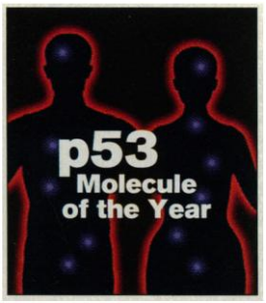
p53 Sweeps Through Cancer Research

A once-obscure molecule takes center stage, as cell biologists and oncologists team up to unravel the secrets of the ultimate tumor suppressor



Back in 1979, when p53 was discovered, almost no one would have nominated it for Molecule of the Year or even of the month. First identified in association with tumor-causing viruses that don't cause cancer in humans, this 53-kilodalton protein was simply one molecule among many, a discovery that might, or might not, be important in cancer.

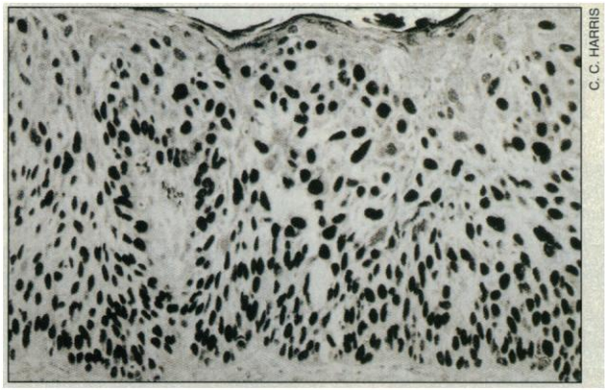
Now, in a classic parable of how basic research can have a profound impact on human disease, this dark horse molecule is blazing a bright trail in cancer research. Found in both inherited and spontaneous cancers, *p53* is to date the most commonly mutated gene in human tumors, and is one of the star members of the tumor suppressor gene family. Of the 6.5 million people diagnosed with cancer each year worldwide, about half have *p53* mutations in their tumors.

The "p" stands for protein, but it could also stand for "prevention," because in normal cells, the actions of *p53* and its protein product help prevent cancer, a property identifying the gene as a tumor suppressor. Sometimes called the "guardian of the genome," *p53* is a leader in the body's anti-tumor army, helping to coordinate a complex system of responses to the DNA damage that might otherwise lead to cancer. Like an emergency brake, wild type *p53* can halt cell growth, or, in some cases, send a cell into a programmed spiral of death. Yet when mutated, *p53* can be doubly dangerous: Mutant forms not only deprive cells of the wild type's beneficial effects but can spur abnormal cell growth.

Picking a single year in which to highlight this gene is unusually difficult, since key discoveries have unfolded rapidly every few months for the past 4 years. But it's clear that in 1993, *p53* research acquired unstoppable momentum. The number of papers has doubled each year since 1979; the 1993 total may hit 1000, as oncologists, cell biologists, virologists, and epidemiologists find common ground in this singular molecule.

From this explosion of research, we offer a few highlights, chosen to illustrate *p53*'s central role in diverse areas, such as cell biology, clinical oncology, and environmental health. In 1993, *p53* linked the basic biology of the cell cycle to the process of tumorigenesis. Also this year, *p53* was found to trigger programmed cell death in response to DNA damage, a finding that has implications for chemotherapy and radiation treatments. A growing stream of papers has correlated p53 status with patient prognosis. Finally, as scientists began to unravel the chain of molecules that interact with p53, they are uncovering a series of tantalizing targets for new drug development.

The dark days. Ironically, for the first 10 years after its discovery, *p53* languished in the backwaters of research. It was thought to act only as an oncogene—a gene that actively promotes tumor growth—because scientists were inadvertently working with a mutant form. And although oncogenes were a hot topic during the 1980s, there were plenty of other genes that seemed more promising than *p53*, which was most often studied in experimental systems. Those who sought *p53* mutations in human tumors by Southern blot techniques were usually disappointed: In contrast to leading oncogenes, *p53* almost always appeared normal.


C. C. HARRIS

Malignant mutant. Nuclei of cancer cells taken from a patient show abnormally high concentrations of p53 protein, suggesting a mutant *p53* gene.

Then, in 1989, *p53*'s fortunes changed. While pursuing the genetic causes of colorectal cancer, researchers found a point mutation in *p53*—a type of mutation not detectable by Southern blots. That same year, other experiments demonstrated that only mutant *p53* promotes abnormal cell growth; the wild type gene suppresses tumors. Suddenly, *p53* became a hot ticket in cancer research.

Myriad mutations. Soon *p53* mutations were cropping up in an astonishing array of cancers. Researchers have documented more than 51 types of human tumors that carry *p53* mutations. The list reads like a catalog of body parts: bladder, brain, breast, cervix, colon, esophagus, larynx, liver, lung, ovary, pancreas, prostate, skin, stomach, and thyroid. Among common tumors, about 70% of colorectal cancers, 50% of lung cancers, and 40% of breast cancers carry *p53* mutations. *p53* is also linked to cancers of the blood and lymph nodes, including Hodgkin's disease, T cell lymphoma, and certain kinds of leukemia.

A continuing stream of clinical studies has reported that p53 status is a good indicator of prognosis. Aberrant forms of the p53 protein are correlated with more aggressive tumors, metastasis, and lower 5-year survival rates. Such reports have emerged for cancers of the colon, lung, cervix, bladder, prostate, breast, and skin.

The power of suppression. How does wild type *p53* suppress tumors? The exact mechanisms are the focus of intense research. But scientists already have demonstrated that *p53* can put the brakes on cell growth and division, push cells into a programmed self-destruct sequence, and prevent the unruly amplification of DNA. All these actions could help suppress the uncontrolled growth that can lead to tumors.

To discover how *p53* performs all these jobs, scientists have probed the protein's biochemical activity and found that p53 acts as a transcription factor, binding specifically to other genes and controlling their expression. For example, this year researchers discovered that one oncogene, *MDM2*, appears to act in a negative feedback loop with *p53*. If this loop goes awry, *MDM2* is amplified and cancer can result: Multiple copies of *MDM2* are seen in about 30% of soft-tissue sarcomas.

p53 seems so important that many assumed that organisms could not function without it. That notion was proven wrong in late 1992, when researchers created a line of genetically altered mice that produce no p53 protein at all. The "knockout" mice are born looking perfectly normal. But *p53's* role soon becomes apparent: After several

weeks, the mice begin to get tumors. By the time they're 6 months old, all have tumors or are dead.

Arresting developments. The most dramatic discovery of the year came only a few weeks ago, when several teams of scientists independently cloned a gene that carries out at least part of *p53*'s tumor suppression mission. The new gene, *WAF1/Cip1*, is turned on by *p53*, and its protein product arrests cells in mid-cycle. In one stroke, scientists had linked years of basic research on the cell cycle—most of it done in frog eggs and yeast—to *p53* and human cancer.

A complex interaction of molecules regulates the cell cycle; the chief players are proteins called cyclins and their associated enzymes, cyclin-dependent kinases. One team of scientists was following this avenue of research, seeking molecules upstream of this pathway. The other team was seeking genes downstream of *p53*. The two met in the middle, cloning and sequencing the identical gene, called *WAF1* or *Cip1* depending on who found it. (To make the story even more interesting, this gene was also cloned by researchers studying how cells age; they called it *sdi1*.)

Apparently, *p53* promotes the expression of *WAF1/Cip1*. The new gene's protein product, p21, binds to cyclin-dependent kinases and inhibits their action. That halts the cell cycle before the cell is committed to divide. Presumably, this gives the cell time to repair DNA before dividing, thus preventing replication of damaged DNA.

WAF1/Cip1 offers an alluring target for drug development, since restoring its function could suppress tumors even if *p53* is mutated. Biotech companies already are moving to exploit the possibilities.

In other *p53* news with clinical relevance this year, scientists continued to explore how human papilloma virus (HPV) and other tumor-promoting viruses make cells susceptible to cancer. HPV, which is implicated in most cases of cervical cancer, targets the p53 protein through a newly identified viral protein, called E6-AP, which is now also a target for drug design.

Suicide cells. This year, in a series of elegant experiments, scientists began to unravel a complex story of how wild type *p53* can push cells into a suicidal track—and how mutant *p53* may derail this programmed cell death, called apoptosis.

Apoptosis is part of normal development and also can be triggered by DNA damage, such as that delivered by radiation and some chemicals, including those used in chemotherapy. In 1993, several groups of scientists showed that after DNA damage, levels of p53 protein and its transcriptional activity rise dramatically. Then, in elegant experiments with knockout mice, scientists this year showed that *p53* is crucial to the apoptotic pathway induced by DNA damage.

All these results strengthened the image of *p53* as a damage-control specialist and have obvious relevance for clinical cancer treatments. Further in vitro work supported this idea, showing that in those cell types in which *p53* triggers apoptosis, mutant *p53* confers resistance to radiation and chemotherapy.

However, the situation is complex, because in some cell types, under some conditions, *p53* seems only to trigger growth arrest, not cell death. Expect more work on this clinically important question in 1994.

Molecular medicine. Thanks to the diversity of mutations, *p53* has helped open up a new field of molecular epidemiology, uniting environmental health studies and the molecular mechanisms of cancer.

Usually, *p53* mutations in tumors are simple missense mutations, which exchange one base pair for another. In the past few years, researchers have found that some carcinogens leave characteristic fingerprints in *p53*. For example, aflatoxin B_1, which contaminates the food supply in some Asian and African countries, causes the amino acid guanine to be replaced by thymine at a particular spot on the p53 protein; some carcinogens in cigarette smoke also cause a characteristic replacement. Such mutational fingerprints can offer clues to a cancer's origin (see Perspective, p. 1980).

Our emerging understanding of *p53* creates a whole crop of potential clinical applications, now being actively pursued by both academic and corporate researchers. For example, one of today's therapeutic dilemmas is the case of patients whose breast cancer has apparently not invaded nearby lymph nodes. Most studies have shown that only a small subset of these women—about 25%—will relapse. That group would benefit from additional chemotherapy, but at this point, there's no method of identifying them. *p53* is a potential biomarker now under investigation.

p53 also opens the door to new therapeutic options. One is simply to restore a good copy of the gene into human tumors. One gene therapy trial has already been approved and 14 patients with advanced lung cancer are expected to receive *p53* gene therapy next year.

Another route is to correct the mutational mistakes. The simple substitution of one amino acid appears to force the p53 protein out of its normal shape. If p53 could be coaxed back into its correct configuration, the restored protein might perform its normal duties—and keep cells from growing out of control.

The well-characterized mutations of *p53* also suggest the possibility of immunotherapy or even a cancer vaccine, which would alert the body's immune system to the mutant forms of the protein.

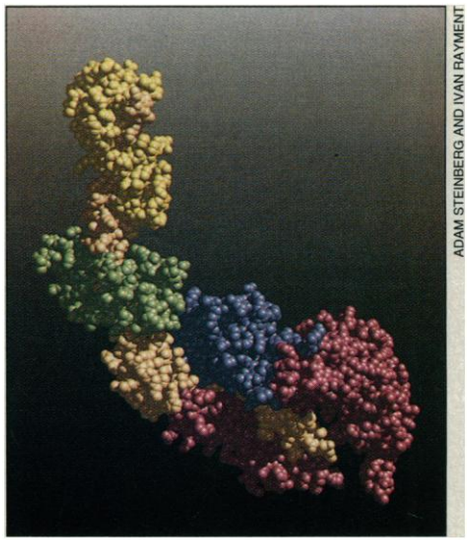
These diverse avenues of research are under active investigation in many pharmaceutical companies. Indeed the effort is worthwhile, because any therapy that restores lost p53 function would have great power to affect a diverse set of cancers.

p53 still bears an obscure label that refers only to the molecular weight of its protein product. Yet thanks to a decade of basic research, this once-modest molecule is pursued by academic and corporate researchers alike, in recognition of its major role in the development of human cancer.

And the runners up are....

In 1993, researchers explored new territory from the atom to the galaxy. Here we highlight nine discoveries—which need not be based on single molecules—that offer a new view of nature, as well as benefits for human society.

Myosin on the move. How does muscle contract? This seemingly simple question has sparked decades of research, much of it centered around the muscle filament myosin and its partner, actin. In 1993, scientists moved to a new level of understanding, by reporting the three-dimensional crystal structure of myosin at a resolution of 2.8 angstroms.



ADAM STEINBERG AND IVAN RAYMENT

Mighty molecule. The three-dimensional structure of myosin, which helps muscle contract.

Nearly 40 years ago, scientists proposed the now-classic cross-bridge model for muscle contraction, suggesting that thick filaments (myosin) and thin filaments (actin) slide past one another in a specific way.

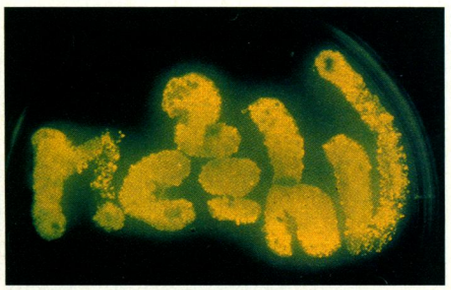
According to the model, myosin dissociates from actin, rebinds at a different angle, and then reverts to its original orientation, thus propelling the actin filament forward.

However, this model has never been proven at the molecular level, nor has such a major conformational change been detected in myosin. Now the three-dimensional struc-

ture of the myosin head, coupled with the actin structure published in 1992, provides the molecular framework needed to test a precise version of this model. For example, a detailed molecular hypothesis published this year suggests that a cleft in the myosin molecule closes during binding.

Research on other motor proteins also marched forward in 1993. The molecule kinesin, which ferries organelles about the cell, was studied with powerful new techniques. By using lasers as "optical tweezers" scientists have measured the distance traveled by a single kinesin molecule, as well as the force it exerts. The field of motor molecules is going full speed ahead.

Tackling tuberculosis. For years, TB was considered a disease of the past, linked to Victorian poets and 1920s sanitariums. But *Mycobacterium tuberculosis* is back with a vengeance, killing 3 million people per year worldwide. In the United States, cases are up 20% since 1985, partly due to TB infection in HIV+ individuals. An alarming proportion of bacteria—33% in New York City in 1991—show drug resistance.



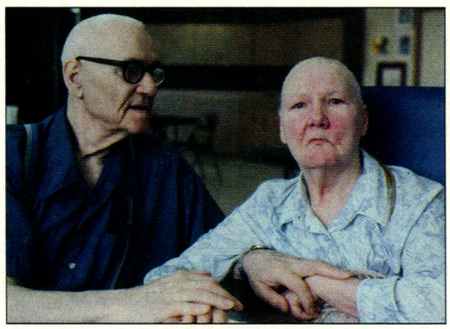
W. R. JACOBS

Shedding light on TB. New screen highlights resistant *Mycobacteria*.

In 1993, the scientific community began to develop new strategies to counter this killer. Most research on the disease stopped after the wonder drugs of the 1950s were developed, but institutions and scientists alike are again beginning to take the disease seriously. NIH's TB budget rose from $4.9 million in 1991 to an estimated $34 million in 1993.

This year, a relatively rapid screen for drug-resistant strains was developed by utilizing the gene for luminescence from the firefly as a marker: Resistant colonies light up the Petri dish. The new test will be valuable to researchers developing anti-TB compounds, as well as to physicians.

There has also been progress in identifying the protein targets of anti-TB drugs. Two years ago, none were known; today, the targets of most of the commonly used drugs are either known or under intense research. In 1993, researchers also identified the gene that allows *M. tuberculosis* to invade the immune cells of its host, a finding that may hold promise for drug and vaccine development. The scientific assault on this ancient plague has only just begun.



RICK BRADY/MEDICHROME

Understanding Alzheimer's. A radical new theory may explain the disease.

Alzheimer's upheaval. Every field of science has periods of exhilarating tumult, when new theories challenge old orthodoxies. This year marked the beginning of just such an era in research on Alzheimer's disease, which affects about 4 million Americans. In 1993, scientists found a new genetic risk factor for the disease and suggested a provocative new theory as to its cause.

For the past 5 years research has focused on the theory that a small protein called β-amyloid, which is found in large plaques amid the debris of decayed neurons in the brains of Alzheimer's sufferers, causes their neurodegeneration. Then in the spring of 1993, researchers reported surprising news: A particular form of a totally different protein called apolipoprotein (ApoE) was a genetic risk factor for late-onset Alzheimer's, the most common form of the disease. Other research teams immediately set about testing the association. It held: People who carry the gene for one form of the protein, ApoE4, are more likely to get Alzheimer's.

In November, the same team of scientists came out with a radical new hypothesis: They suggested that Alzheimer's develops because the ApoE4 variant deprives cells of their normal protection against neurodegeneration. However, the controversy still rages, and the β-amyloid theory is by no means disproven.

Signal events. When cells respond to extra-cellular signals, they receive the message at the cell membrane but often respond from the nucleus. How does the news travel through the cell? This year, researchers explored not one but two answers to that question, mapping out two nearly complete signal transduction pathways used by growth-inducing factors.

Perhaps the best-known intracellular circuit is the Ras pathway, centered around the protein product of the prototype oncogene, *ras*. In 1993, two gaps in the Ras pathway were filled by several teams of scientists. One new step involves a protein called GRB2, which physically couples activated receptors on the cell membrane to another protein called SOS; in turn, SOS is capable of activating Ras.

Other scientists filled the next gap, showing that activated Ras binds to a protein kinase called Raf. Raf then initiates a chain reaction called the MAP kinase cascade, which apparently brings the pathway to the nucleus.

The other newly mapped route from membrane to nucleus is more direct, with only two intermediate steps. Enzymes called tyrosine kinases apparently pick up the signal from receptors in the membrane. The kinases then activate latent cytoplasmic proteins, which complete the loop by moving to the nucleus and binding DNA.

Work this year showed that a number of different growth factors rely on a single protein subunit, p91, as part of the DNA-binding complex. So a surprising number of different growth inducers, such as interferons and some interleukins, use similar signalling pathways. Eventually, understanding these cellular information circuits may help correct the garbled messages that can sometimes cause cancer.

How low can you go? In 1992, most scientists thought Antarctic ozone had hit bottom. But in autumn 1993, according to balloon-borne instruments and satellite measurements, the hole sank even deeper, thinning the protective ozone shield over Antarctica to less than one-third its normal thickness.

These unusually low levels were probably due to sulfate aerosols produced by the explosion of Mt. Pinatubo in 1991. The effect is not just a polar problem: Satellite data also revealed that average total global ozone was at record lows in late 1992 and early 1993.

Ironically, 1993 was the year pundits chose to pick on what they called the ozone "hoax." But those who study the stratosphere continued to insist that there's plenty of evidence to link chlorofluorocarbons (CFCs), ozone loss, and increased ultraviolet (UV) radiation.

This year, some evidence emerged that as ozone dropped from 1989 to 1993, ultraviolet-B radiation—the kind that can

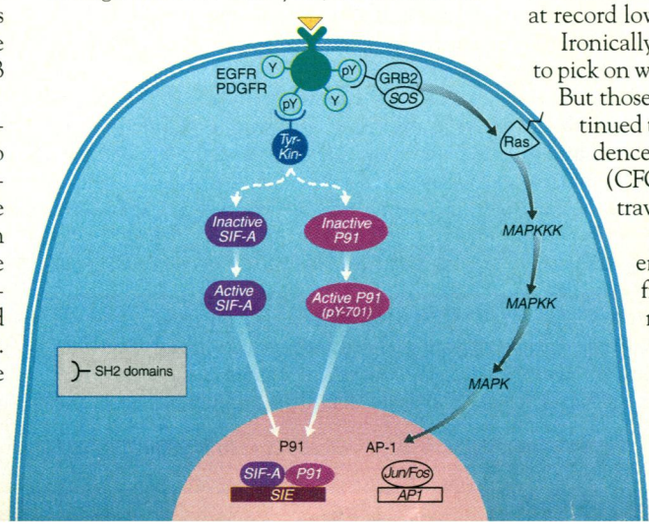


ILLUSTRATION: K. SUTLIFF

Completing the circuit. Two roads lead to the nucleus.

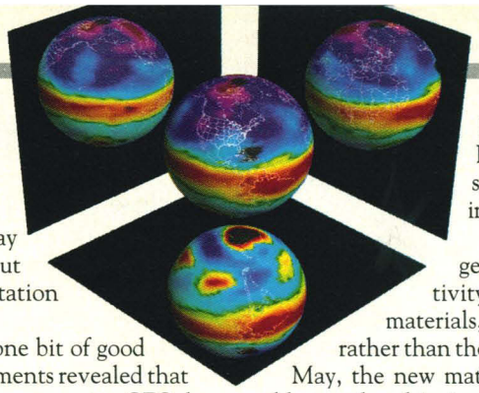


PHOTO: NASA

Global low. Stratospheric ozone sinks even lower.

cause skin cancer—may have increased by about 5% at one measuring station in Toronto.

The year brought one bit of good news: Global measurements revealed that the rate of increase in ozone-eating CFCs has slowed. If present trends continue, global atmospheric chlorine will hit a maximum sometime before the year 2000, and then slowly decline.

Supply meets demand. Since the 1980s, the anticancer drug Taxol® has had a supply problem: Its source was the bark of slow-growing Pacific yew trees. As more and more clinical data demonstrated Taxol's power against breast, ovarian, and other cancers, medical scientists needed more drug—and a more environmentally friendly source.

In 1993, a multi-disciplinary scientific effort, including unusual partnerships between foresters and pharmaceutical scientists, broadened the supply line with renewable sources.

GARY STROBEL

Healing yew. Twigs and needles supply taxol too.

This year, scientists perfected the technique of synthetically creating one part of the prized compound and extracting the rest from the needles and twigs of the yew tree, rather than its bark. The result is a semisynthetic version that doesn't require cutting down trees. Other scientists continued clinical tests of Taxotere®, another semi-synthetic compound similar to Taxol.

Also this year, plant biologists went seeking a fungal producer of taxol and found one in the interstices of the bark of a yew tree in a remote and untouched Northwestern forest. As only 5% of all fungi have been systematically studied, their search was an educated guess that paid off. And although *Taxomyces andreanae* now produces only tiny amounts of taxol, it may in time be coaxed into becoming a fungal factory for the drug.

Meanwhile, demand for Taxol is likely to grow. The drug was approved as a treatment for ovarian cancer just one year ago, and approval for breast cancer may come soon.

Super developments. From 1986 to 1988, scientists made heady progress in developing high-temperature superconductors, ceramic materials that can conduct electricity without resistance. But from 1988 to early 1993, the maximum superconducting temperature remained stuck at 125 degrees kelvin (−148 Celsius). Finally, this past spring, the long-standing record was broken.

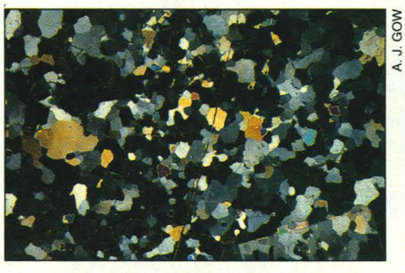
In March, scientists generated superconductivity in a new family of materials, a mercury compound rather than the standard thallium. In May, the new material nudged past the old record, achieving superconductivity at 133 K. In September and October, researchers had success with another tack, and found that under the intense pressure of 235,000 atmospheres, the onset of superconductivity began at more than 150 K.

And in the most stunning find of all, published only last week, scientists reported evidence of superconductivity in yet another new material—one with multiple layers of copper oxides—at a balmy 250 K. That number approaches the grail of 300 K, room temperature. Other researchers working in similar compounds had previously reported possible traces of superconductivity. But the new signals are more long-lasting and apparently reproducible.

If the finding is confirmed—and that's still a big if—then 1993 will mark the beginning of a new era in superconductivity. For now, the result provides an electrifying end to an encouraging year.

Ice Age lessons. A detailed record of past climates lies buried in the Greenland Ice Sheet, and in the past 2 years, scientists have drilled two narrow cores through 3 kilometers of ice to get it. In 1992 and 1993, they reported that Earth's climate, at least in the region around Greenland, is capable of startlingly rapid change. That finding may help us understand the forces that affect modern climate conditions.

The twin ice cores, drilled only 30 kilometers apart, preserve dust, oxygen isotope ratios, and other climatic clues. The evidence shows that as Earth emerged from the last Ice Age, the climate lurched unsteadily toward warmer conditions. For example, a lengthy cold snap called the Younger Dryas struck from 12,900 to 11,600 years ago. At the end of that time, the climate abruptly jumped to warmer, wetter interglacial conditions, and the rate of snow accumulation doubled in just 3 years. Such rapid climate shifts were fairly common during the Ice Age.

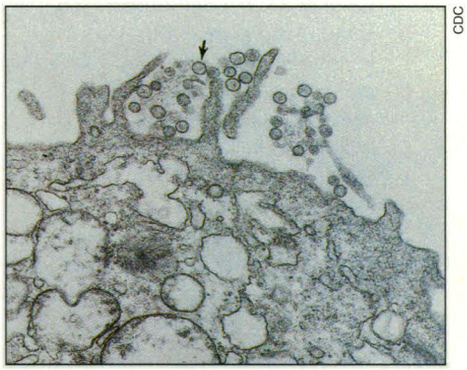


A. J. GOW

Core memory. Ice core crystals record past climate change.

Early work on the older layers of one core, deposited 110,000 to 130,000 years ago, suggested that the last interglacial also was marked by sudden changes in climate. However, since there are two cores, scientists could compare results—and they found that beyond 110,000 years ago, the cores don't match. At least for now, the earlier record is suspect, and the cores provide a cautionary lesson in the value of having a sample size greater than one.

Emerging infections. Grim news struck the Four Corners area of the Southwestern United States in May 1993, when a mysterious and lethal pulmonary illness erupted. So far, the outbreak has killed 29 people, and it serves as a classic example of an emerging pathogen, one that suddenly sweeps through human populations. Health officials have just begun to recognize the danger of such pathogens, of which HIV is the best-known example.



CDC

Viral villain. Lethal strain of newly identified hantavirus *(arrow)*.

In some respects at least, the Four Corners episode may serve as a model of an effective response to such emergent killers. Scientists at the Centers for Disease Control and Prevention immediately tackled the disease but were unable to isolate live virus from patients. Instead, they used the tools of molecular biology, specifically the polymerase chain reaction (Molecule of the Year in 1989) to amplify bits of viral DNA from patients' tissue. By 9 June, scientists had discovered that the mysterious pathogen was a type of hantavirus, named for the Hantaan River in Korea where a prototype strain struck in the 1950s.

By matching the viral DNA to that found in deer mice, CDC scientists also identified these rodents as the carriers of the virus. There is still no known cure for the disease. However, an epidemic appears to have been averted, in large part because officials were able to warn people to avoid contact with deer mice—an indication of our increasing sophistication in fighting infectious diseases.

–Elizabeth Culotta and Daniel E. Koshland Jr.