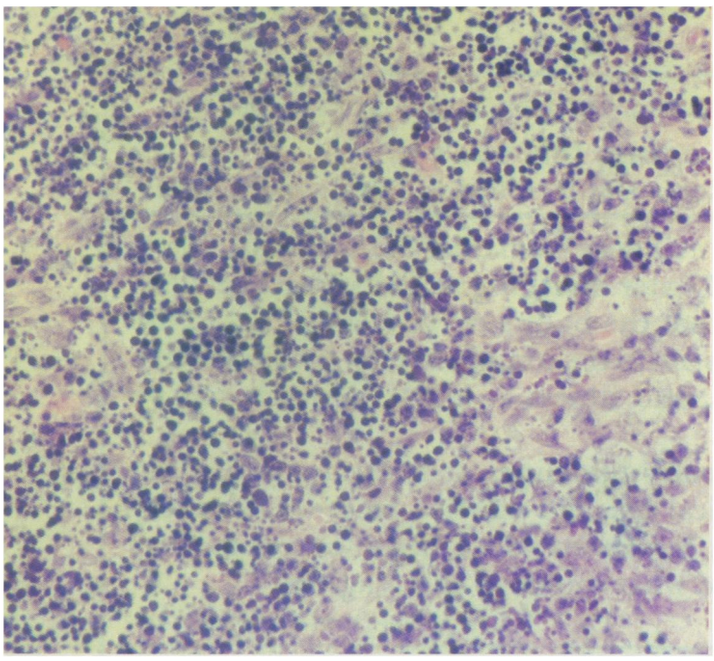

GUARDIAN GENES

How cells use genes to resolve matters of life and death

By GABRIELLE STROBEL

In the early 1970s, Andrew H. Wyllie revived talk about an obscure phenomenon called apoptosis. What the Scottish scientist didn't foresee was that his work would spawn one of today's liveliest fields of biological inquiry (SN: 11/21/92, p.344).

Apoptosis is the orderly self-destruction of cells. It can obliterate millions of cells in mammals, and scientists think it regulates the number of cells in many tissues. Tantalized by apoptosis' links to ailments ranging from cancer to autoimmune and neurodegenerative disorders, researchers in universities and biotechnology companies are racing to unravel the mechanisms that steer this dramatic event.

"It is becoming increasingly clear that the rate at which cells die is just as importantly controlled [by genes] as the rate at which new cells arise," says Craig B. Thompson, a Howard Hughes Medical Institute researcher at the University of Chicago.

During both embryonic development and adult life, many cells receive death sentences in the form of biochemical or physiological signals. In response, these cells don't just waste away, they activate a genetic death program, diligently manufacturing proteins they will use to dismantle themselves. Normally, adjacent cells promptly devour the dead cell's remains, removing all evidence of the process — which explains why apoptosis went largely unnoticed until Wyllie took a closer look.

For several years, scientists suspected that genes orchestrated this programmed cell death while at the same time keeping a tight rein on it. A mammalian death-inducing gene was discovered only last November. As yet, researchers know little about this "killer" gene, but they have learned a lot about a "savior" gene, discovered roughly a year earlier (SN: 10/10/92, p.229). This human gene, bcl-2, counters some cells' programmed demise.

"The identification of bcl-2 brought big insight to the field," says Thompson. "Bcl-2 prevents the cell from inducing its suicide program — at the time, that seemed to be a great explanation" for how genes direct apoptosis.

Yet bcl-2 raised as many questions as it answered. Just how important is it during development? Is it really a universal foe of cell death? How does it protect cells? Does it act alone or as part of a gene "squad?"

While programmed cell death occurs in many different parts of the body, it plays one of its most important roles in the maturation of the immune system. How bcl-2 fits into that process, though, may be more complicated than scientists thought a year ago, argues Dennis Y. Loh in the Sept. 17 SCIENCE. Loh is an immunologist and Howard Hughes Medical Institute researcher at Washington University in St. Louis.

In embryonic and newborn mammals, the bone marrow churns out millions of immune-cell candidates. Before being accepted into the immune system, however, these nascent cells are purged in the thymus. There, several waves of apoptosis do away with cells that might turn against the body and cells deemed benevolent but useless, explains Loh. Only immune cells, or lymphocytes, that recognize the body's molecular signature but don't attack the body are chosen to join the army of pathogen-fighting cells.

If the sorting goes awry, the result may be autoimmunity or other major health problems, says Loh. "Much of basic immunology revolves around these selections."

How the thymus tells good from bad is not clear, but recent studies have suggested that bcl-2 may be involved. These studies show that the gene was shut down in immature, death-prone lymphocytes but turned on in long-lived, mature cells that patrol the body.

"Several groups correlated these observations and proposed that bcl-2 plays a critical role in lymphocyte development," says Loh. "There were no hard data for that link, so we decided to test it by getting rid of bcl-2." If the gene is indispensable to lymphocyte survival, there should be no such cells without it, he reasoned.

Loh's group created mice that carried a defective bcl-2 gene in their immune system cells. "Surprisingly, these mice initially developed a seemingly normal repertoire of lymphocytes and thus disproved the hypothesis that bcl-2 is absolutely necessary for early development," holds Loh.

Weeks later, though, the mouse immune systems foundered, indicating that they need bcl-2 to function. At around 4 weeks of age, the lymphocytes began dying off prematurely and the production of new lymphocytes fell precipitously.

Hard on the heels of that report followed a second describing another strain of genetically altered mice. Raised in the laboratory of Stanley J. Korsmeyer, also a Howard Hughes researcher at Washington University, these mice lacked the bcl-2 gene in all of their organs, not just the immune system.

These animals also appear normal at birth, Korsmeyer and his colleagues wrote in the Oct. 22 CELL. That finding extends to all mouse organs Loh's conclu-

***Above:** Most lymphocytes of bcl-2-deficient mice die several weeks after birth. In this thymus, dead cells (dense purple spots) vastly outnumber living cells (large, light pink).*

sion that bcl-2 is not required during the early development of the immune system.

But after a week, this second strain of mice also began to show problems. They suffered such sudden and severe apoptosis that "immune organs, such as the thymus and the spleen, practically dissolved," Korsmeyer says.

Besides crippling the immune system, the lack of bcl-2 caused kidney disease, which killed many mice after several weeks.

For the roughly 25 percent that survived those initial onslaughts, another surprise was in store. These mice suddenly turned gray, almost as though trapped in an accelerated aging process.

The first grizzled black or brownish coats of the bcl-2-deficient mice seemed normal enough. But during puberty, when mice grow new hair, gray patches appeared. About three weeks later, the piebald creatures were completely gray.

Exactly what goes wrong in the hair follicles of these mice eludes Korsmeyer and his co-workers. Logic suggests that manufacturing enough hair pigment somehow depends on bcl-2. The biochemical synthesis of that pigment generates plenty of cell-damaging free-radical molecules, notes Korsmeyer, hinting that in normal mice, bcl-2 might help detoxify these hazardous by-products.

David M. Hockenbery, a former member of Korsmeyer's team, examined this idea in detail, intrigued by the finding that most of the protein made from bcl-2 resides in the membranes of mitochondria. These tiny power plants inside cells host biochemical reactions that produce free radicals.

"We wondered what . . . mitochondrial functions might have to do with apoptosis," recalls Hockenbery, now at the Fred Hutchinson Cancer Research Center in Seattle. Learning how bcl-2 can safeguard cells may also shed some light on another basic mystery of apoptosis — the cellular dysfunction of which doomed cells ultimately die.

If bcl-2 rescues cells by siphoning off free radicals, then antioxidant chemicals should be able to do the same, Hockenbery and his colleagues reasoned. Indeed, some antioxidants blocked apoptosis almost as well as bcl-2 does, they wrote in the Oct. 22 CELL. Moreover, cells with added bcl-2 withstood the ravages of artificially increased concentrations of free radicals better than cells not so modified.

In a separate study in the Nov. 19 SCIENCE, a research team led by Dale E. Bredesen of the University of California, Los Angeles, reports similar findings.

Hockenbery and his co-workers also found that lipid peroxidation — a type of damage perpetrated by rampant free radicals — occurs in dying cells. When testing for lipid peroxidation, which leads to membrane rupture, "we found that it happens early on in apoptosis and progresses dramatically when the classical symptoms of apoptosis appeared," Hockenbery said. "We thought that was pretty strong evidence that free radicals could have early effects in the [destruction] process and are not just a secondary phenomenon."

But exactly where does bcl-2 come into play? Hockenbery says that rather than throttle down the generation of free radicals in the first place, the bcl-2 protein seems to stop them from wreaking havoc in the cell — at least in the examples of apoptosis they studied. In contrast, Bredesen's group, using a different approach, noted a decrease in free-radical formation as well as damage control.

"Currently, we are trying to find out whether [protection from free-radical damage] also happens in other models of cell death," Hockenbery says. "Maybe this is a general mechanism."

A general mechanism is sorely needed. In recent years, numerous groups have observed programmed cell death in a bewildering variety of cells and physiological situations. For instance, electric stimulation spells death for some cells, while exposure to hormones sounds the death knell for others. Still others die when denied various types of life-support molecules, such as nerve growth factor or interleukins.

"We are all looking for a unifying theme that might funnel all these different models into a common death pathway," says Hockenbery. He and his co-workers speculate that — no matter what the specific death signal or cell type — once a cell is bent on dying, it does so via free radicals, turning loose a chain of ruinous chemical reactions that bcl-2 can counter.

However exciting, bcl-2 isn't the only player in the drama of cell death. Some researchers have scratched their heads from the outset over why this gene rescues some cells but not others. Similarly, if bcl-2 didn't influence the selection of suitable immune cells, as Loh's and Korsmeyer's groups suggest, what did?

Several newly discovered genes suggest that cells contain at least two independent pathways to death. One of these genes, bcl-x, may block a different pathway from that blocked by bcl-2, report Thompson and his colleagues in the Aug. 27 CELL.

What's more, bcl-x seems to be a double-edged sword. When expressed in one form, it hinders cell death "at least as well as bcl-2 does, if not better," says Thompson. "In contrast, when expressed in the [other] form, bcl-x directly inhibits bcl-2's ability to keep the cells from dying."

As described in CELL, the life-prolonging form of bcl-x appeared in long-lived cells, such as neurons in the brain, while the other form occurred in short-lived cells, such as immature lymphocytes.

"These data fit a very nice pattern in humans, but in mice it doesn't seem to be that simple," Thompson cautions. "The body clearly has rules on how to regulate these genes in a subtle way, but we don't know these rules yet."

Another study indicates that imbalances in the concentrations of proteins antagonistic to each other can decide a cell's fate. A protein dubbed bax can bind to bcl-2 and thus suppress its action, scientists led by Korsmeyer reported in the Aug. 27 CELL. They suggest concentration-dependent interactions in which, as in a tug-of-war, a prevalence of bax would push a cell over the cliff, whereas a prevalence of bcl-2 would save it.

The genetic codes of bcl-x, bax, and two other recently discovered genes all resemble the code of bcl-2, pointing to the existence of an entire family of genes that together determine a cell's destiny. "I suspect we will find a complex cascade of gene and protein interactions doing this," notes Thompson.

Researchers are only beginning to work out these interactions, sorting through a web of sometimes confusing and irreproducible results. Not to worry, says Loh, "that's how science works — if it is definitive, it's not interesting." □

Deborah Veis *et al.*/Washington University

In puberty, gray spots (left) first appear in mice lacking bcl-2. Two weeks later, the mice have turned completely gray.