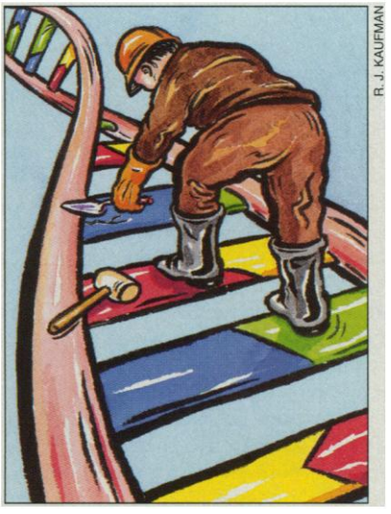
DNA Repair Works Its Way to the Top

The hard-working DNA repair enzymes preserve genetic information, guard against cancer, and unite basic cell biology, cancer research, and toxicology

In 1994 we salute not one molecule but many: The enzymes of the DNA repair systems, which protect and maintain the information in the genetic code. Every second that you read this, the DNA in each cell of your body is being damaged. Chemical bonds are breaking, DNA strands are snapping, and nucleotide bases are flying off. Each cell loses more than 10,000 bases per day just from spontaneous breakdown of DNA at body temperature. Meanwhile, many cells are dividing and therefore copying DNA, and each copy introduces the possibility of error. Exposure to carcinogens adds to the injury and causes strange new forms to sprout from the double helix.


R. J. KAUFMAN

If such damage were allowed to persist, cells would cease to work properly, mutations would accumulate, and the chances of tumors developing would soar. Fortunately, the DNA repair enzymes are on the job. Like a well-trained maintenance crew, they continually scan DNA for mistakes, slice out damaged pieces, and patch up gaps.

In 1994, experiments revealed the molecular nuts and bolts of the repair machinery, unveiling a system of surprising versatility and power. This year, many different avenues of research converged on repair enzymes. Biologists who study basic cellular business such as gene expression suddenly found themselves studying repair as well, as the same molecular players turn up in each process. Geneticists discovered at the end of 1993 that a common form of colon cancer is due to flaws in one repair system, mismatch repair. Now cancer researchers who 5 years ago hadn't even heard of mismatch repair are seeking its footprints in a variety of tumors. Repair research also holds promise for a better understanding of environmental carcinogenesis and the potential pitfalls of using model organisms to test chemical toxicity.

Given the current hubbub of research, it now seems ironic that DNA repair enzymes were once thought to be important only in unusual circumstances such as radiation damage or in rare forms of human disease. In 1994, decades of painstaking labor on various repair pathways finally paid off, firmly establishing the central role of DNA repair in the life of the cell.

Copy editing. There are multiple DNA repair pathways, and each specializes in a certain kind of damage. In late December 1993, the mismatch repair pathway was catapulted into the limelight when independent teams reported that defects in a mismatch repair gene, *hMSH2*, caused a significant proportion of cases of a common type of cancer, hereditary nonpolyposis colorectal cancer (HNPCC). Researchers estimate that one in every 200 people in Western populations may carry a predisposition to this disease.

Mismatch repair specializes in errors made when DNA is copied. It scans newly made DNA for mispaired bases, cuts out mistakes, and fills in the gaps with the correct sequences (see Perspective by Modrich on p. 1959). This year, researchers cloned three additional genes (*hMLH1*, *hPMS1*, and *hPMS2*) that are defective in other cases of HNPCC. The pieces of the puzzle are assembling with astonishing speed, chiefly because the molecular details have already been worked out in *Escherichia coli*. Indeed, the mismatch repair breakthrough represents the rewards of decades of basic research on the genetic maneuvers of *E. coli* and fungi.

The *hMSH2* gene was found quickly in part because researchers suspected mismatch repair might be at fault and went hunting for genes similar in sequence to the known *E. coli* genes. And the interactions of the mismatch repair proteins are emerging rapidly in higher organisms, again thanks to earlier work in *E. coli*. In a flurry of papers this year, biologists were able to establish the basic roles of several eukaryotic mismatch repair genes, showing where they imitate the *E. coli* pattern and where they differ.

In 1994, researchers showed that in yeast, three proteins begin repair by forming a complex at the site of the mismatch, which is a more complicated variation on the theme in *E. coli*. Also this year, biologists demonstrated that human cells may have a mismatch repair technique that bacteria lack: Humans appear to correct unpaired DNA loops of five bases or more, while in bacteria, mismatch repair handles loops of only three bases.

Scientists are now working on diagnostic tests for defective mismatch repair genes, which could save lives by allowing early cancer detection and treatment. Clues implying defects in this pathway have also been found in some spontaneous tumors, so flaws in mismatch repair may turn out to be important in a wide spectrum of cancers.

Indeed, the link between mismatch repair and colon cancer provides concrete support for a hypothesis put forward years ago: that tumors arise when a cell starts generating a raft of mutations. In the past, scientists have focused on particular genes whose mutations led to cancer. Now, there is renewed emphasis on how and why the cell accrues so many mutations in the first place.

Damage control. Normal cellular activities injure DNA through oxidation and other common reactions. To repair such damage, cells rely on another pathway, called base excision repair, which targets single damaged bases. This year, biologists published the detailed three-dimensional structure of one of the key base excision repair enzymes, DNA polymerase beta.

Meanwhile, outside agents such as ultraviolet light and chemicals also inflict damage on DNA. Many of these lesions are handled by a third repair pathway, called nucleotide excision repair (NER), which recognizes and repairs large, bulky lesions in DNA (see Perspective by Sancar on p. 1954). NER is crucial to humans: Individuals who lack it have the rare genetic disease xeroderma pigmentosum and are so sensitive to sunlight that they often develop skin cancer before age 10.

Recently, years of research on this disease and on repair have coalesced, allowing scientists to tease out the identities and functions of many of the molecular players in this complex pathway. This year, aided by an in vitro system that allows repair to occur in a test tube rather than a cell, researchers showed that proteins work together to excise damaged DNA, making two cuts, one on each side of the lesion. Last year, the first knockout mice lacking an excision repair gene

(*ERCC1*) were created; other groups have now knocked out different genes and are working with new strains.

All this painstaking labor yielded remarkable rewards. In 1994, biologists have been stunned by how often newly identified excision repair proteins crop up in unexpected places. So-called "repair" enzymes are now known to participate in DNA replication, the control of the cell cycle, and the expression of genes. Enzymes that can slice chromosomes perform this duty in both recombination and repair; molecules that unwind DNA do so as part of the process of gene expression as well as repair.

Indeed, the research community has been amazed by the strong connections between repair and transcription, the process by which the genetic information encoded in DNA is transmitted to RNA, to be eventually expressed as a protein. For example, those who study transcription have long been familiar with a large protein complex called TFIIH that helps begin the process. In the past year or two, biologists have shown that many TFIIH components are actually DNA repair proteins, and that the whole complex itself functions in repair. Just a few weeks ago, yet another TFIIH component was identified as a protein that helps regulate the cell cycle; thus transcription, repair, and the cell cycle are linked in a single complex. This new research sketches a more integrated picture of the cell, in which all processes relating to DNA are coordinated and the same molecular toolkit is used for different tasks.

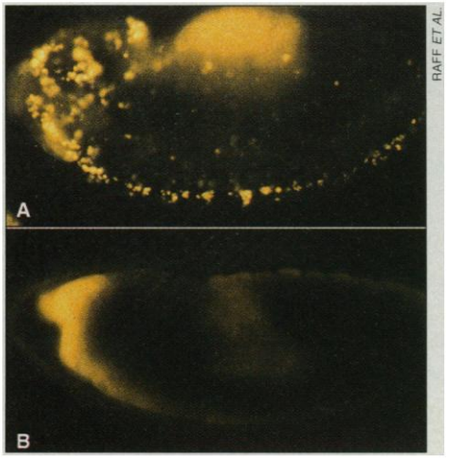
Risk assessment. In addition to the versatility of molecular tools for cutting, patching, and binding DNA, work on excision repair has uncovered another link to transcription: Damage in transcribed genes (those which will be expressed as protein) is repaired faster than damage in nontranscribed genes (see Perspective by Hanawalt on p. 1957). This makes sense from a cell's point of view, because damage in transcribed genes might prevent synthesis of key proteins, while damage in nontranscribed DNA will have few immediate consequences, although it may lead to cancer in the long run.

This subpathway of NER, called transcription-coupled repair, is ubiquitous, found in bacteria, yeast, rodents, and humans. Furthermore, research during the past few years has suggested that rodents are less efficient than humans are at repairing certain kinds of damage in nontranscribed DNA. Thus rodents may be more sensitive than humans are to some carcinogens. Because most carcinogen testing is done on rodents, such findings have important implications for risk assessment. Indeed, a better understanding of the details of DNA damage and repair will help pave the way to risk assessment based on the mechanics of carcinogenesis.

DNA repair is also linked to other clinically relevant areas. For example, researchers continue to probe the theory that a decline in DNA repair is part of the biology of aging. With repair enzymes at last showing off their power, new researchers are flocking to the field, while longtime repair workers savor the fruits of their labors. In 1994, as the accomplishments of repair enzymes were widely recognized, DNA repair took its rightful place at the center of cell biology and cancer research.

And the runners-up are ...

Science surged forward in many fields in 1994, on scales ranging from the subatomic to the galactic. We celebrate achievement in nine areas that offer valuable benefits to society, as well as a record of spectacular scientific progress this year.



Death wish. Many normal cells self-destruct (A), while mutants refuse to die (B).

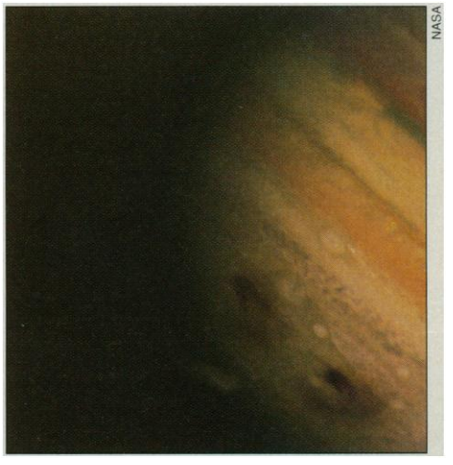
Live and let die. Humans may want to live forever, but many cells know better. At prescribed times they execute a suicide program and die with characteristic death rattles. This altruistic act, called programmed cell death or apoptosis, allows nearby cells to thrive and is crucial for development and immune function. In 1994, an avalanche of papers detailed additional genetic and protein players that execute this suicide program in organisms as diverse as nematodes and humans.

In 1994, researchers identified a vertebrate gene family, homologous to the gene *ced-3* in the roundworm, that may take part in the suicide program. Also this year, the fly entered the cell death scene with the cloning of the *reaper* gene, necessary for apoptosis in the fruit fly *Drosophila*. Without *reaper*, fly embryos have extra cells in the wrong places and die before hatching.

Researchers also explored the processes that keep the self-destructive urge in check, such as the molecular couplings of the *ced-9* gene product, which protects against cell suicide in the roundworm, and its human homolog, the *bcl-2* gene product. Such research has a direct bearing on cancer biology, because cancerous cells find a way to avoid apoptosis. New evidence this year supports the notion that radiation and certain anticancer drugs work because they induce apoptosis via the protein product of the tumor suppressor gene *p53*, 1993's Molecule of the Year. Other new research suggests tantalizing links between apoptosis and neurodegenerative diseases. As apoptosis genes and their functions are similar in organisms from nematodes to humans, it appears that programmed cell death, like taxes, is universal.

Lasting impact. The July collision of comet Shoemaker-Levy 9 with the giant planet Jupiter offered an unexpectedly dazzling display to observers around the globe—and delivered a hefty scientific payload of new insights into the nature of comets and of Jupiter itself. Some early forecasts of the impact predicted a dud of a show, in part because the 21 speeding comet fragments slammed into the planet's backside. Instead, the Earth-bound audience linked by the Internet and several spacecraft was treated to a premier astronomical performance. Plumes of debris soared thousands of miles above Jupiter, and a chain of soot-filled hot spots marked the fragments' collisions with the planet.

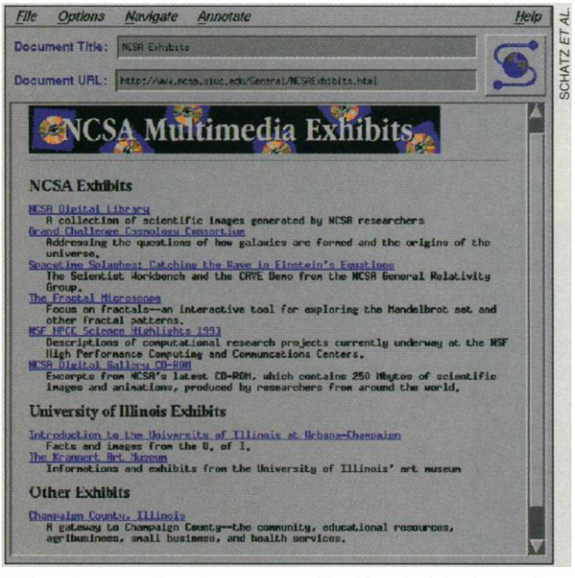
Researchers are still puzzling over whether the fragments were large or small and how deeply they penetrated the Jovian atmosphere. In any case, Shoemaker-Levy 9's dramatic breakup and observations of its death offer new support for the image of comets as fragile assemblages of dust and ice, held together only by their own weak gravity. The impact also provided a glimpse into Jupiter, by blowing parts of its atmosphere literally sky-high. In the aftermath, researchers found sulfur above the Jovian clouds, providing strong confirmation that the planet's middle cloud



Crash site. The comet left a pall of debris and lingering wounds on Jupiter.

layer is indeed composed of sulfurous compounds. Others tracked the speed of Jupiter's high-altitude winds using the debris plumes. With gigabytes of data to pore over, scientists will be studying the event for years to come.

The devastating power of the impact also brought home a basic astronomical lesson: It could happen here. As Jupiter slowly began to heal its bruises, Congress urged a willing National Aeronautics and Space Administration to search the heavens for asteroids and comets that might menace our own planet.



SCHATZ ET AL.

Network news. Popular programs like Mosaic linked sound and video, and fueled the Internet boom.

Public wire. Fading stereotypes conjure up the image of the scientist as an ivory-tower loner, but in 1994, one of science's chief contributions to society was the world's latest communications miracle, the Internet. Created by researchers, the Internet was originally shaped by the scientific culture. But this year there could be no doubt that the Internet has gone public, becoming a dynamic arena for work and play not only for scientists, but for millions of people around the world.

Mosaic software, made widely available in late 1993, made it simple to chart a course to information, sound, and images on other computers worldwide. Ever-increasing transmission speeds allowed data, graphics, video, and sound to zip through the network at up to a billion bits per second. Thanks to such advances, it's estimated that more than 20 million people have now discovered the joys of surfing the net, and their numbers increase at a rate of more than 10% per month. Current traffic over the NSF backbone is 10,000 times greater than at the start of 1993.

Applications have also grown with breathtaking speed, as more and more users bend cyberspace to their needs. Virtually every scientific lab and institution is on-line, making electronic collaborations, shared databases, and on-line journals a snap. The fastest growth, however, may be in commercial users, which now comprise nearly one third of the total. This has brought culture clash, as the breezy informality of the electronic frontier is eroded by new types of settlers, such as politicians, 12-step support groups, and lawyers advertising their wares. Growing pains include junk e-mail, security concerns, and the specter of new pricing strategies. With economists plotting a new electronic currency and Mick Jagger rocking live on-line, it's clear that the Internet is now firmly in the public domain.

Universal time. How old is the universe? Astronomer Edwin Hubble asked that question back in the 1920s. In 1994, new data from the Hubble Space Telescope allowed researchers to improve their estimates. They concluded that the universe may be a mere 8 billion years old—which is younger than some of the stars within it. As that's clearly impossible, the new evidence may herald a dramatic shift in theories of how the universe evolved.

Most cosmologists agree that the universe began with a primordial explosion, the Big Bang, and has been expanding ever since. Since Hubble's time, astronomers have been chasing the so-called Hubble constant (defined as the speed at which a galaxy is receding from Earth divided by the distance to that galaxy). The constant pins down the rate of expansion of the universe, and so constrains its age. But a firm number has proved maddeningly elusive, because it requires accurate estimates of vast reaches of space.

This year, two groups made the best estimates yet for the distance to stars in the Virgo constellation. One team peered through the blur of Earth's atmosphere with high-tech image enhancers; another relied on the crystal-clear vision of the Hubble telescope. Both teams converged on an estimate of 80 or more for the constant.

According to current theory, those numbers mean the universe is definitely less than 12 billion years old, and perhaps as young as 8 billion. Yet well-accepted theories on stellar evolution date some stars at 13 to 16 billion years old.

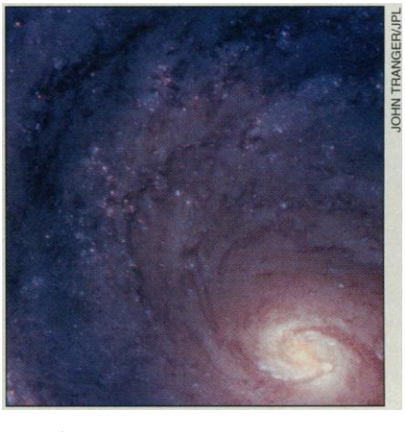
With such a paradox facing cosmology, some still doubt the new results, and Hubble telescope researchers continue to gaze at distant stars to shore up the estimate. Meanwhile, cosmologists are already pondering the addition of a fudge factor first posed by Albert Einstein, the cosmological constant, that could make the age discrepancy vanish.

Finding our roots. Who were our ancestors? In 1994, the answer to that question changed dramatically with the discovery of a new, more primitive species on the evolutionary line leading to *Homo sapiens*. Unearthed near the village of Aramis in Ethiopia, the new find pushes back our knowledge of human history by about half a million years and provides a tantalizing glimpse of the most apelike of our known ancestors.

Called *Australopithecus ramidus* (from the word meaning "root" in the language spoken near Aramis), the species fills a crucial gap in the fossil record. Biochemical evidence has long suggested that the human lineage split from that of the African apes between 4 and 6 million years ago. Until recently, however, hominid fossils from the crucial time and place were scant.

Evidence from pollen, magnetostratigraphy, and other fossil animals at the site shows that *A. ramidus* lived and died in a woodland environment about 4.4 million years ago. The species is now known from 17 fragments of teeth and bone, including arm bones and a child's jaw with a milk tooth. The remains were scavenged by carnivores, but even so, they sketch a creature clearly more chimplike than the next youngest species in our lineage, *A. afarensis*, which includes the famous 3.2-million-year-old skeleton "Lucy." Like chimps, *A. ramidus* has relatively small molars and large canines, but it is placed on the human line by virtue of the shape of its canines and the apparent orientation of its vertebral column.

So far, the new finds from Ethiopia are silent on the all-important question of whether this early hominid walked upright. If future finds include a pelvis or leg bones, expect more excitement out of Africa in 1995.

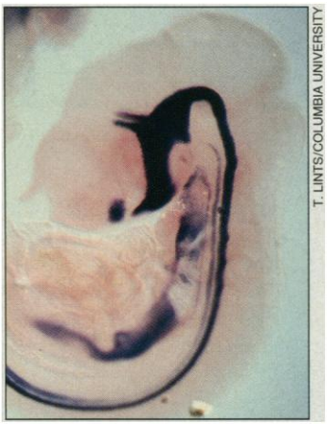

JOHN TRAUGER/JPL

Stargazing. The distance to stars in the galaxy M100 led to the age of the universe.

Holy hedgehog! Developing embryos somehow transform a mass of undifferentiated cells into a differentiated organism. This year, a rapid-fire barrage of papers highlighted a single family of molecules—*hedgehog* and related genes—that helps pull off the job in or-

ganisms ranging from flies to mice.

Classic experiments in developmental biology showed that certain key areas of embryonic tissue direct the patterning of cells around them, but no one knew exactly how this happened. Then at the end of 1993 and throughout 1994, a series of clever molecular experiments showed that in the fruit fly *Drosophila*, the Hedgehog protein (named for the bristly, stunted fly embryos that form when the gene is knocked out) is the molecule that carries the signal from one cell to another. For example, Hedgehog is secreted by cells in one compartment of the *Drosophila* larva and regulates specific genes in other compartments; the resulting pattern of gene expression specifies positional information. Hedgehog also helps pattern the eye of the adult fly and segments of the embryo.



T. LINTS/COLUMBIA UNIVERSITY

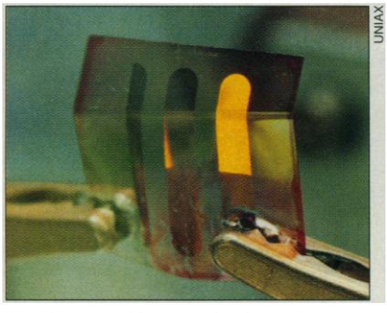
***Hedgehog* territory.** Chick embryos express the gene.

Meanwhile, other teams of scientists uncovered versions of this powerful gene in vertebrates. One variety, *sonic hedgehog*, gives directional information to other cells in the nascent limb and neural tube of fish, chicks, mice, and rats.

With key molecules identified, mechanistic work is moving ahead. A few weeks ago, biologists showed that the Hedgehog protein cleaves itself in two, and each part may have a different function. By popping up everywhere from chick wings to fly eyes, *hedgehog* has brought molecular, cell, and developmental biology together—and provided another elegant example of how evolution conserves its valuable creations.

Electric plastics. Plastics were the growth industry of the 1960s, but this year some organic polymers with the ability to conduct electricity made a splash as promising new materials. In 1994, researchers created the first all-plastic transistors and made better optical devices from polymers, setting in motion a new wave of plastic electronics.

Most common polymers are insulators, but plastic may also be crafted to serve as a semiconductor or conductor. Such conducting polymers have the virtues of light weight, flexibility, and low cost. For example, the two kinds of plastic transistors built this year can be bent and twisted and rolled—and they keep on conducting. Created by thin-film or printing techniques, the polymers can in principle be produced cheaply.



UNIAX

Lighting up. New conducting polymers make flexible electronic devices.

Researchers are also doing creative chemistry to improve plastic light emitting diodes (LEDS), the ubiquitous little red lights found on electrical equipment. This year, scientists developed a new way to make a single polymer material light up in different colors. One big problem remains: The glow of today's plastic LEDS fades with time, a flaw that will have to be overcome before the devices sweep the market.

Also this year, scientists improved the quality of nonlinear optical organic materials, which can be used to make instant holograms. Potential applications include optical data storage and image processing. If conducting polymers live up to their promise, another golden age of plastics may be just around the corner.

Top candidate. The hunt for the top quark, the lone missing member in the pantheon of subatomic particles, passed a milestone in 1994, as researchers at last reported a probable sighting of their elusive quarry. One of six fundamental building blocks of matter, the top quark is vital to physicists' theoretical view of matter, called the Standard Model. The other five quarks have been observed, but the top is so heavy that it has repeatedly evaded the grasp of physicists seeking to create it in collider experiments. If the top did not exist, or if its mass lay outside certain limits, then theoretical physics would be in for drastic revision.

This spring, researchers smashing protons against antiprotons at extremely high energies at the Fermi National Accelerator Laboratory reported the first direct evidence of the top quark. Such subatomic particles are too ephemeral for a clear sighting, but physicists detected a cascading spray of other particles that fits the signature of a top quark. The particle's mass was estimated at about 174 GeV, plus or minus 10%—about the mass of an atom of gold and within range of theoretical predictions.

Because the evidence is not conclusive, physicists are still watching showers of particles erupt in their collider. Once the top is firmly in hand, particle physicists will focus on another long-sought particle, the Higgs boson. The target of the doomed Superconducting Super Collider, the Higgs is still out of reach, but for now, physicists can take pride in their glimpses of the top.

Mind matters. In 1994 a drug called Prozac introduced millions of Americans to the power of the neurotransmitter serotonin. By emphasizing the biological basis of mood, Prozac sparked a wide-ranging public debate on the role of drugs in psychiatry. Meanwhile, in the scientific arena, neuroscientists continued to draw tighter links between brain chemistry and behavior, particularly in animal models.

Prozac, or fluoxetine, is one of five known drugs that inhibit reuptake of serotonin. By effectively increasing the amount of serotonin processed by nerve cells, the drug gives users more of what seems to be a good thing. Although it works much like its predecessor antidepressants, Prozac has fewer side effects. Devotees report that it can lift the mantle of clinical depression and boost failing self-confidence, and it has become the most widely prescribed drug in American psychiatry.

Some saw the public's embrace of Prozac as a turning point in psychiatry, solidifying the shift from "talking cures" like psychotherapy to biological ones. Proponents foretell the dawn of a new era in psychopharmacology, in which all sorts of mental disorders are treated by adjusting the chemistry of the brain. Critics worried about overuse of a personality-altering drug and pointed out that so far no one knows exactly how fluoxetine works its magic. But despite philosophical differences, most agreed on Prozac's power over mind and mood.

TOM LYLE/MEDICHROME

Popular pill. Prozac enhances the action of the neurotransmitter serotonin in the brain.

Meanwhile, neurogeneticists passed a milestone in exploring the link between serotonin and aggression in mice. They created a strain of mice lacking one of the 14 serotonin receptors and found that this particular flaw rendered mutants unusually aggressive: They attacked other mice with twice as much fervor as normal. In 1994, serotonin united biology and behavior in both the scientific and public theaters.

–Elizabeth Culotta and Daniel E. Koshland, Jr.