Cooperation Between Oncogenes

Investigators focus on cooperation between two or more oncogenes to help explain the multistep development of human cancers

Research on oncogenes has taken a new turn within the past few months, as investigators have begun to focus on requirements for oncogene cooperation in the development of malignancies. The results may help to bridge the gap between what clinical experience has taught, namely, that human cancers develop slowly, in many steps, and laboratory experience with the 20 or so known oncogenes that appear to cause malignant transformation in a single step.

A recent example of possible oncogene cooperation, which is being investigated by Philip Leder and Kathleen Kelly of Harvard Medical School and Brent Cochran and Charles Stiles of Harvard's Dana-Farber Cancer Institute, was reported at the annual meeting of the Institute of Medicine, held in Washington DC on 26 October. Leder described new data indicating that one of the oncogenes, which is designated *myc* because it was first identified in the virus MC29, is turned on by growth factors, including platelet-derived growth factor (PDGF).

Earlier this year, PDGF was found to be at least closely related, if not identical, to the product of another oncogene, this one called *sis* because it was first found in simian sarcoma virus (*Science*, 15 July, p. 248). The results described by Leder in Washington show how two potential oncogenes might cooperate in the normal growth of a cell and how they might act on one another in malignant transformation.

Myc and many of the other oncogenes were originally identified in viruses that cause cancers in laboratory animals. Subsequent study showed that the oncogenes were derived, in whole or in part, from cellular genes that had somehow been picked up by the viruses during the course of infection. This raised the question of how the cellular genes, which probably help to regulate normal cell division and differentiation, might become activated to produce the uncontrolled growth and abnormal differentiation characteristic of cancer. Such activation may occur not just when the cellular sequences are acquired by appropriate viruses, but also in the cell itself.

The activation of *myc* often appears to result from altered control that causes increased expression of the gene product. But, Leder notes, although the expression may be greatly increased in some tumor cells, in others it is little higher than in normal cells. "It isn't enough to convince us that this alone is responsible for transformation."

Leder postulates that production of the *myc* gene product at the wrong time in the life cycle of the cell may be more important for effecting transformation than the amount of product made. After cells divide, they enter a stage of relative quiescence, which is designated G1 and lasts about 10 hours. This is then followed by a period of about 9 hours during which DNA is synthesized, a prequisite for cell division.

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Leder and his colleagues took three types of cells that had been arrested in the G1 phase, stimulated their division with cell specific growth factors, and measured the effect on transcription of the *myc* gene into messenger RNA, the first step of protein synthesis.

They used two types of immune cells, T and B cells, and also fibroblasts. "If you take resting B cells or resting T cells, the amount of the *myc* message is very low," Leder told the meeting participants, "but if you hit them with a mitogen, within 1 to 2 hours the amount of *myc* message increases by an order of magnitude." These cells were stimulated with artificial, nonphysiologic mitogens. But for the fibroblasts, the investigators used PDGF. Here Leder continues, "There was a 40-fold increase in the amount of *myc* message."

The growth factor PDGF acts on receptors located on the surfaces of cells. The protein product of the *myc* gene is located in the nucleus, where it may help regulate other genes needed for cell division. The finding, Leder concludes, "links in a regulatory way two potential oncogenes, the one for PDGF and another coding for a nuclear protein that could be at the remote end of a chain that was set in motion by PDGF." It implies that oncogenes may fit in a cascading hierarchy where the action of one controls another.

Other work, such as that showing that tumor cell lines may contain more than one activated oncogene, has already suggested that two or more of the genes may cooperate in transformation. For example, Geoffrey Cooper of the Dana-Farber Cancer Institute and Paul Nieman of the Fred Hutchinson Cancer Research Center in Seattle found that DNA from chicken lymphoma cells transforms cultured cells of the NIH 3T3 line.

Although activation of the *myc* gene apparently contributes to the development of the chicken lymphoma, the transforming gene identified by gene transfer turned out not to be the *myc* gene, but a member of a new family of oncogenes that have been designated Blym because they were discovered in B cell lymphomas. Burkitt's lymphoma is another B cell lymphoma in which *myc* activation is thought to be important. Many Burkitt's lymphoma cells also carry a Blym transforming gene, according to the Cooper group.

In addition, Robert Weinberg and his colleagues at the Massachusetts Institute of Technology (MIT) have found that a human leukemia cell line and a Burkitt's lymphoma line carry an activated gene of the *ras* family in addition to the activated *myc*.

Experiments in which investigators have detected oncogenes by gene transfer to cultured cells have been criticized because the NIH 3T3 cells that are used as the recipients are already at least partially transformed. They have been immortalized and are capable of growing

indefinitely in culture. Primary cell lines, which have not been immortalized, are not transformed by transfer of a single oncogene.

However, Weinberg, with Hartmut Land and Luis Parada of MIT, and, independently, H. Earl Ruley of Cold Spring Harbor Laboratory, have recently shown that primary cells can be transformed by two genes acting in concert. The MIT workers found that primary rat embryo fibroblasts are completely transformed to malignancy by a member of the *ras* oncogene family if it is transferred with the *myc* gene. Neither of these two genes can accomplish this result separately. In a similar fashion, Ruley showed that the same *ras* gene would transform a primary line of rat kidney cells in combination with a gene, designated E1A, from adenovirus.

Adenovirus and polyoma, both of which have DNA genomes, transform animal cells in a minimum of two steps. The E1A gene and the large T (for tumor antigen) of polyoma virus are needed to immortalize the cells. Then additional viral genes confer the characteristics of complete transformation and tumorigenicity on the immortalized cells.

The growing view is that transformation generally requires the activation of at least two oncogenes. "The results suggest," Weinberg says, "that the reason why carcinogenesis is multistep is a requirement for activating sequentially multiple genes." Some of the genes, including *myc* and E1A, appear to be needed for immortalization, whereas others, including *ras*, work later in the transformation pathway. The proposed role for *myc* in immortalization is consistent with the Leder group's finding that this gene is turned on by growth factors, implying that the *myc* product acts to facilitate cell division in some fashion.

In addition, chemical carcinogens that immortalize cultured cells may pave the way for the later action of oncogenes, according to Robert Nerbold and Robert Overell of the Institute of Cancer Research, Pollards Woods Research Station in Chalfont St. Giles, England.

Although investigators have shown that two oncogenes can collaborate to transform primary cells in culture, the results do not necessarily mean that just two events are sufficient. Additional, as yet unidentified, steps may also be required. Nevertheless, the experiments on oncogene cooperation begin to address some of the complexities of how gene changes may contribute to the multistep development of cancer.

—JEAN L. MARX

Isotopes Add Support for Asteroid Impact

Osmium isotope analysis supports an asteroid impact 65 million years ago but cannot exclude a huge volcanic eruption

Recent analyses of osmium isotopes deposited on the earth's surface 65 million years ago support the contention that an asteroid impact contributed to mass extinctions at the end of the Cretaceous Period, including that of the dinosaurs. The analyses reported in this issue (p. 613) appear to eliminate once and for all the possibility that over millions of years geochemical processes concentrated iridium, osmium, and other exotic elements from seawater or ground water. The newly determined isotopic ratios most likely resulted from some catastrophe, presumably an asteroid impact, but they do not eliminate the possibility of a mammoth eruption that spewed iridium-laden volcanic debris derived from the mantle. Other evidence weighs against such an eruption, but a consensus has not formed yet on that question.

High concentrations of iridium, osmium, and other platinum group metals in the sediments deposited at the boundary between the Cretaceous Period and the subsequent Tertiary Period prompted the suggestion that an asteroid struck the earth and caused mass extinctions at the end of the Cretaceous. Luis Alvarez, Frank Asaro, and Helen Michel of Lawrence Berkeley Laboratory and Walter Alvarez of the University of California at Berkeley began the current debate over the Cretaceous-Tertiary extinctions in 1980, when they argued that the iridium in the boundary layer must have come from an impacting body because no geological process had ever managed to bring much iridium from the iridium-rich mantle to the surface. Crustal rocks contain very little iridium, they noted, and meteorites are rich in the element. Skeptics wondered whether geochemical processes might have concentrated the dilute iridium supplied by crustal rocks and cosmic dust drifting down from space (*Science*, 20 November 1981, p. 896). The chemistry of the sediment layer at the boundary somewhat resembled that of meteorites, the skeptics conceded, but how many ways are there to make a sediment layer that chemically resembles meteorites?

Geochemical reactions cannot separate one isotope of a heavy element from another the way they can separate and enrich different elements, so researchers are turning to isotopic analysis for less equivocal tests of the impact hypothesis. Jean-Marc Luck and Karl Turekian of Yale University, whose report appears in this issue, chose to measure the ratio of osmium-187 to osmium-186. Like elemental ratios, this isotopic ratio does change. With time, rhenium-187 decays radioactively into osmium-187. And geochemical processes do change the proportion of rhenium to osmium and thus ultimately give different rocks different osmium isotope ratios. But this ratio should still provide a good test, Luck and Turekian reasoned. The present typical osmium isotopic ratio of meteorites is about 1 and that of continents is thought to be about 10, an easily distinguished difference. Rhenium decays exceedingly slowly—it takes 46 billion years for half of the rhenium-187 atoms initially present to decay to osmium-187.

Luck and Turekian's first problem was to demonstrate that if geochemical processes had indeed concentrated osmium, the osmium still carried the high isotopic ratio characteristic of the continents. To do that, they measured the osmium isotopic ratio in deep-sea manganese nodules, which can concentrate dissolved metals from seawater. The osmium-187/osmium-186 ratio in seven manganese nodules from the major ocean basins of the world ranged from 6.0 to 8.4, lower than expected for a purely continental source but well above the value of about 1 for meteorites.

Presumably, in addition to the osmium they received from the continents, re-