Oncogenes Reach a Milestone

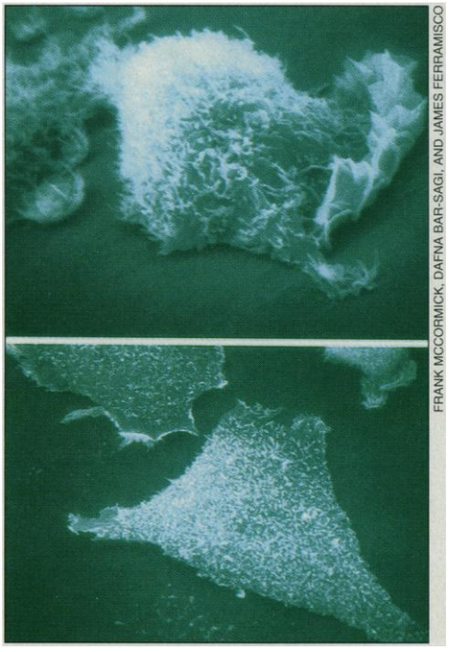
In the 20 years since genes with oncogenic potential were discovered in cells, the research has grown explosively, extending far beyond cancer causation to normal cell biology

A little more than two decades ago, researchers who wanted to understand the molecular basis of cancer were operating largely in the dark. There was a plethora of theories to explain how normal cells become cancerous, but little experimental evidence to support any of them. "You name it, there was a theory," recalls an early worker in the field, Steve Martin of the University of California, Berkeley. But a rapid series of developments was about to bring light to the darkness.

In 1970, researchers showed that Rous sarcoma virus (RSV), which causes cancers called sarcomas in chickens, carries a gene that can transform a normal cell into a cancerous one. Then in work done 5 years later (but published in 1976), Michael Bishop, Harold Varmus, and their colleagues at the University of California, San Francisco, showed that this supposedly viral "oncogene" is present in normal cells—indicating that normal cellular genes, somehow gone awry, hold the solution to the mystery of cancer. Bishop and Varmus's discovery—for which they shared the 1989 Nobel Prize—was a "startling revelation," says Inder Verma of the Salk Institute, and to celebrate that revelation, Verma has co-organized a 20th birthday party for the oncogene, to be held next month under the auspices of the famed Keystone symposia.

This will be a birthday party with much to celebrate. In the past 20 years, some 70 positively acting oncogenes have been identified. Researchers have also identified about a dozen tumor suppressor genes whose loss or inactivation may also contribute to cancer development, the most recent example being the long-sought breast cancer susceptibility gene *BRCA1* (*Science*, 7 October 1994, p. 66). And that followed hard on the heels of the discovery that a common hereditary form of colon cancer is caused by defective DNA repair genes, a finding lending further credence to the idea that cancer is fostered by gene mutations (see "DNA Repair Works Its Way to the Top" on p. 1926). "In 1994, there's incontrovertible evidence that cancer is a genetic disease," says Varmus.

But the impact of these remarkable and rapid developments extends far beyond cancer to cell biology generally. "We've uncovered a gold mine" is how one longtime oncogene researcher, George Vande Woude of the National Cancer Institute–Frederick Cancer Research and Development Center, puts it. Researchers have found that the pathways by which cells recognize and respond to growth factors, hormones, and other regulatory molecules are liberally paved with the normal protein products of oncogenes. Consequently, oncogene research is now illuminating areas of biology that at one time were thought to be completely unrelated to cancer genetics, including developmental biology, neurobiology, and immunology.



FRANK MCCORMICK, DAFNA BAR-SAGI, AND JAMES FERRAMISCO

Subduing Ras. Inhibiting Ras makes transformed cells *(top)* become more like normal.

Rousing start

Although the evidence that cancer is a genetic disease is now incontrovertible, that wasn't the case when Bishop and Varmus began working together in 1970. At that time, researchers had suspicions that genetic mutations might play a role in human cancers, but there was no direct proof for the hypothesis. Some researchers thought the uncontrolled growth and other characteristics of cancer might not be due to permanent gene mutations but to "epigenetic" changes affecting other cell components. Proteins might be damaged or altered after being synthesized, for example, or the cell's energy-producing machinery might be deranged.

The RSV studies changed all that. Beginning in about 1970, researchers including Martin, Peter Vogt, then at the University of Southern California in Los Angeles, and Peter Duesberg, also of Berkeley, obtained evidence from RSV mutants that the virus carries a gene, called *src* (for sarcoma), that is responsible for RSV's ability to make cells cancerous.

But what really got the world of cancer research excited about *src* was Bishop and Varmus's Nobel Prize–winning discovery, made with Vogt and Dominique Stehelin, then a postdoc of Bishop's, that chicken cells carry their own *src* gene. This meant that the cancer-causing gene had been in the avian cells to begin with, and that RSV had picked it up from infected chicken cells. This discovery was crucial, says Salk's Verma: "For the first time, it told us that the cell contains genes that have oncogenic potential."

Even more intriguing for understanding biology generally was the finding from the same researchers that mammalian cells also carry the *src* gene. These studies showed that the structure of the gene had been highly conserved during evolution, indicating that the gene in its normal state has an important cellular function.

The discovery of the cellular *src* gene raised two separate, but related, questions: What does the gene do normally, and how does it malfunction to cause cancer? Efforts to answer the first question pointed the way to important insights into how cell division is turned on and off in the healthy organism. As Philip Leder of Harvard Medical School in Boston notes, "The beautiful thing [about the oncogene work] is that it's taught even more about cell biology than it has about cancer."

One of the "beautiful things" to emerge from analysis of the normal function of the *src* gene was a whole new class of important regulatory enzymes: the protein tyrosine kinases. The first clue to their existence came from Ray Erickson's group, then at the University of Colorado, Boulder, as well as from the Bishop and Varmus group, both of which reported in 1978 that the protein made by the *src* gene is a protein kinase, an enzyme that adds phosphate groups to other proteins. And that step pointed the way to a fundamental insight into how some cellular oncogenes normally function. "Finding that Src

was a kinase really introduced the concept that this was a regulatory protein," says Joan Brugge of ARIAD Pharmaceuticals in Cambridge, Massachusetts, who worked on Src as a postdoc in the Erickson lab.

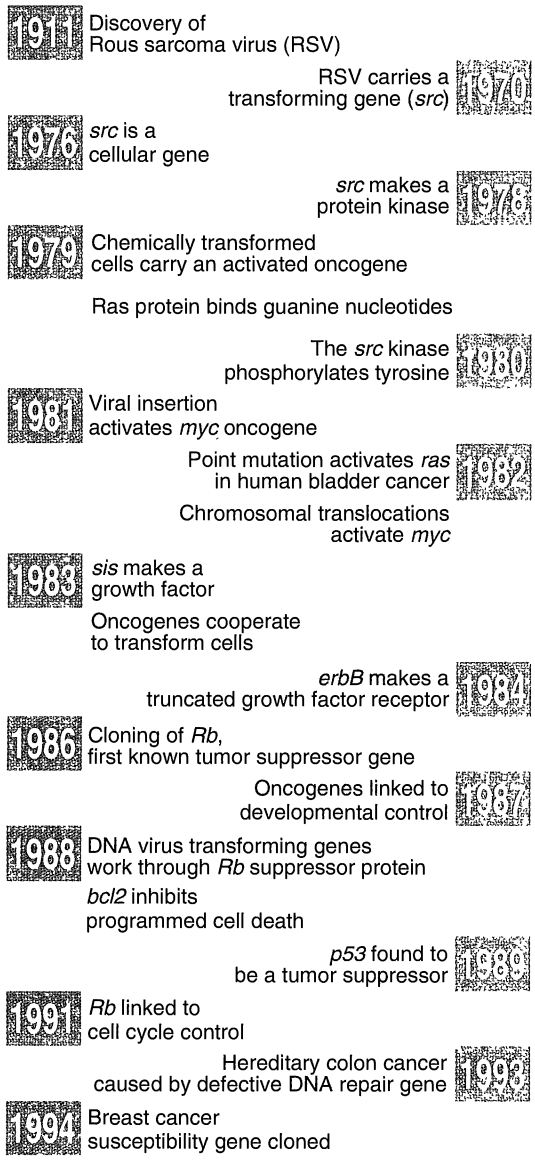
Brugge was referring to the fact that such phosphate additions had already been shown to be an important way that enzymes control the activities of their target proteins. But there was a twist to the way Src carries out this function. At the time, the other known protein kinases added phosphates to residues of the amino acids serine and threonine. But in 1980, Tony Hunter and Bart Sefton of the Salk Institute found that Src puts its phosphates on another amino acid—tyrosine. This discovery was made serendipitously, says Hunter, because he happened to use some outdated buffer for the experiments. The *pH* of the "aged" buffer had dropped from 1.9 to 1.7, which allowed the phospho-tyrosines to separate from the phospho-serines and therefore be detected. Despite this accidental beginning, "everybody was very excited" to find that Src protein was a tyrosine kinase, says Verma. "It was another handle for identifying transforming genes as something special."

And, even before Hunter and Sefton's discovery, researchers in the field suspected that the cellular oncogenes would be special because they are crucial regulators of normal cell growth. But direct proof of that hypothesis did not come until 1983, when two teams independently demonstrated that the *sis* gene, which was originally found in simian sarcoma virus, encodes part of a growth factor, in this case, platelet-derived growth factor. That was followed a year later by the discovery that the *erbB* transforming gene (from avian erythroblastosis virus) encodes a truncated growth factor receptor with tyrosine kinase activity. Those discoveries "really gave the field a tremendous conceptual boost," says Bishop, because they put oncogene products directly on the cell's growth control pathways.

Once placed on those pathways, oncogene products assumed a progressively larger role. Of the 20 or so oncogenes identified so far that encode tyrosine kinases, some produce receptors for picking up growth factor signals and transmitting them into the cell. Others of the protein products, including Src, work just under the membrane or in the cytoplasm to transmit growth-factor signals. In fact, the proteins encoded by the great majority of the 70 or so known oncogenes have proved to be components of the cell's growth and other regulatory pathways, acting all the way to the nucleus, where some are transcription factors that regulate gene activity.

As knowledge of the role of oncogenes in the normal cells was expanding, parallel efforts to find out what causes the *src* gene of RSV to misbehave and make cells cancerous were also paying off. One early clue to the difference between the normal gene and its transforming counterpart was the finding that the viral gene is much more active than the normal cellular gene at producing its Src protein. In addition, structural comparison of the viral and cellular genes by Hidesaburo Hanafusa's group at Rockefeller University revealed that the viral gene had undergone mutations that altered the protein, making it a more effective tyrosine kinase. Together, these quantitative and qualitative changes provide a powerful growth stimulus to cells infected with RSV.

Still, there is an irony to the *src* story. Despite its seminal influence on the development of the oncogene field, "*src* itself is not so heavily involved in human oncogenesis," Hanafusa points out. That role has fallen to other oncogenes identified in the flood tide of the last two decades—especially the one known as *ras*.



Some cancer gene milestones. While they are too numerous to list them all here are a few.

Making the link

The *ras* oncogene has had a major influence on the field, in part because it was one of the first oncogenes to be linked directly to human tumors. A key step on the way to making that link came in 1979, when Robert Weinberg's team at the Massachusetts Institute of Technology showed that cells transformed by a chemical carcinogen carry an active oncogene, suggesting that oncogenes were a target for the chemical. Because the cells had never experienced a viral infection, the result also suggested that oncogene activation might be a common theme in cancer development, not just in virally induced tumors. Indeed, Weinberg characterizes this discovery as "by far the most important thing that I have done in my career."

Later work by several groups, including Weinberg's, Michael Wigler's at Cold Spring Harbor Laboratory on Long Island, and Marianno Barbacid's at the National Cancer Institute, showed that *ras* is activated in both chemically transformed cells and human bladder cancer cells, thus making the link to a specific tumor. And it doesn't take much to activate the oncogene—a change in a single amino acid will do. "Finding that *ras* genes were mutated in human tumors was a major step forward. It was an indication that oncogene mutations might be important in human tumors," says Hunter. Since then, researchers have amassed evidence that *ras* is the most commonly activated oncogene, contributing to the development of perhaps 30% of human cancers, including such common ones as colon, bladder, and pancreatic cancer.

While work done in the 1980s showed that a change in a single amino acid is enough to turn *ras* on, researchers have found that there are several other ways of activating oncogenes. One involves the chromosomal abnormalities called "translocations," in which two chromosomes exchange segments. In 1982, several teams found that the *myc* oncogene is located at or near the translocation breakpoints in cells of the cancer called Burkitt's lymphoma; this shift in the oncogene's position leads to its activation.

And that isn't the only way translocations can lead to creation of an active oncogene. Also in 1982, researchers found that the *abl* oncogene is located at a translocation breakpoint in certain leukemia cells. Subsequent work showed that *abl* had fused with another gene, *BCR*, at the breakpoint, resulting in production of an ab-

Rapid Growth Can Influence a Field's Sociology, Too

The explosive growth of oncogene research hasn't just changed science's understanding of cancer. It's also dramatically changed how science gets done in the field—and some of those changes cause some of the current leaders to worry about the next generation of young researchers.

As far as the current generation goes, the influence of oncogenes has been so great that many early workers in the field have been catapulted into totally new areas of research—such as gene control, or developmental biology, in species ranging all the way from yeast and the fruit fly *Drosophila melanogaster* to mammals. One consequence of this branching effect, says the Salk Institute's Inder Verma, whose work includes oncogenes and gene therapy, is increased cooperation, as investigators have to seek advice and reagents from researchers in other fields. "Ira Herskowitz [of the University of California, San Francisco] works on yeast, [Berkeley's] Gerry Rubin works on *Drosophila*," Verma says, "and all of us now sit down in the same room" to discuss our research.

Cooperation across disciplinary boundaries is stimulating. Still, working in a large and growing field also carries frustrations. Several researchers interviewed by *Science* who were in the field when it was small expressed regret that it's grown so big that they can't keep up with it all. Says *src* pioneer Steve Martin of Berkeley: "One has to work on a much smaller aspect of the field. This is rather frustrating. I would like to work on everything involved with *src*."

The size of the field also means it's easier to be scooped, notes the U.K. Medical Research Council's Terrence Rabbitts. When he started, he says, "you knew who your rivals were and the kinds of things they were doing," and so could avoid duplicating their efforts. "Nowadays," he says, "everything you are doing replicates what someone else is doing."

Harold Varmus, director of the National Institutes of Health since 1993, worries that the sheer size of the field now could inhibit researchers, especially young ones, from taking a chance on exploring uncharted waters. In his talk this year at the Tenth Annual Oncogene Meeting in Frederick, Maryland, he said: "In the areas of light, there is so much to do, and with so much light, it's difficult to be attracted to areas of darkness—areas where the most remarkable discoveries are waiting to be made."

– J.M.

normal fusion protein.

Cancer researchers didn't need any more than those hints to see that translocation breakpoints were a rich field for study, and focusing on them has "allowed a whole range of other oncogenes to be identified that weren't previously known," says Terrence Rabbitts of the MRC Laboratory of Molecular Biology in Cambridge, U.K., who was an early contributor. Those involving fused genes, which are cropping up in solid tumors as well as in the leukemias and lymphomas, are particularly interesting, he adds, "because these create proteins that don't exist outside the tumor and might be good targets for therapy."

Tumor suppressors move ahead

As researchers link more and more oncogenes to human cancer and study their mechanisms of action, they have begun creating a picture—at least in broad strokes—of how human cancer is caused. "We're very close to having a framework for understanding how a normal cell becomes malignant," says Cold Spring Harbor's Wigler, although he hastens to add that "we're not quite there." One essential piece of the sketch that has only recently begun to be filled in concerns the tumor suppressor genes. These genes are, in a sense, the "opposite numbers" of oncogenes, because their normal role is to inhibit, not stimulate, cell growth.

For most of the 20-year history of oncogenes, they have overshadowed the tumor suppressors. [The first known tumor suppressor, the retinoblastoma gene (*Rb*), wasn't cloned until 1986, for example.] But in recent years the suppressor genes have come into their own, with demonstrations that one of them—*p53*—is mutated in up to 50% of all human cancers, as well as discoveries that a series of inherited cancer susceptibility genes encode tumor suppressors.

As the tumor suppressor studies merge with two decades of intense research on oncogenes, the developing picture suggests that cancer develops as the result of a series of genetic insults over time, some of which turn on oncogenes, while others knock out tumor suppressors. This view fits neatly with a great deal of evidence that had been previously acquired in carcinogenesis and epidemiological studies indicating that cancer is a multistage disease that takes many years to develop.

The last two decades have been a wild and exciting scientific ride in the cancer research community. And there is clearly a great deal to laud at the upcoming birthday party. But one sobering note, almost all in the field say, is that despite the remarkable progress made in understanding the genesis of cancer, the work so far has had little impact in the clinic. "The disappointment has been the lag between the understanding of basic mechanisms and applications," says Martin.

"It's been a real blast over the past 10 years, pulling out all the genes and figuring out what they do. But the next challenge will be to put the information to work," agrees Frank McCormick of Onyx Pharmaceuticals in Richmond, California, one of the new biotechnology companies that have sprung up to tackle the problem. And that challenge could well occupy the agenda for the next 20 years of oncogenes.

–Jean Marx

SOME ONCOGENES AND THEIR FUNCTIONS

Oncogene	Product Location in Cell	Function
sis	Secreted	Growth factor (PDGF)
erbB	Cell membrane	Part of epidermal growth factor receptor
neu/c-erbB2	Cell membranes	Protein tyrosine kinase
src	Cell membrane	"
raf/mil	Cytoplasm	Protein serine kinase
ras	Cell membrane	GTP binding/GTPase
myc	Nucleus	Transcription factor
fos	Nucleus	"
jun	Nucleus	"
bcl-1 (cyclin D1)	Nucleus	Activates cell cycle kinases
bcl-2	Internal membranes	Antagonizes programmed cell death

Additional Reading

H. E. Varmus, "Oncogenes and retroviruses I," in *Les Prix Nobel: The Nobel Prizes 1989* (Almqvist & Wiksell International, Stockholm, 1989), pp. 194–211.

J. M. Bishop, "Oncogenes and retroviruses II, *ibid.*, pp. 220–238.

T. H. Rabbitts, "Chromosomal translocations in human cancer," *Nature* **372**, 143 (1994).

J. A. Witkowski, "The inherited character of cancer—An historical survey," *Cancer Cells* **2**, 229 (1990).