A Time to Live, a Time to Die

Biologists probe the genetics of programmed cell death

By CAROL EZZELL

"First, you murder," Michael O. Hengartner forthrightly told a horde of expectant faces. "Next, you get rid of the body. Then, you hide the evidence," he explained, pacing back and forth in the dimly lit room.

Hengartner wasn't instructing a group of apprentice hit men. Instead, the Massachusetts Institute of Technology (MIT) biologist was addressing a gathering of cancer researchers, detailing the functions of a recently identified set of genes that controls life's only inevitable process: death.

Together, Hengartner and his MIT colleagues constitute one of scores of research teams around the world who are reviving scientific interest in the molecular mechanisms of a phenomenon called apoptosis, or programmed cell death. Among other things, this phenomenon (pronounced apa-tosis, with the second "p" silent) prevents humans from having webbed fingers and eliminates cells of the immune system that can't tell "self" from "nonself." It also underlies metamorphosis – the magic wand that turns caterpillars into butterflies and tadpoles into frogs. In adults, it phases out old body cells so they can be replaced by new ones.

Over the past year, biologists from a range of disciplines have uncovered evidence that this seemingly salutary process has a dark side. Several new studies suggest that apoptosis can play roles in AIDS and autoimmune diseases; others indicate that disruptions in the usual orderly progression of apoptosis lead to the uncontrolled cell growth of cancer.

Apoptosis – which means "dropping off" in Greek – was first described in 1951 as a step in animal development. The process takes its name from its appearance as it unfolds under the microscope: Within minutes, cells undergoing apoptosis shrink and shed tiny, membranous blebs that neighboring cells quickly gobble up, mirroring Hengartner's colorful description. In contrast, during necrosis – cell death arising from injury – cells swell for hours and then burst, spraying their contents about as a chemical signal that attracts immune-system cells to fight the injurious microbe or substance.

In the late 1960s and early 1970s, researchers began gathering evidence that apoptosis occurs as part of the normal turnover and replacement of worn-out tissues in adult organisms. They discovered that apoptosis resembles suicide in some ways: Old cells actively participate in their own demise by turning on genes and making new proteins that will shortly cause their death.

Since the mid-1980s, cell biologists and geneticists have started sorting out the causes and implications of apoptosis in a wide range of animals, including humans. Last spring, they began reporting evidence of the role played in apoptosis by the cancer-causing c-myc gene – named for its initial discovery in myelocytomas, tumors consisting of tightly packed bone marrow cells.

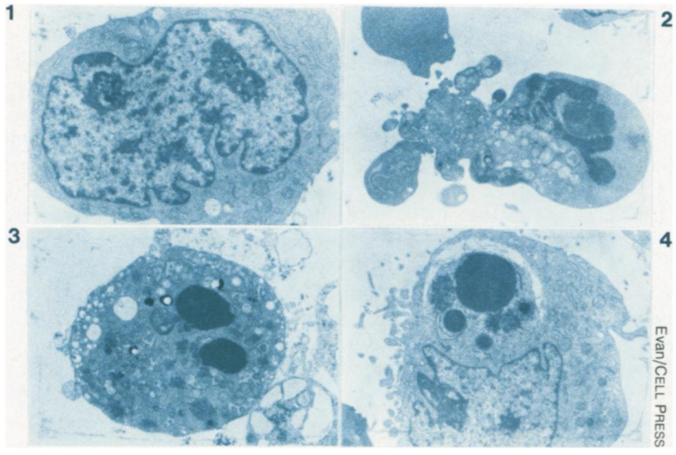
The c-myc oncogene becomes overactive in a wide range of mammalian tumors, including human cancers of the breast, bladder, colon, lung, and cervix (SN: 6/1/91, p.347). In many cases, c-myc's hyperactivity begins when a cell inexplicably creates extra copies of the gene, reproducing it over and over within the cell nucleus.

Because cells with such c-myc amplifications grow and divide nonstop – and further, because the c-myc gene encodes protein-containing regions that can bind to DNA – scientists hypothesize that c-myc regulates other genes involved in cell division. Ironically, Gerard I. Evan of the Imperial Cancer Research Fund Laboratories in London and his colleagues reported in the April 3 CELL that c-myc can also cause apoptosis under certain conditions.

Evan's group found that while laboratory-cultured cells with hyperactive c-myc genes can grow faster than cells with less active c-myc genes, they also die faster than those cells when deprived of growth medium. Moreover, the researchers noted, the cells with overactive c-myc genes died with all the visible hallmarks of apoptosis.

Evan and his co-workers conclude that c-myc functions as a two-edged sword: While it usually acts to keep a healthy cell dividing, it can also trigger cell death if outside conditions aren't right for continued cell proliferation or if the cell has become genetically damaged. In this way, c-myc can function as a built-in cellular self-destruct mechanism.

So how does c-myc cause cancer? According to a model developed by Evan and his co-workers, damage to the c-myc gene – caused either by slips in the DNA-repair machinery or by environmental injury – usually results in cell death. But some cells sustain such genetic damage and go on to develop a mutation that activates, or turns on, a second gene. This second gene somehow overrides c-myc's death command, allowing the cells to grow into tumors.



A cell undergoing programmed cell death, or apoptosis, usually dies within half an hour. First, DNA in the cell's nucleus coagulates into clumps (dark splotches, top left panel). Then, the cell dissolves, shedding blebs of membrane (top right and bottom left panels). Finally, neighboring cells gobble the dead cell's remains (bottom right panel).

Two papers in the Oct. 8 NATURE provide evidence that this second gene is bcl-2, an oncogene named for its initial discovery in human immune-system cancers called B-cell lymphomas. In the first paper, a team led by Douglas R. Green of the La Jolla (Calif.) Institute for Allergy and Immunology reports that death-prone cells containing extra c-myc genes survive much longer following insertion of an activated bcl-2 gene, which produces a protein with unknown function.

"In the absence of bcl-2, c-myc induces death," summarizes Green, "but in the pres-

ence of bcl-2, there's no death." Cancer results, he asserts, "not just because the [mutated] cells grow faster, but also because they die more slowly."

In the second paper, a team led by the Imperial Cancer Research Fund's Evan reports similar results and provides evidence suggesting that the bcl-2 mutation can help cancer cells resist the deadly effects of chemotherapeutic drugs. Many such drugs kill cancer cells by causing them to undergo apoptosis.

Evan's group administered the anticancer drug etoposide, also known as VP16, to death-prone rat cells genetically engineered to contain the activated bcl-2 gene. The researchers found that the bcl-2 gene prevented many of the cells from undergoing apoptosis and delayed its onset in others.

Further evidence that bcl-2 increases the resistance of cancer cells to chemotherapy is published in the Oct. 1 CANCER RESEARCH. Toshiyuki Miyashita and John C. Reed of the University of Pennsylvania School of Medicine in Philadelphia inserted copies of the activated human bcl-2 gene into mouse lymphoid tumor cells. They found that the genetically engineered cells survived a dose of the steroid drug dexamethasone roughly 100 times larger than that required to kill cells lacking the bcl-2 gene. Moreover, the cells resisted death induced by several other chemotherapeutic drugs, including the widely used cancer therapies vincristine and methotrexate.

The findings "may open the door to a whole new approach for the treatment of cancer," says Reed, who is now at the La Jolla (Calif.) Cancer Research Foundation. "If you could use drugs to reduce the expression of bcl-2 [in cancer cells], you might make the cells more sensitive to existing chemotherapeutic drugs," he suggests.

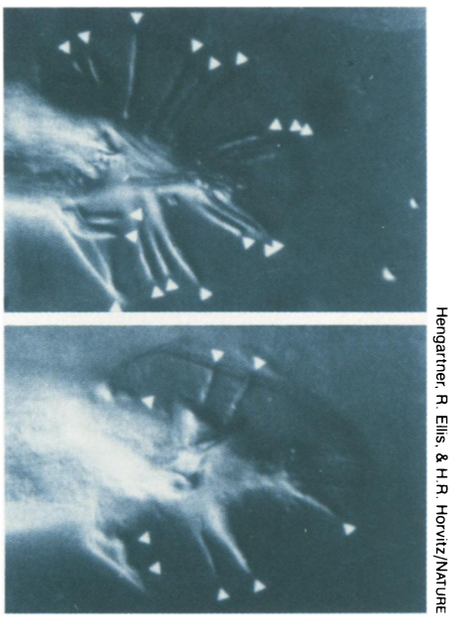
Reed and his colleagues are working with Genta, Inc., a San Diego-based biotechnology company, to develop so-called antisense drugs to block the activity of bcl-2. Antisense drugs — which consist of the same chemical building blocks that make up the genetic material DNA — turn off specific genes by binding to and inactivating messenger RNA, the intermediate compound that genes use to tell a cell to make a given protein (SN: 2/16/91, p.108).

Reed says initial tests in laboratory-cultured cells show that antisense drugs that target bcl-2 make cancer cells more vulnerable to apoptosis induced by chemotherapeutic drugs. "We're hoping to get our [bcl-2] antisense drug into clinical trials soon," says Reed. "We'd love to see if we could get it to work [in cancer patients]."

"There's a possibility that in all [the processes that turn cells cancerous] there may be mechanisms that favor cell death," adds Green. "If other genetic changes override that, you get full-scale transformation [into a cancer cell]."

In the meantime, MIT's Hengartner has found that the tiny roundworm *Caenorhabditis elegans* has a gene that resembles human bcl-2. He reported last month that the structure of bcl-2 is similar to that of a roundworm gene called ced-9, for *C. elegans* death (SN: 10/10/92, p.229). Moreover, like bcl-2, ced-9 protects cells from programmed cell death, Hengartner and his colleagues reported in the April 9 NATURE.

Hengartner says that ced-9 regulates the activity of two other genes, ced-3 and ced-4, that actually cause cells to undergo apoptosis. When ced-9 is "on," it shuts off ced-3 and ced-4, allowing a cell to live.



Hengartner, R. Ellis, & H.R. Horvitz/NATURE

The tail of a normal male roundworm has 18 appendages called rays (white arrowheads, top panel) that the worm uses to sense and position hermaphrodites during mating. A male lacking functional ced-9 genes, which prevent cell death, has only eight rays (white arrowheads, bottom panel) because some of the cells that form each ray failed to survive during embryonic development.

But when ced-9 is inactivated by a mutation, ced-3 and ced-4 start up, prompting a cell to commit suicide.

This feedback mechanism ensures that so-called stem cells in a developing roundworm die when they are no longer needed, says Hengartner. Scientists know that the minuscule roundworm generates 1,090 cells during its embryonic development. However, 131 of these cells die, so an adult roundworm consists of exactly 959 cells.

Hengartner's team has shown that roundworms with an abnormally activated ced-9 gene develop superfluous body parts, presumably because the extra 131 cells never die (see cover). In contrast, the researchers report, the offspring of roundworms lacking functional ced-9 genes die as embryos, evidently because the ced-3 and ced-4 genes functioned unchecked, killing all of the young organism's cells prematurely.

"Ced-9 is the switch between life and death" in the developing roundworm, concludes Hengartner.

Two studies published earlier this year demonstrate that the mammalian immune system may employ a similar set of cell-death genes. In the first study, a group led by Shigekazu Nagata of the Osaka Bioscience Institute in Osaka, Japan, has found that mice genetically predisposed to an affliction resembling the human autoimmune disease systemic lupus erythematosus (SLE) have defects in a protein required for apoptosis in white blood cells.

Accordingly, Nagata and his colleagues report in the Mar. 26 NATURE, the mice fail to purge themselves during embryonic development of white blood cells that attack their own tissues. As a result, the animals develop the swollen lymph glands, lethargy, and tissue damage characteristic of lupus.

The results reported by Nagata's team "are the first hint of a cell-death link with a real disease model," comments Green. "It looks like a gene that is involved in the programmed cell death process is defective in this strain of mouse with horrendous autoimmune problems."

In the second paper, which appeared in the July 10 SCIENCE, a group led by Frank Miedema of the University of Amsterdam in the Netherlands reports evidence that AIDS resembles the other side of the same coin. Miedema and his colleagues took white blood cells called T-cells from male AIDS patients. When they stimulated the cells' CD3 receptors using antibodies, up to one-fourth of the cells committed suicide by apoptosis. In contrast, the antibody treatment failed to induce significant levels of apoptosis in T-cells isolated from men not infected with the AIDS-causing HIV virus.

Miedema and his colleagues suggest that HIV infection "hyperactivates" T-cells, giving them a hair-trigger tendency toward suicide. They say this mechanism may explain why AIDS patients show a decrease in all types of T-cells, not just those bearing the CD4 receptor that HIV uses to enter and infect some T-cells.

Developments such as these signal renewed interest in the study of cell death among researchers from a variety of fields, say many biologists. "This will be a very fruitful area of research for some time to come," predicts Reed. □