, the two proteins share 28% of their amino acids, and CED-3 is identical to the ICE protein in a 5-amino-acid stretch thought to be the active site responsible for ICE's protease activity.

Yuan and her colleagues quickly decided to see whether ICE in fact participated in cellular self-destruction. They transferred the ICE gene into rat cells in culture and showed that production of the ICE protein killed the cells. To test whether the death was really ICE-induced, they tried several mutant ICE genes that coded for inactive enzyme, and found that those genes did not kill cells. Finally, they showed that the ICE-induced death could be blocked both by the known protective gene, *bcl-2*, and by *crmA*, a cowpox virus gene whose protein product inhibits ICE's protein-splitting activity. These results show that overexpression of ICE can cause cell death, and that this effect depends on its protease activity, says Yuan. But, she adds, "it doesn't show whether ICE may be acting in vivo to cause cell death during development."

To test that possibility, Yuan and her colleagues turned to a cell system more developmentally relevant than the connective tissue cells used for the earlier experiments: cultured neurons deprived of growth factors. This system mimics what happens during normal nervous system development when nerve cells die because they fail in the competition for life-sustaining growth factors. In this issue of *Science*, the Yuan team reports that, while control neurons died as expected when their growth factors were removed, neurons expressing *crmA* were protected against death. Because the protein made by *crmA* is a specific inhibitor of ICE, "this shows that ICE or something very related to ICE is acting in [these] neurons to cause death," say Yuan.

Yuan's finding is important, says Martin Raff, a cell-death researcher at University College, London, "because it is yet another possible indication that the death pathway is highly conserved [among species]. And it gives you a clue that proteases may be involved in the pathway." But, Raff adds, the findings so far give no hint as to where CED-3 or ICE fits into the pathway leading to death, an important question to be answered if ICE's role is to be understood.

Also unknown is how these suicide proteins interact with the protective proteins made by *ced-9* or its mammalian equivalent *bcl-2*, although studies with *C. elegans* may shed some light on this issue, Yuan says. Work in the roundworm shows *ced-9* is necessary for survival only when the suicide genes *ced-3* and *ced-4* are functional. That suggests that *ced-9* works by somehow preventing *ced-3* and *ced-4* activity. Something similar may be happening between Bcl-2 and ICE in mammalian cells, says Yuan: "If Bcl-2 levels are high, the condition in which to activate ICE doesn't exist."

But the mere presence of Bcl-2 apparently isn't enough to save cells from mortality. Stanley Korsmeyer and his colleagues at Washington University recently discovered that Bcl-2 must first win a sort of hand-to-hand competition with a death-promoting twin that they call Bax. The researchers found Bax while searching for proteins that might bind to Bcl-2 and help it protect cells from death. Instead they came up with Bax, which binds to Bcl-2, and is closely related in amino acid sequence as well—but, like an evil twin brother, actually contributes to cell death rather than inhibiting it.

Korsmeyer posits that the Bcl-2-Bax pair constitutes a "pre-set rheostat within cells," with the ratio of Bcl-2 to Bax determining whether a cell receiving a death order accepts it or ignores it. If the Bcl-2 protein is in excess in a cell, it binds up all the Bax, and the rest of the Bcl-2 molecules pair with each other. Under these conditions, the cell survives. On the other hand, if Bax predominates, it grabs all the Bcl-2, and forms Bax-Bax pairs as well. In that case, the result is death. During development, says Korsmeyer, that means that the Bcl-2/Bax ratio should drop at times when cells need to be weeded out by apoptosis. And, at least in some cases, that is what happens. For example, Bcl-2 levels are high in immature T lymphocytes, but drop precisely at the point in development when signals come in to kill off self-reactive cells.

That's a nice piece of support for the notion that the life-death struggle of Bcl-2 and Bax can control the route to cellular suicide. Yet those two genes clearly aren't the sole controllers of cell death. Korsmeyer's group made knockout mice missing *bcl-2* and found that cell death is, as expected, excessive in some of the animals' tissues, including the developing kidneys and the thymus, where T lymphocytes develop. Other tissues, however, including the nervous system, seem to develop normally, implying that other genes like *bcl-2* may protect against programmed death. One candidate has already been found. Last August, Craig Thompson and his colleagues at the University of Chicago reported their discovery of a gene related to *bcl-2*, which they named *bcl-x*.

Thompson's group found that the *bcl-x* gene makes two proteins: a long form that protects cultured lymphocytes from death, and a short form that seems to promote death. Thompson suggests that these two forms of the Bcl-x protein may make up a competing pair like Bcl-2 and Bax. Whether the Bcl-x proteins might govern death in cells of the nervous system and other tissues in living animals isn't clear yet, says Thompson, but his group is making *bcl-x* knockouts that should help answer that question.

One remaining mystery in the Bcl family, Korsmeyer says, is whether both members of these protein pairs have biochemical functions, or whether only one does, while the other serves as a "handcuff" that keeps its competitor from doing its job. And beyond the question of which partner has a biochemical function is the broader question of what that function might be.

Recent work from Korsmeyer's group and Dale Bredesen's lab at the University of California, Los Angeles, might provide a clue on that score. They've found that highly reactive oxygen radicals may be involved in triggering apoptosis, and that Bcl-2 may block

INTRACELLULAR PROTEINS INVOLVED IN PROGRAMMED CELL DEATH

C. elegans	Mammalian	Role in Cell Death	Function
CED-9	Bcl-2	prevents	Opposes Bax
?	Bax	promotes	Opposes Bcl-2
?	Bcl-x (long)	prevents	Opposes Bcl-x (short)
?	Bcl-x (short)	promotes	Opposes Bcl-x (long)
CED-3	ICE	promotes	Protease
CED-4	?	promotes	?

either the formation of oxygen radicals or their effects. Bredesen and his colleagues found that Bcl-2 seems to protect cells not only from apoptotic death, which may be triggered by modest amounts of oxygen radicals, but also from the nonprogrammed necrotic death that occurs under conditions that normally make oxygen radical levels soar. If those results hold up, they could widen the potential for eventual therapeutic uses of Bcl-2, since the death of cells during strokes, trauma, and some degenerative diseases may be predominantly necrotic.

While some cell death researchers are excited by the oxygen-radical connection, others are skeptical. "It's getting a lot of hype at the moment, but I must confess I don't find it very convincing," says Raff, of University College, London, who notes that apoptosis aims to take cells apart "neatly, and in minutes." Reactive oxygen radicals, he thinks, seem "a little too uncontrolled" to kick off that kind of process.

Washington University's Johnson says he finds the reactive oxygen link "intriguing" but also a bit unsettling. And he points out that there are alternative biochemical means through which Bcl-2 may be working: For example, a team at Onyx Pharmaceuticals in Richmond, California, recently reported that Bcl-2 associates with a protein called R-ras, which is related to the *ras* oncogene product, and is thought to be involved in intracellular signaling in ways that are as yet unknown.

Bcl-2 and ICE are clearly only two parts of the cell death puzzle, and while the clues they provide are important pieces to have on the table, the puzzle will not be complete until researchers fill in the story of how cell surface molecules receive the death command and transmit its message to the cell. Others are also following up leads suggesting that cell death may have some elements in common with the cell-division cycle.

Ultimately, researchers may find that they can manipulate cell death, turning it on to destroy cancer cells or turning it off to prevent the loss of neurons in neurodegenerative disease. But for now, the main goal is to find all the puzzle pieces. "We're not to the point yet where everything makes sense," says Washington University's Johnson, "but I think we've got a lot of the pieces that will turn out to be important."

–Marcia Barinaga

Additional Reading

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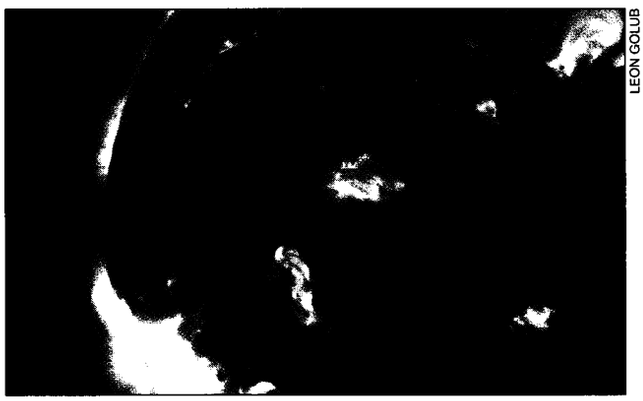
SPACE PHYSICS

Solar Physicists Peer Into a Mysterious Furnace

During a total eclipse, the solar corona is an awesome sight: a halo that streams out into space for several times the sun's diameter. The spectacle is more than awesome, though; it's a brazen challenge to astrophysicists. This halo of glowing gas can fight off the sun's gravitational pull and extend so far into space only by maintaining a temperature of several million degrees Kelvin—200 times hotter than the solar surface. The very existence of the corona thus seems to flout standard thermodynamics, which predicts that temperatures will drop off with distance from the sun's energy-producing core, just as the air in an old farmhouse cools as you move away from the stove. "The question is, Why on earth is [the corona] there?" says solar physicist Keith Strong of the Lockheed Palo Alto Research Laboratories.



LEON GOLUB

Close scrutiny. An x-ray image made at the onset of an eclipse reveals fine structure in the magnetic loops, suggesting that the corona might be heated by electrical resistance.

Somehow, most researchers believe, mechanical energy from the churning of the sun's outer layers gets pumped up into the corona and turned into heat. Learning how this happens would not only solve a key riddle posed by the sun and other stars; it would also unmask the driving force of the solar wind, an extension of the corona that affects terrestrial matters such as communications and, some researchers speculate, even the weather. With so much at stake, solar physicists have had no shortage of theories. But until recently, they had few facts to test those theories against. Now, says Jack Zirker of the National Solar Observatory, new space-based observations of the sun are starting to take researchers "into the guts of the physical processes."

Once there, researchers hope to pick out the best of a series of proposed mechanisms, many of which center on the bundles of magnetic field lines that arch over the sun's surface. Pockets of turbulence in the bundles or wavelike motions in the lines might generate heat; "nanoflares"—sparklike releases of magnetic energy from the bundles—might heat the corona by spewing high-energy particles; electric currents flowing along the field lines could turn them into giant toaster filaments. To tell these mechanisms apart, researchers need detailed images of the magnetic bundles, which are outlined by hot, x-ray-emitting gas.

Such images are now arriving from two new instruments, the Soft X-Ray Telescope (SXT) on board the Japanese satellite Yohkoh and the Normal Incidence X-Ray Telescope (NIXT), flown on sounding rockets by Leon Golub of the Harvard-Smithsonian Center for Astrophysics and his colleagues. The SXT, which has been watching the sun since Yohkoh's launch in August 1991, can take pictures in much quicker succession than previous instruments, yielding movies that reveal bursts of heating. NIXT takes only snapshots, but it can reveal detail three times finer than SXT. While the new images haven't pointed to a clear winner among the competing theories, a vision of heating by electric currents seems to be on the upswing, while a long-time favorite, nanoflares, appears to be fading.

Proposed and developed by Eugene Parker of the University of Chicago during the 1980s, the nanoflares theory holds that as the footpoints of the magnetic bundles shuffle around on the solar surface, the field lines in the bundles get stretched and braided—something like maypole streamers wound up by disorderly dancers. The process is "like stretching rubber bands," says Roger Kopp of Los Alamos National Laboratory, and it results in a buildup of magnetic energy. Parker suggested that this energy gets released when adjacent field lines suddenly reconnect, releasing tension. When the process takes place on a large scale, it is thought to power solar flares. Large flares are too rare to heat the corona, but Parker proposed that tiny bursts of reconnection take place a few times a second in each bundle, triggering enough nano-