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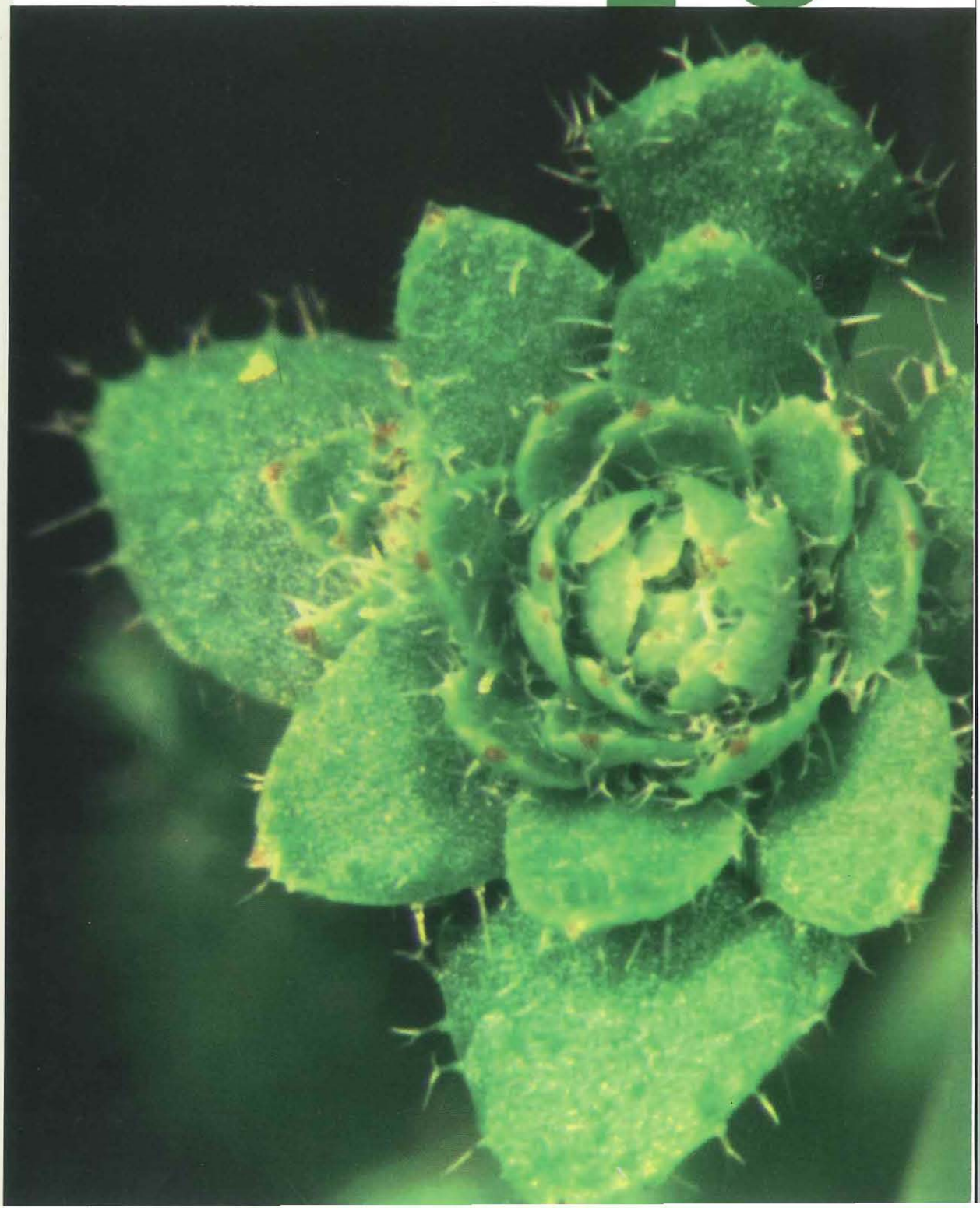
E&S

ENGINEERING AND SCIENCE



Volume LX, Number 4,
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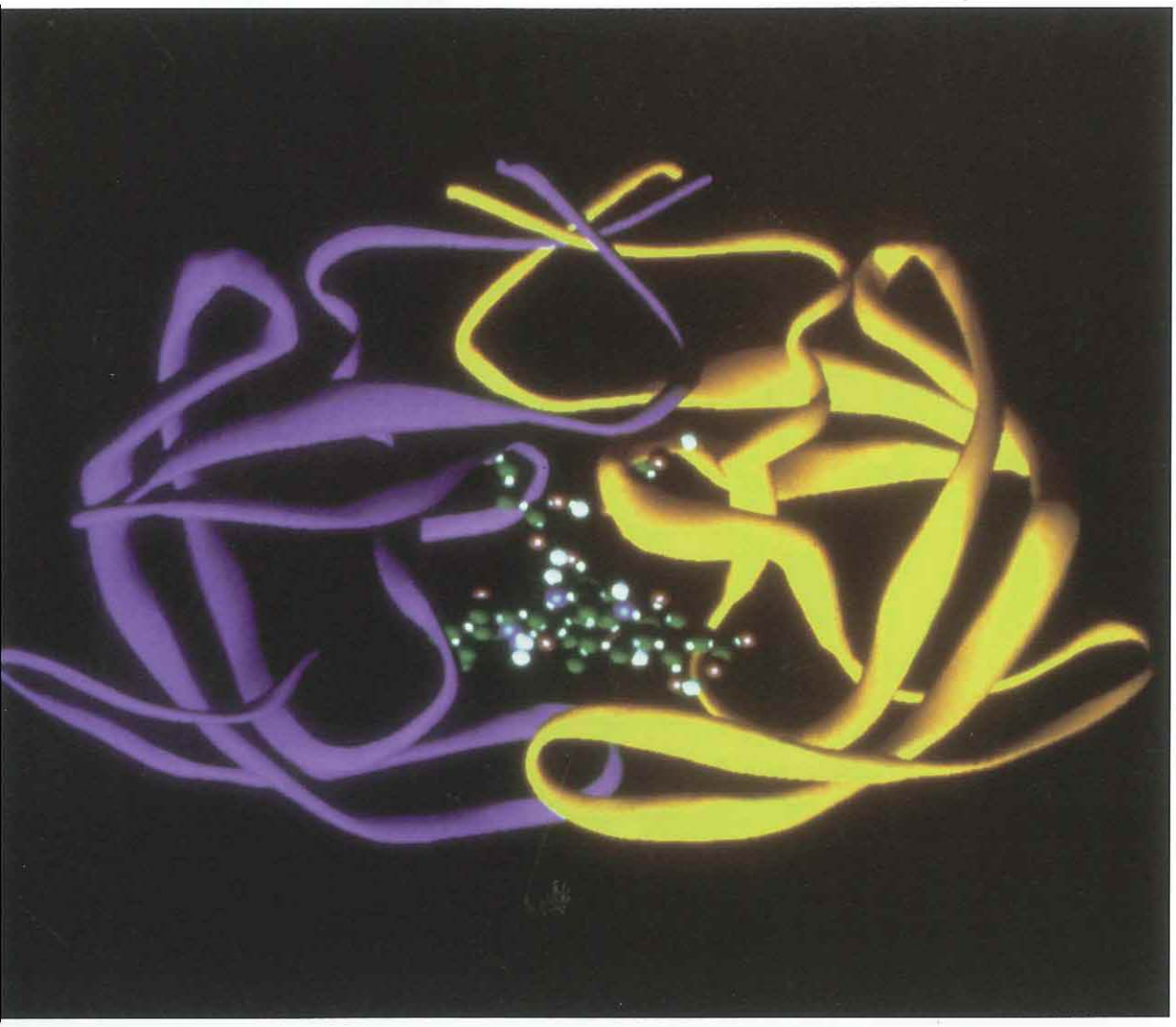

One of the proteins that make up the AIDS virus and are essential for its replication is the HIV protease. The molecule consists of two subunits (here represented by the purple and yellow ribbons), which cut other viral particles into the smaller bits necessary for replication. Those particles have to fit neatly into a particular site in the protease. Biologists have worked out the atomic structure of the protease and designed small molecules to substitute in the site, thus hindering the protease's function. This and other advances in the battle to subdue AIDS are described in an article adapted from talks at Caltech's 1997 Biology Forum, beginning on page 24.

California Institute of Technology

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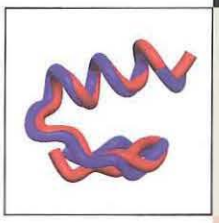

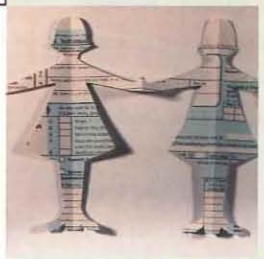

On the cover: An all-leaf, triple mutant of the mouse-ear cress (*Arabidopsis thaliana*) flower was created by disabling all three groups of genes that govern organ identity in the flower's four whorls. Without these genes, leaves replace the sepals, petals, stamens, and carpels. Elliot Meyerowitz has adopted *Arabidopsis* as a research organism, using its conveniently compact genome to research how a flower is made. An article about his work begins on page 8.

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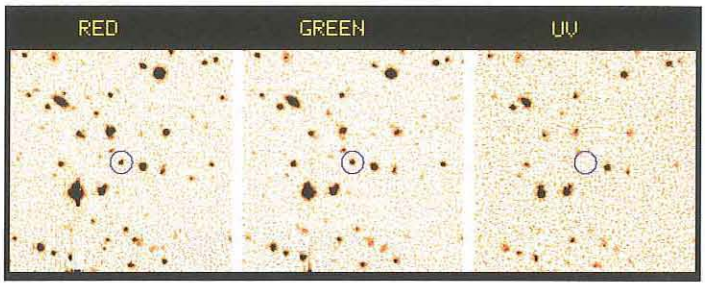
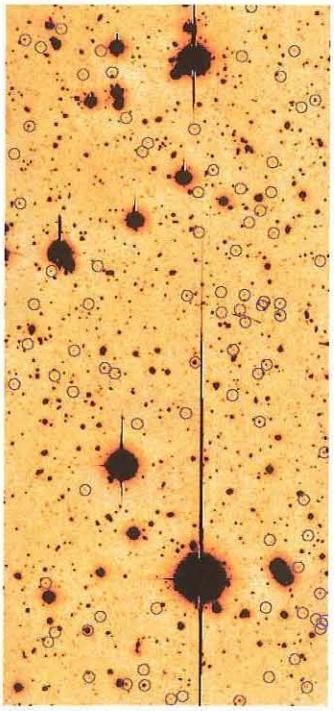
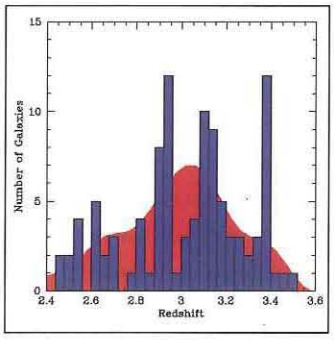






Associate Professor of Astronomy Charles Steidel (PhD '90) and colleagues have found several large clusters of galaxies that had begun to aggregate when the universe was only about one-tenth of its present age, long before gravity would have had time enough to pull them together. These clusters therefore reflect the distribution of matter in the universe soon after the Big Bang. Since a galaxy's redshift is a proxy for its age, the astronomers used the 200-inch Hale Telescope at Caltech's Palomar Observatory to search for galaxies whose light has been shifted so far to the red that they are invisible at ultraviolet wavelengths (top). This portion of a typical field from the Hale Telescope (middle) contains some 2,000 galaxies and about 75 "ultraviolet dropouts" (circled). The redshifts of these dropouts were then measured at the twin 10-meter telescopes at Caltech's W. M. Keck Observatory in Hawaii. The blue bars (bottom) show the clumpiness of the dropout galaxies' measured distribution; the red curve in the background shows how the data would look if the galaxies were distributed randomly in space.

That Was a Quick Decade

The January 1998 broadcast of *AirTalk: The Caltech Edition* on KPCC (89.3 FM) marks the 10th anniversary of that monthly live radio program. The *Caltech Edition* is a special installment of the regular *AirTalk* series, which is hosted every weekday evening by Larry Mantle. Mantle devotes *AirTalk* to interviews of political figures, celebrities, academic personalities, authors, and others from a wide variety of backgrounds. Listeners have the opportunity during the show to call in and talk to Mantle or the guests directly. KPCC, licensed to Pasadena City College, has the strongest signal of any NPR-affiliated station in Los Angeles and Orange Counties, and, in general, can be heard from Santa Barbara to San Diego.

Caltech President David Baltimore and Robert O'Rourke, Caltech's associate vice president for institute relations and the originator of the idea for the series, are the 10th-anniversary guests. Past *Caltech Edition* guests have included Kip Thorne, an expert on gravitation and a longtime collaborator with Stephen Hawking; Ed Stone, who holds a joint appointment as director of Caltech's Jet Propulsion Laboratory (JPL) and as vice president at Caltech; Donna Shirley, the head of JPL's Mars exploration program; Christof Koch, an expert on neural networks and computers who has spoken on the cloning controversy; seismologists Kate Hutton and Lucy Jones; and planetary scientist Andy Ingersoll, who is an expert on global warming and the El Niño phenomenon. □

Caltech senior Rowena Lohman, geology major, flutist, actress (seen here as Eva in last fall's production of *Thieves' Carnival*), and rock climber, has been named one of *Glamour* magazine's Top Ten College Women for 1997. The award includes a trip to New York City, where honorees are introduced to professionals in their field.

Caltech Beats the Big 10, Big 12, Big West, Big East, SEC, Ivy League. . .

No, it's not the fantasy of a frustrated football coach, but the results of a very different nationwide sporting event—the Fourth Annual Collegiate Championship of Amateur Radio Clubs. The Caltech Amateur Radio Club (CITARC) took first place, winning by commanding margins the two lesser events that make up the championship. In both events, the club, which holds the call sign W6UE, attempted to contact other amateur radio operators. Scoring is based on the number of contacts completed times the number of sections contacted. (For these contests, Canada and the United States—including Alaska, Hawaii, Puerto Rico, the U.S. Virgin Islands, and the Pacific Territories—are divided into 79 sections, some of which are quite difficult to contact because of their distance or sparse population. The idea is to reward the stations that reach the widest areas; otherwise stations in densely populated regions could win on purely local contacts.) The first event, held on the first weekend of November, was conducted in Morse code; the other, on the third weekend of November, was in voice mode. In both events, Caltech made a clean sweep of all 79 sections. (For more information about CITARC, check their Web site at http://www.cco.caltech.edu/~w6ue/)

There's a serious side to all this—in the aftermath of an earthquake or other natural disaster that brings down telephone lines and computer networks, amateur radio provides a vital communications link. Contests such as these hone operators' technical skills and test the station's equipment, much like emergency-preparedness excercises.

Some two dozen colleges participated, including Penn State, the University of Texas, the University of Nevada–Reno, Virginia Tech, the University of Arkansas, and Harvard. Oh, and That Other Institute of Technology? We beat them, too. □—*DS*

Hudson Bay Bounces Back

While Earth's gravitational field is commonly thought of as constant, in reality there are small variations in the field as one moves around the surface of the planet.

These variations have typical magnitudes of about one ten-thousandth of the average gravitational attraction, which is approximately 9.8 meters per second per second. A global map of these variations shows large undulations at a variety of length scales. These undulations are known as gravity anomalies.

There are many such anomalies in Earth's gravity field, but one of the largest negative gravity anomalies (implying the attraction of gravity being a little less than average, or in other words, a mass deficit) is centered on Hudson Bay, Canada.

Using a new approach to analyzing planetary gravity fields, Assistant Professor of Geophysics Mark Simons at Caltech and Bradford Hager at MIT have shown that incomplete glacial rebound can account for a substantial portion of the Hudson Bay gravity anomaly.

With this new information, Simons and Hager were able to place new constraints on the variations in strength of the materials that constitute the outer layers of Earth's interior (the crust and mantle). Their work appeared in the December 4 issue of the journal *Nature*.

About 18,000 years ago, Hudson Bay was at the center of a continental-sized glacier. Known as the Laurentide ice sheet, this glacier had a thickness of several kilometers. The weight of the ice bowed Earth's surface down. The vast majority of the ice eventually melted at the end the Ice Age, leaving a depression in its wake.

While this depression has endured for thousands of years, it has been gradually recovering, flattening itself out like a vacated sofa cushion. (The term "glacial rebound" refers to this tendency of land in formerly glaciated areas to rise after the ice load has disappeared.) The coastlines located near the center of the former ice sheet have already risen several hundred meters, and will continue to rebound.

"The rate at which the area rebounds is a function of the viscosity of Earth," says Simons. "By looking at the rate of rebound going on, it's possible to learn about the planet's viscosity."

Simons says that geophysicists have known for some time about the Hudson Bay gravity anomaly, but have hitherto been uncertain how much of the gravity anomaly is a result of glacial rebound and how much is due to mantle convection or other processes.

The gravity anomaly is

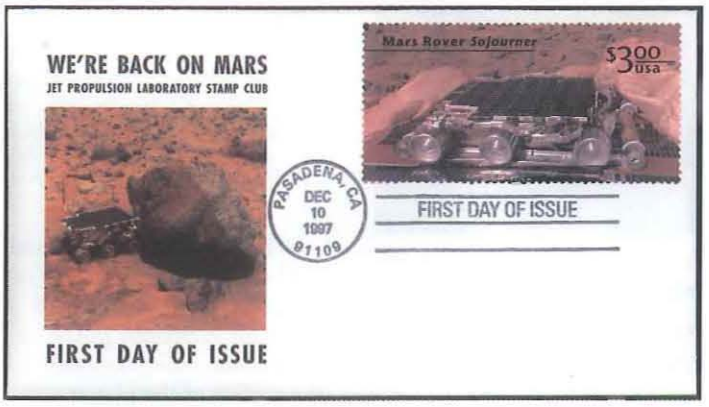
measured from both the ground and from space. Simons and Hager use a gravity data set developed by researchers at NASA's Goddard Space Flight Center.

However, knowing how much of an anomaly exists at a certain site on Earth is not sufficient to determine the pliability of the materials beneath it. For this, Simons and Hager developed a new mathematical tool that looks at spatial variations of the spectrum of the gravity field.

In many instances, this approach allows one to separate the signatures of geologic processes that occur at different locations on Earth. In particular, Simons and Hager were able to isolate the glacial-rebound signature from the signatures of other processes, such as manifestations of plate tectonics, that dominate that gravity field but are concentrated at other geographic locations.

Having an estimate of incomplete postglacial rebound allowed Simons and Hager to derive a model of how the viscosity of the mantle changes with depth. Simons and Hager proposed one such model that explains both the gravity anomaly as well as the uplift rates estimated from the coastlines.

Their favored model suggests that underneath the oldest parts of continents (some of which are over 4 billion years old), the viscosity of the outer 400 kilometers of Earth's interior is much stiffer than it is under the oceans. Therefore, these continental keels can resist the erosion by the convective flow that drives plate tectonics. □—*RT*



On December 10, the Mars Pathfinder mission was honored by the U.S. Postal Service with the issuance of a $3 priority-mail stamp. Fifteen million of the stamps, which bear a portion of the first panoramic image returned after the July 4 landing, have been printed.

The Cartoon Guide to Geophysics

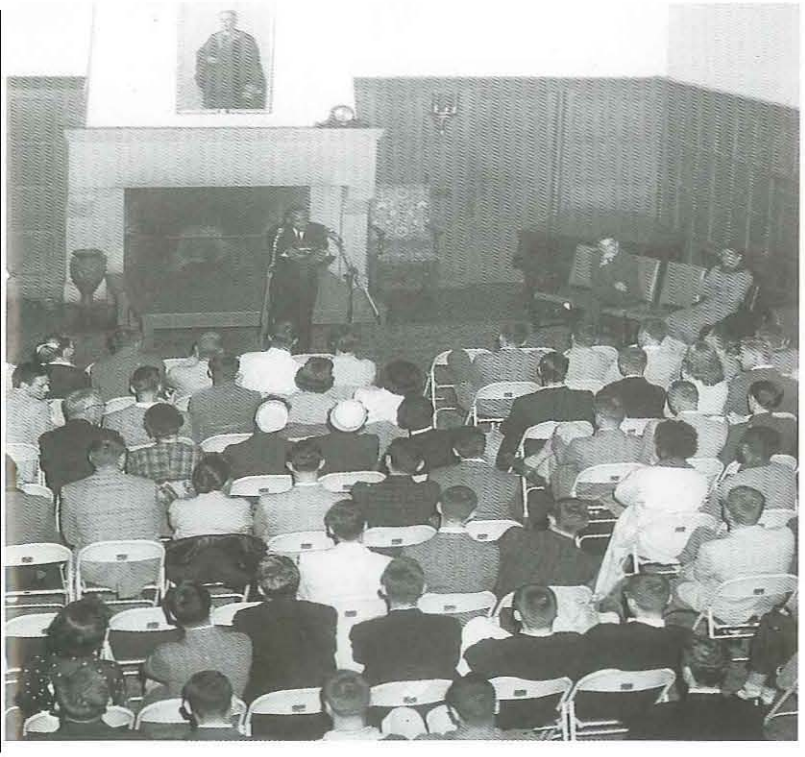
As every Bugs Bunny fan knows, the laws of physics in the cartoon universe are rather different than in our own. But can cartoon physics inspire real discoveries? The "Feedback" section of the October 11, 1997, issue of *New Scientist* noted, "Where do scientific ideas first appear? Looking back 35 years reveals one possibility. In the early 1960s, American TV aired a popular weekly cartoon called the *Rocky and Bullwinkle Show*. In one sequence, the dimwitted hero, Bullwinkle J. Moose, notices that his normally frozen home town of Frostbite Falls, Minnesota, is starting to thaw much earlier than usual.

"No, it isn't global warming arriving early. Our hero discovers that the North Pole has become so top-heavy with ice that it is slipping toward the equator, taking Frostbite Falls into sunnier climes and wreaking general environmental havoc..." (This polar deep freeze was caused by arch-nogoodnik Boris Badenov's scheme to displace the North Pole, and with it Santa Claus, into the Pacific Ocean. Boris, operating from the new North Pole, planned to take over Christmas, carrying gifts up the chimney instead of down.)

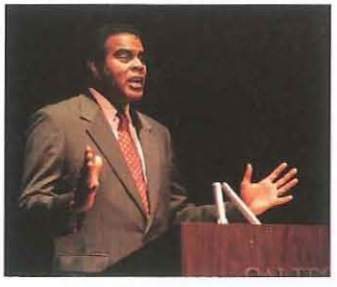
The article went on to compare this plot line to recent speculations by Professor of Geobiology Joseph Kirschvink (BS, MS '75) that some 534 million years ago, a mass imbalance in the mantle beneath the supercontinent of Gondwanaland (which had formed from the fusion of several lesser land masses only 20–30 million years earlier) caused Earth to become rotationally unstable. Gondwanaland, which was straddling the South Pole at the time, suddenly lurched 90 degrees northward as a result, shifting the excess mass to a more stable equatorial location. (See "Atlas Shrugged" in the Random Walk section of the last *E&S*.)

The item concluded, "Kirschvink's proposal earned him considerable ink in the science press... Nowhere, however, was there any recognition of the key contribution to the theory made by Bullwinkle J. Moose."

Kirschvink, who loved *Rocky and Bullwinkle* as a kid, has a more prosaic explanation. "The theoretical possibility of true polar wander has been known for over 50 years. Then, back in the early '60s, a bunch of papers came out saying that Earth's moment of inertia is about half a degree off from its spin axis, and is moving back toward it. This was presumed to be due to deglaciation [see Hudson's Bay Bounces Back]. Some writer for the show probably saw it in the newspaper somewhere and said, 'Hey, cool. I can use that!'" □—*DS*



The Reverend Martin Luther King, Jr., was on campus February 25–27, 1958, as the second visitor in the Caltech Y's Leaders of America program. He arrived in Los Angeles with his wife, Coretta Scott King, and the two were driven to campus from the Beverly Hilton by then-sophomore Kent Frewing (BS '61). For three days, King met informally with a large number of students, faculty, and staff, and presented formal lectures at the Athenaeum and Dabney Hall (above). He was also the guest of honor at several meals in the undergraduate residential houses. Forty years later, basketball-star-turned-media-star Tommy Hawkins, vice president of communications for the Los Angeles Dodgers, was the keynote speaker at Caltech's King Day observances (below). In a moving speech, Hawkins described his experiences as the first black player on the University of Notre Dame's basketball team, crediting King, Jackie Robinson, and Notre Dame president Rev. Theodore Hesburgh, C.S.C., for making it possible.



10-4, Copy That

Caltech biologists have pinpointed the sequence of reactions that triggers the duplication of DNA in cells.

In companion papers appearing in recent issues of the journals *Science* and *Cell*, Assistant Professor of Biology Raymond Deshaies and his colleagues describe the chain of events that lead to the copying of chromosomes in a baker's yeast cell. Baker's yeast is often used as a model for human cells, so the research could have implications for technology aimed at controlling cell reproduction, such as cancer treatments.

"We've provided a bird's-eye view of how a cell switches on the machinery that copies DNA," says Deshaies. "These principles can now be translated into a better understanding of how human cells proliferate."

The group's research keys primarily on how cells copy and segregate their chromosomes during the process of duplicating one cell into two. The new papers are concerned with how cells enter the DNA synthesis phase, during which the chromosomes are copied.

For years, cell biologists have tried to determine precisely which chemical events set off these reactions. The cell cycle is fundamental to the growth and division of all cells, but the process is somehow ramped down once the organism reaches maturity.

The paper appearing in *Science* describes how DNA synthesis is turned on. In the preceding stage (known as G_1), proteins named G_1 cyclins trigger the destruction of an inhibitor that keeps DNA synthesis from beginning.

This inhibitor sequesters an enzyme referred to as S-CDK (for DNA synthesis-promoting cyclin-dependent kinase), thereby blocking its action. Once the S-CDK is released, it switches on DNA synthesis. The S-CDK is present before the copying of DNA begins, but the DNA copying is not turned on until the S-CDK is freed of its inhibitor.

The Deshaies group has shown that several phosphates are attached to the S-CDK inhibitor. These phosphates act as a molecular Velcro, sticking the inhibitor to yet another set of proteins called SCF.

The *Cell* paper essentially picks up the description of the cell cycle at this point. The SCF, which acts like a molecular "hit man," promotes the attachment of another protein, ubiquitin. (See *E&S*, Spring 1995.) Ubiquitin in turn attracts the cellular garbage pail, prote-asome. The inhibitor is dis-posed of in the proteasome, thereby freeing the S-CDK, which goes on to stimulate DNA duplication.

The process described above is quite complicated even in this condensed form, and actually is considerably

more complicated in its technical details. But the detailed description that Deshaies and his colleagues have achieved is important fundamental science that could have technological implications in the future, Deshaies says.

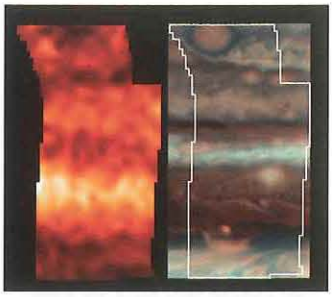
"This traces the ignition of DNA synthesis down to a relatively small set of proteins," he says. "Any time you figure out how a part of the cell division machinery works, you can start thinking about devising new strategies to turn it on and off."

It is a precise turning on and off of DNA replication, many researchers think, that will someday be the key to better and more specific cancer-fighting drugs. Because a tumor is a group of cells that literally never stops the cell duplication cycle, a greater understanding of the cycle itself is almost certain to be a factor in further medical advances in cancer treatment.

"It could be five to 10 years, but this work could point the way to new cancer-fighting drugs," Deshaies says. "It is much easier to begin a rational approach to developing new treatments for cancer if you are armed with fundamental insights into how the cellular machinery works."

The other authors on the paper in the October 17 issue of *Cell* are Caltech grad student R. M. Renny Feldman, postdoc Craig C. Correll, and Kenneth B. Kaplan, a postdoc at M.I.T.

The other authors of the *Science* paper from the October 17 issue are Rati Verma, a senior research fellow at Caltech; Gregory Reynard, a Caltech technician; and R. S. Annan, M. J. Huddleston, and S. A. Carr, all of the Research Mass Spectrometry Laboratory at SmithKline Beecham Pharmaceuticals in King of Prussia, PA. □—*RT*



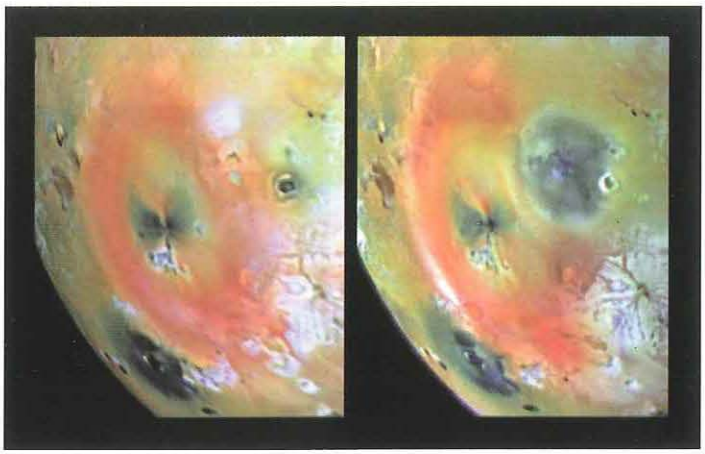
Above: A high-resolution map of Jupiter's temperatures (left) and a Hubble Space Telescope view of the same area (right), taken within 10 hours of the Galileo map. The visually bright spots are generally colder than their surroundings, indicating that rising gas is cooling and forming reflective condensates.

Above: This one-minute exposure of a piece of Jupiter's night side, which was taken in the moonlight of Io, has been colored red for dramatic effect. The white patches near the top are lightning storms, made visible by multiple bolts during the exposure. As befits the king of the gods, Jove's lightning bolts are hundreds of times more powerful than terrestrial ones.

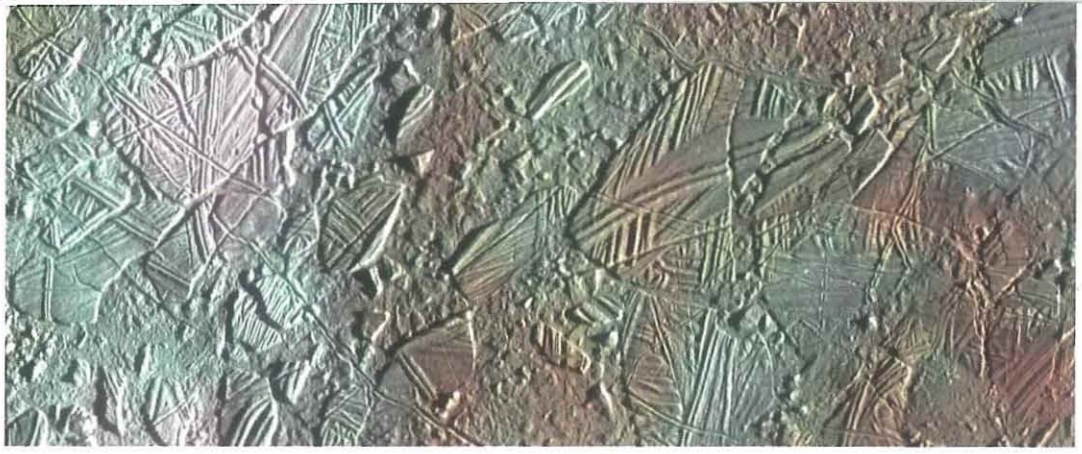
Galileo: Primary Mission Accomplished

JPL's Galileo spacecraft ended its two-year, 11-orbit primary mission to Jupiter on December 7, 1997. The program will henceforth be known as the Galileo Europa Mission, or GEM. Galileo will swoop past Europa eight times in the next two years, looking for hints as to whether a liquid ocean lurks under Europa's fractured, icy crust. Four loops by Callisto will follow, which will slow the spacecraft and alter its course for Io. If the intense radiation in Io's neighborhood doesn't prove fatal, Galileo will then make two close passes by Jupiter's pizza-faced moon for a detailed look at its volcanic surface.

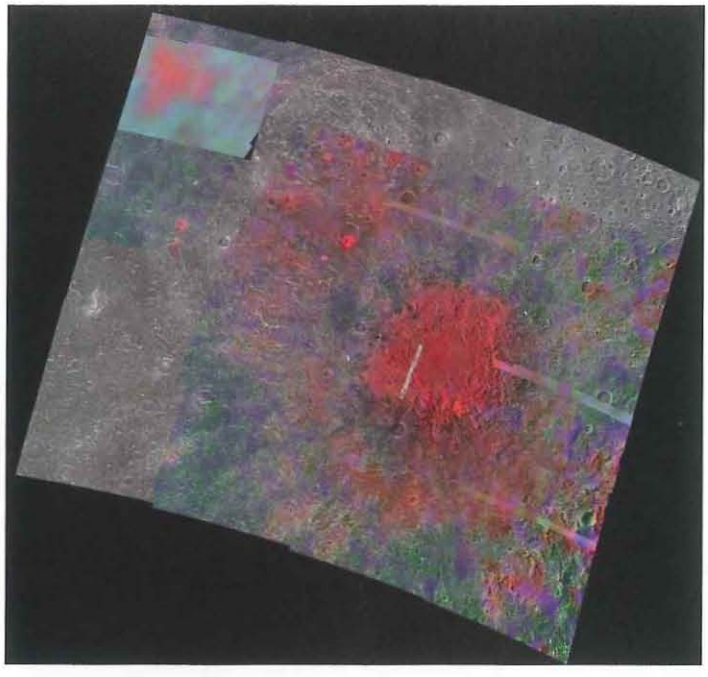
During its primary mission, Galileo beamed back roughly a billion bytes of data, enabling such nonphotogenic discoveries as the tenuous atmospheres around Ganymede, Callisto, and Europa; the metallic cores within Io, Europa, and Ganymede; and Ganymede's magnetic field. The spacecraft also returned more than 1,800 pictures of Jupiter and these four moons. Here are some recent highlights.



Above: Between April (left) and September (right), 1997, an Arizona-sized dark spot formed on Io around a caldera, or volcanic depression, called Pillan Patera. (The red ring is deposits from Pele, another volcano.)

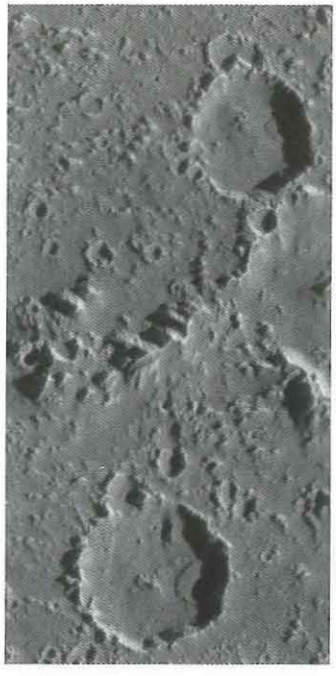


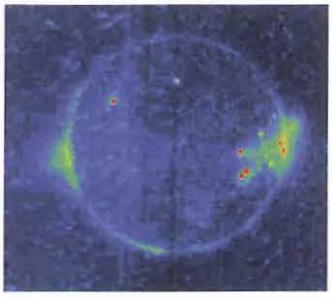
Above: In this color-enhanced view of Europa, the blues and whites come from a dusting of fine ice particles ejected by the impact that formed a 26-kilometer-diameter crater some 1,000 kilometers to the south. The rest of the surface has been painted reddish-brown by mineral contaminants that escaped from beneath the crust when it fractured. Europa's original color was probably the deep blue seen elsewhere over large areas of its surface.

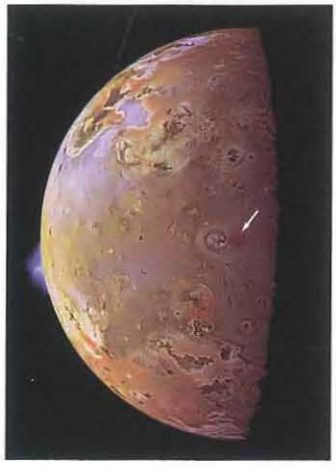


Right: This false-color image of part of Callisto's southern hemisphere combines visible and infrared data. Red indicates icy areas, while regions with less ice are blue. The big red blotch in the center is an unnamed, 200-kilometer-diameter impact crater; several of the other red blotches correlate with lesser craters, suggesting that an icy subsurface underlies a thin coating of darker material.

Far right: The pancake-shaped deposits on the floors of these two craters are landslides from the crater walls. Each landslide is about 3–3.5 kilometers long. The fact that they traveled such large distances may indicate that Callisto's surface material is very fine-grained.







Far left: Io glows in the dark. This false-color image was taken in visible light when Io was in Jupiter's shadow. Red marks the most intensely glowing regions (lakes and flows of hot lava), with dimmer areas trailing off through yellow and green into blue. Like our moon, Io always keeps the same side facing its planet. The point closest to Jupiter is at the right-hand edge of this image, where a field of hot spots can be seen. Jupiter's tidal influence pulls Io's surface some 50 meters out of round at this point, squeezing and heating the magma. The diffuse glow on Io's left limb hangs over a volcanic vent named Prometheus, and extends some 800 kilometers into space, although the visible plume is only about 75 kilometers tall. Left: In this true-color image, the plume on Io's limb is the first one ever seen emitted by Pillan Patera. The reddish-brown shadow of Prometheus's plume is marked by the arrow. (The vent itself is in the center of the adjoining dark ring.) Prometheus, which was discovered by Voyager 2 in 1979, may have been continously active for more than 18 years.

What makes a flower? What makes these organs, and what makes them appear in the same sepal-petal-stamen-carpel order, time after time, species after species?

Late Bloomer: *Arabidopsis* Arrives

by Rebecca Rothenberg

Professor of Biology Elliot Meyerowitz is surrounded in his meat locker cum nursery by his charges—hundreds of *Arabidopsis* mutants in various stages of development, some just beginning to germinate, some gone to seed.

What makes a flower? A seed, dirt, sun, water, Miracle-Gro, and a little luck, right?

Okay, let's put it another way: what makes *up* a flower? Ah. Well, petals, of course. Those little green leaves that enclose the bud and remain outside the petals—the sepals. The long filaments in the middle of the petals—the stamens, or male, pollen-carrying organs. The other, female, structure at the center—the carpel, which contains the single or compound ovary that becomes a seed.

In fact, as it turns out, almost every flower in the world, from rose to camellia to carnation to wild mustard, has exactly the same parts, or organs, in exactly the same pattern: concentric whorls of—from the outside in—sepals, petals, stamens, and carpels. Check it out in your garden. (The aster, or composite, family, such as daisies, dandelions, and sunflowers, in which each "petal" is actually a complete flower, has different terminology, but the rule holds nonetheless.)

So let's return to our first question: what makes a flower? What makes these organs, and what makes them appear in the same sepal-petal-stamen-carpel order, time after time, species after species? And what tells each plant of a given species to make the right *number* of organs and with the right spacing in between (for example, in the wild, *Arabidopsis* almost always has four petals and six stamens, whereas members of other plant families have different numbers of organs).

Professor of Biology Elliot Meyerowitz thinks he knows the answers to some of these questions. He's identified the sequence of master regulatory genes that turns on the instructions to make flower organs appear in the appropriate whorl. In fact, Meyerowitz can make a flower that's all sepals. Or a lush (but sterile) bloom comprising four whorls of petals. Or what he jokingly calls "a manly thing," consisting only of stamens.

Meyerowitz's work has unfolded in his lab in the northwest wing of Church, which at first glance looks like every other biology laboratory: the Ikea kitchen section run amok. Endlessly replicated shiny counters are covered by glassware and machinery of unknown purpose. But make a wrong turn and suddenly you're in a closet-turned-potting shed, garden spade leaning up against 20-pound sacks of planting mix, green garden hose coiled at your feet. Cross the hall and you're in another familiar milieu, a garden nursery. Actually, Meyerowitz explains, it's a fluorescent-lit meat locker modified into a nursery: refrigerator shelves designed for shrink-wrapped pork chops instead hold hundreds of flats of weedy little plants. Some are barely germinating, some are flowering, some are very strange-looking indeed, with strap-like structures instead of stems, and flowers growing higgledy-piggledy up the sides instead of in an orderly pattern. Some have gone to seed, sending up scaffolds of dry seedpods that give the room the forlorn look of a vacant lot.

The protean *Arabidopsis thaliana* can be classy (right) or funky (below).

Yet these homely weeds—mouse-ear cress, or, more properly, *Arabidopsis thaliana*—are the heart of the lab, and one of Meyerowitz's most important contributions to contemporary genetics.

Arabidopsis thaliana is a diminutive member of the mustard family. It stands about five inches high, has a rosette of leaves at the base of its stem and a stalk of tiny, four-petalled white flowers. In the wild it looks like, and is, a scruffy cousin to sweet alyssum; it was named for Johannes Thal, a 16th-century herbalist who first described it. "Not," says the soft-spoken Meyerowitz in his office behind the closet/potting shed, "for the Greek muse of comedy," Thalia. Though there *is* something comic and endearing about the little plant. It inspires metaphors—"the people's plant" and "the Hyundai of plants"—and pranks: Meyerowitz has a slide of a chia pet furry with sprouting *Arabidopsis*, the gift of Mike Nasrallah, a Cornell colleague.

When Meyerowitz set out to determine which genes tell a particular cell in a plant's growing shoot, or apical meristem, to become part of a sepal, as opposed to, say, a petal or carpel, he knew he would begin in classic Mendelian fashion, by looking at mutations in the plant's phenotype and inferring information about its genotype. Unlike Mendel, however, who had to wait for nature to produce those interesting mutations, 20th-century geneticists can induce mutations by soaking seeds in a mutagenic agent like ethyl methanesulfonate.

But at the outset Meyerowitz was faced with a fundamental decision. What would he use as the experimental organism? Peas, like Mendel? Maize, like Barbara McClintock? Some cash crop, like wheat, tomatoes, or tobacco? And here Meyerowitz made a very canny choice, grounded in his instincts and training as a molecular biologist. He chose *Arabidopsis.*

Before Meyerowitz, *Arabidopsis* was not unknown in the lab. It had obvious research advantages: small size, short generation time (four to six weeks), prolific seed production, and the sheer tenacity to flourish in fluorescent-lit labs. As early as 1907, in fact—just about the time zoologist Thomas Hunt Morgan was introduced to an obscure little "fruit" (technically vinegar) fly, *Drosophila melanogaster,* by a colleague at Cold Spring Harbor—a German graduate student named Friedrich Laibach determined the chromosomal content of *Arabidopsis thaliana.* But while *Drosophila* rapidly climbed the biological charts, producing fascinating mutations, many PhDs, one Crafoord and two Nobel Prizes for Caltech professors—most recently in 1995, for Morgan Professor of Biology, Emeritus, Ed Lewis—and, finally, its own on-line arcade game and Web site (http://flybrain.uni-freiburg.de/), *Arabidopsis* remained a wallflower, pretty much sitting out the 20th century.

It was heard from briefly in 1943, when the loyal Laibach returned to his early research and once again extolled the virtues of *Arabidopsis* as a research organism. But plant geneticists continued to work with the useful or the beautiful: familiar species like petunias, tobacco, tomatoes, and maize.

But advances in molecular biology were beginning to promote a whole new approach to genetics: rather than simply inferring a gene's function from its expression in the organism's phenotype, researchers were beginning to understand, and to be able to manipulate, the chemistry of the gene itself. The new techniques were tested and developed using simple animals with few genes—the bacterium *E.coli*; the roundworm *Caenorhabditis*; and of course, *Drosophila*—but by the early 1970s a few forward-looking, or long-memoried, plant geneticists began to take a second look at *Arabi-*

dopsis. Those five chromosomes Laibach had counted in 1907 were the smallest of any known flowering plant, pointing to a modest, manipulable genome.

So when University of Missouri agronomist George Redei in a 1976 review article once again took up the banner of *Arabidopsis*, which he referred to, charmingly, as "our beloved organism," the scientific world was almost persuaded. Chris Somerville, now director of the Carnegie Institution of Washington Department of Plant Biology at Stanford, began to use the plant to investigate the genetics of photorespiration. But his work did not, he has remarked, trigger the groundswell of *Arabidopsis* research he expected. In fact, when Maarten Koornneef and colleagues at the Agricultural University of Wagenigen in the Netherlands put together a linkage map of *Arabidopsis*, the paper had difficulty finding a publisher due to lack of interest.

Then, in the early 1980s, mouse-ear cress got lucky. It caught the attention of Elliot Meyerowitz.

Meyerowitz was an unlikely champion. Though he had attended plant genetics seminars in graduate school at Yale, he had never taken a botany course and to this day claims to be fuzzy on the particulars of plant physiology. He was a fly man: his postdoctoral work at Stanford dealt with *Drosophila*, and at Caltech, which he joined in 1980 as an assistant professor, he investigated the regulatory effects of steroids on a gene that produces a glue-like protein in *Drosophila.*

But sometime in the early '80s he became interested in the developmental genetics of plants. It was a relatively unstudied field and it "seemed like fun," he says. "People were beginning to look at individual genes in animals—at their genetic and genomic structures. I got really curious to know how different plants were." To investigate this he would need a plant that was small, easy to grow—and, in order to take advantage of those new techniques in molecular biology, possessed of a lean-and-mean genome. He would need. . . *Arabidopsis*.

So Meyerowitz and his colleagues set out to realize the research potential of Laibach's organism. In a 1985 paper in *Science* he and Caltech graduate student Robert Pruitt laid out a campaign. First, they determined just how small *Arabidopsis's* genome was. They reported about 70,000 kilobase pairs—that is, 70 million letters of DNA; current reports are somewhat higher, but the essential estimate of about 20,000 genes remains. If this sounds daunting, maize, another staple of plant genetics, has about 2,500,000 kb pairs and 30,000 genes. The ratio of these two genomes to number of estimated genes hints at another fact about *Arabidopsis's* genome: very little of it is "junk DNA"—DNA that does not translate into proteins, much of it mysterious repetitive sequences, like stutters in a genetic statement, that serve no known purpose other than to bedevil molecular biologists. Meyerowitz calls the elaborate work he had to do to establish these basic facts "a piece of history"; in a field that progresses as rapidly as genetics—when today's graduate students can buy kits from mail-order catalogs to clone genes, isolate DNA, or radioactively label probes—15-year-old methodology seems as archaic and cumbersome as grinding your own flour to make a cake.

In the same 1985 paper Meyerowitz proposed to construct an RFLP (restriction fragment length polymorphism) map, in order to be able to isolate and clone specific genes. Building on Koornneef's work, he published his map in 1988. Now Meyerowitz (and others—the map and the DNA library were made generally available) could begin to exploit *Arabidopsis* to investigate some research questions.

And the question Meyerowitz eventually came

Plant breeders have known for centuries how to produce such beautiful floral mutants as the camellias above, in which petals have turned into stamens (center) and stamens into petals (bottom). They have not been particularly interested in the scraggly mouse-ear cress (posing at right with the far lovelier poppy, which also happens to have four petals), but the humble little weed has a beauty of its own as a research organism.

By charting the patterns of inheritance over several generations, geneticists can puzzle out the order in which the genes lie on the chromosome.

Of Mouse-Ear Cress and Maps

Alfred Sturtevant invented linkage mapping in about 1913. Later he became one of the original faculty members of Caltech's Division of Biology, founded in 1928. Sturtevant was Ed Lewis's adviser in the '30s, and, like Lewis, worked with Drosophila. But, after his retirement, he also did genetic tests with irises, descendants of which are planted in a memorial garden behind Parsons-Gates.

Genes are strung in a fixed order on the chromosome, so the idea of mapping is to determine where exactly a gene lies. In theory, this could go down to exact numbers—a gene lies at letters 36,504 to 37,391, say, in chromosome 5. Creating such maps is one goal of genome sequencing projects. In the meantime, researchers are trying to figure out which genes are close to each other—drawing what are called linkage maps, of which restriction fragment length polymorphism (RFLP) maps are one kind.

Like so many other things, linkage maps start with sex. Ordinary cells have two complete sets of chromosomes—one from each parent. During the early stages of meiosis, the process by which sperm and egg cells are generated, the chromosomes pair off and trade genetic material back and forth. The chromosomes, which look like capital Xes, high-five each other, and wherever the arms (or legs) of the two Xes touch, they swap. It's as if two people bumped elbows and each came away from the encounter wearing the other person's forearm instead of their own. This genetic shuffling determines whether you get your mother's hair and your father's eyes (or your father's petals, if you're a plant), and in the longer term drives variations within a species and, ultimately, evolution. Each egg or sperm gets one set each of the new, mixed'n'matched chromosomes, so when they combine, the fertilized egg has the normal complement of two sets of chromosomes.

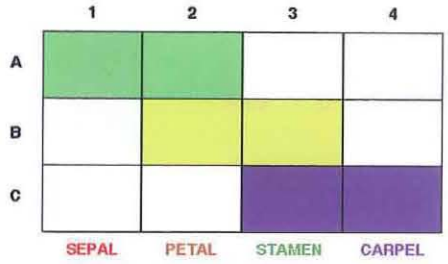
The DNA that crosses over is generally hundreds or even thousands of genes long, so if two genes are close to each other on the chromosome, the odds are they'll stay together during the trading session—either both of them will move, or neither will. This is the linkage in linkage mapping. But as they become separated by longer and longer stretches of DNA, they begin to behave more independently. So the frequency with which genes migrate together is a proxy for how close they are. By charting the patterns of inheritance over several generations, geneticists can puzzle out the order in which the genes lie on the chromosome.

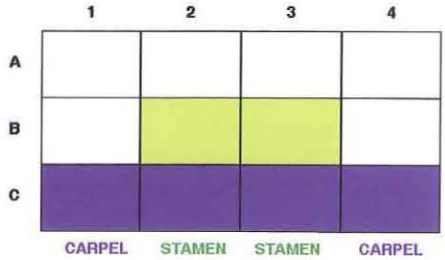
In RFLP mapping, the chromosome is treated with a restriction enzyme, which recognizes a four- to eight-letter stretch of DNA code and cuts the DNA wherever that code appears. This gives an assortment of fragments of various lengths. A process called gel electrophoresis sorts them by length—longer fragments are heavier and don't move as far from the point of origin. A series of other treatments eventually makes the fragmentation pattern visible as a set of dark blobs.

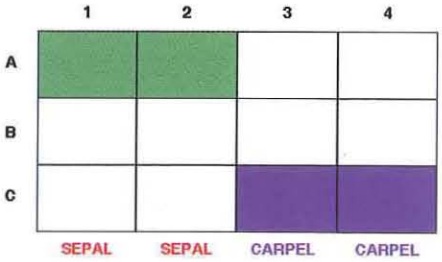
And here's the nub: many genes have subtle variations within their DNA sequences—just a letter or two here or there—that don't affect their functions, but alter one or more sites where the restriction enzyme should cut them. Thus, two individuals with different variants of the gene will have different fragmentation patterns—the site that should have been cut but wasn't will now be part of a longer fragment that won't move as far. (Hence the name restriction fragment length polymorphism—polymorphism is a five-dollar word meaning "many forms.") These patterns, again, are inherited with the DNA, and since RFLPs are very common, the odds are good that there'll be one reasonably near the gene you're trying to map. Furthermore, there are hundreds of known restriction enzymes, each of which recognizes a different sequence of letters, and new ones are being discovered all the time. □

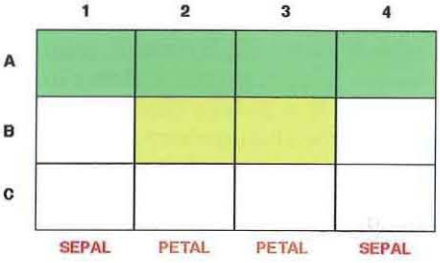
—DS

The normal, or wild-type, *Arabidopsis* contains (from outside whorl to inside) four sepals, four petals, six stamens, and two carpels.









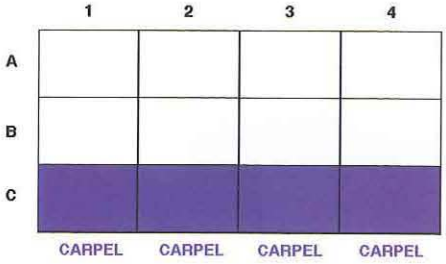
In the graphs above, the numbers along the top indicate the whorl (position from outside to inside), and the organ types run along the bottom axis. Along the vertical axis are the groups of master regulatory genes that specify organ identity; "A" controls the organs in whorls 1 and 2, "B" in 2 and 3, and "C" in 3 and 4. In the wild-type flower at top, the A genes produce sepal and petal; B, petal and stamen; and C, stamen and carpel. Knocking out the A genes (second from top) gives rise to a bizarre flower with carpels and stamens where the sepals and petals should be. When the B genes are disabled, a flower of sepals and carpels emerges. And C-class mutants (bottom) consist of only sepals and petals. In each case, the number of organs in each whorl can remain the same as in the wild-type flower.

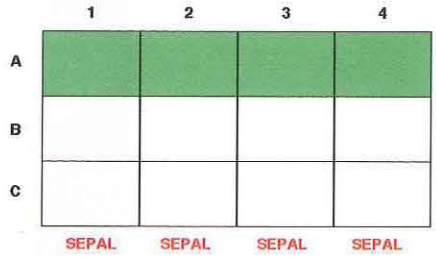
to ask of *Arabidopsis* was the one we began with: How do you make a flower? In other words, what Ed Lewis had done with *Drosophila*—looking at such mutations as an extra thoracic segment or misplaced set of legs to determine which genes were homeotic, that is, regulators of organ identity and position—Meyerowitz proposed to do with *Arabidopsis*.

In retrospect, flowers, with their simple, familiar, nearly ubiquitous pattern, seem ideal for this kind of research. Meyerowitz displays a German text from the 1930s that scrupulously documents and catalogues mutations in snapdragons. "It's hard to understand why they didn't go on to the next step, to try to look at flower development," he says with a kind of bemusement. "They had all the mutants but they never made the theories."

But Meyerowitz did. Patiently knocking out genes in the *Arabidopsis* seeds and observing the results in the nursery, he and his team eventually demonstrated that three groups of genes govern the four whorls of flower organs in an overlapping fashion: group "A" specifies organ identity in whorls one and two (normally sepal and petal); "B" in two and three (petal and stamen); and "C" in three and four (stamen and carpel). A and C are also mutual antagonists: the action of one suppresses the other. So in A-class mutants, that is, mutants in which the "A" genes have been disabled, carpels replace sepals and stamens replace petals, and carpel-stamen-stamen-carpel flowers develop; B-class mutants create sepal-sepal-carpel-carpel flowers; C-class, sepal-petal-petal-sepal. If all three groups are missing, a flower consisting entirely of leaves is produced.

Meyerowitz is at a loss to explain why no one did this work earlier, since this part of his work, which resulted in the construction of the A-B-C model, is simple in concept—"not 'deceptively simple,'" he insists, "just simple"—and was accomplished with the techniques of classic genetics. Certainly there were no technical limitations;





These examples of *Arabidopsis* are both double mutants. Knocking out the B and C genes produces a "flower" that is all sepals (above, right), and when the A and B genes are disabled, you get all carpels (right).

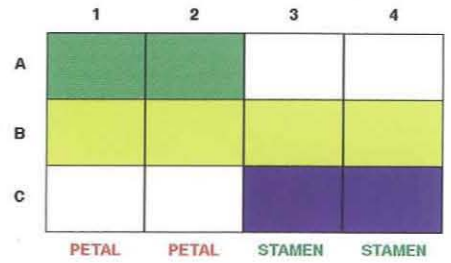
about this Meyerowitz is adamant. His model was constructed with 50-year-old techniques and classic, zap-it-and-see-what-happens methodology; that is, induce mutations, observe phenotypic changes, infer genetic changes, cross and back-cross to identify mutated gene. He sometimes wonders if somebody *did* do the work before him; "Maybe I'll come across it in the library one day, somebody's PhD thesis done decades before I did it," he says. In fact, at about the same time, similar, and complementary, work was being done with snapdragons by Enrico Coen of the John Innes Institute in Norwich, UK.

But the definitive tests of the Meyerowitz model, the tests that moved his work beyond classical genetics, involved actually isolating the genes of interest, cloning them, and reinserting them into plants in which they'd been knocked out, to confirm that they did in fact perform the predicted function. This capability didn't exist before the early 1980s, and it didn't exist for all organisms. But because Meyerowitz had done the initial legwork with *Arabidopsis,* he was able to perform the final tests—with the labor and collaboration of many, many graduate students and postdocs, he emphasizes.

In fact, the *Arabidopsis* genes performed the same regulatory functions when inserted into other plants—in petunias, for example, and in tobacco. Transgenic tests like these are perhaps the most persuasive arguments in modern genetics, and the most profound. They demonstrate the conservation of genes down through the evolutionary process; or, as Meyerowitz has said more eloquently, "the unity of life—one of the great, satisfying conclusions of modern genetics."

Chatting with Meyerowitz in his office, where he is self-effacing—describing himself as "father and couch potato"—informal, digressive, and wryly humorous, you might not think him capable of such ringing statements. But in formal talks, such as last year's Watson and Bi 0.1 lectures, he is passionate and lucid, and has a gift for communicating the Byzantine, recursive complexities of current genetic theory in concrete language. It is perhaps this articulateness (not to mention the photogenicity of *Arabidopsis* itself), that has led to his work being recognized not only by his peers—Meyerowitz is a member of the American Academy of Arts and Sciences and the National Academy of Science, and has recently won the Medal of the Genetics Society of America, the Mendel Medal of the UK Genetical Society, Japan's International Prize for Biology, and the "Science pour l'Art" Prize of LVMH Moet Hennessy·Louis Vuitton—but by the popular press as well. He and his work have been profiled in *Newsday, Discovery, Mosaic,* and *The New York Times.*

Meanwhile, both in his own lab and as former chair of the Multinational Arabidopsis Genome Research Project (similar to the Human Genome Project), Meyerowitz continues both to map *Arabidopsis* and to put *Arabidopsis* on the map,



Activating the B genes throughout the flower leads to replacement of sepals with petals and of carpels with stamens, creating a flower with two whorls of petals, for a total of eight, and a set of extra stamens where the ovary would be.

coordinating an effort that is, at least relative to other sequencing projects for cereal crops such as corn and rice, a model of international data sharing.

And Meyerowitz continues to explore the mysteries of the regulation of cell division in developing flowers. "The organ identity stuff was nice," he says; "it came to a pretty simple set of answers"—(and some possible practical applications for agriculture: an all-carpel flower, for example, might produce several times the usual number of seeds)—"but it raised a series of more complex questions." You might call them the "downstream questions": what is happening to the genes that the master regulators regulate—the genes that control organ *number*, for example? Despite the shuffling of organ identity produced by the manipulations of the A-B-C model, *Arabidopsis* produced a normal *number* of organs in each whorl: four in the first, four in the second, six in the third, two in the fourth.

However, Meyerowitz's lab has identified a class of genes—the CLAVATAs, so-called for the "club-shaped" mutations they produce—that regulate not organ identity but organ number. CLAVATA1, for example, seems to set up the apical meristem, the plant's growing tip, which forms the substrate for flower organs. When mutant, this gene produces the strap-like structure with too many flowers that's growing in the nursery. Jenn Fletcher, a postdoc, is on the verge of isolating CLAVATA3, and grad student Mark Running has isolated PERIANTHIA, which actually makes an *Arabidopsis* with *five* petals—a taxonomic disaster for botanists, who rely on characteristics like number of petals and sepals to classify plants.

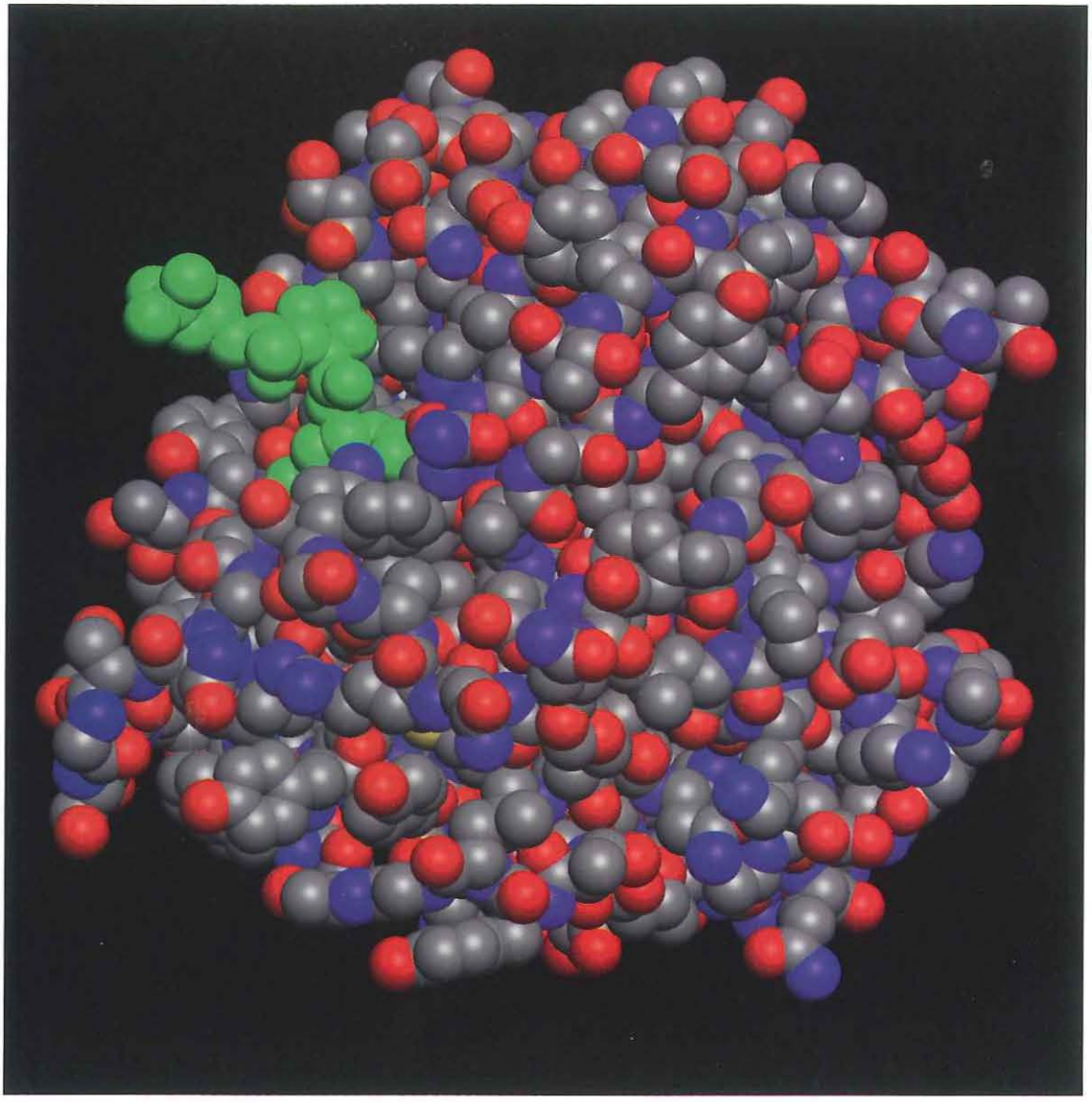
And the lab has discovered something about a gene involved in the regulation of the number of stamens. A mutant plant missing the gene may produce a dozen or more stamens—not as "manly" as the all-stamen flower Meyerowitz produced by tinkering with the master regulatory genes, but still a "superman." But Meyerowitz and postdoc Steve Jacobsen have discovered a way to merely modify the activity of the gene through DNA methylation, producing less macho "clark kent" mutations. Methylation is of particular interest to biologists, since it appears to play a role in cell memory, and, in mammals, in the inactivation of one of the two X chromosomes in females. This work sheds light on the process, since it shows that overall disruption of methylation to *Arabidopsis* is accompanied by hypermethylation in certain sequences of the plant's genome—a discovery that may have implications for medical research, since certain cancer tumors have been associated with overmethylation of genes.

In a glorious finale to a century of relative obscurity, Astronaut *Arabidopsis* is about to ride a space shuttle in an experiment that will provide insight into the effect of gravity (or no gravity) on root growth. And if a plan to convert the abandoned USDA greenhouse at the corner of Del Mar and Michigan into greenhouses for Meyerowitz is approved, his *Arabidopsis* may be leaving its meat locker for swankier digs.

So Elliot Meyerowitz has been good to mouse-ear cress, and vice-versa. But Meyerowitz dismisses any special fondness for "our beloved organism." "Look around," he says, gesturing at the walls of his office; "do you see any needlepoint of *Arabidopsis*? Any statues?" Well, no. At the time there were a couple of classy botanical posters from the Huntington Library, and a large tapestry of dogs playing poker—a running joke, which has since been replaced by a painting presented to him by Maya Lin, designer of the Vietnam Memorial in Washington, DC, and a fellow winner of *Science pour L'Art*. No monuments to *Arabidopsis*. It's simply a vehicle, he says; if there were another organism that served his research purposes better, he'd use it.

But you have to be careful with *Arabidopsis*. After about 60 or 70 years, it grows on you. □

A protein automatically twists its backbone around until it curls itself up into its proper shape—it's as if you could thread all the parts of a turbocharged big-block Chevy V-8 one by one onto a piece of twine, throw the twine into a tub of water, and pull out the fully assembled engine.



No Assembly Required

by Douglas L. Smith

Proteins are the machinery of life, and they work over an astonishing range of conditions—from subzero Antarctic waters, where the fish have protein antifreeze in their blood, to geothermal vents where steam-scalded bacteria live in ambient temperatures above 150° C. Increasingly, proteins are the machines of industry as well. And not just designer drugs (or even generic drugs, such as insulin), although that's the high-profile end of the business, but such humble products as the stain-removing enzyme in your laundry detergent. (An enzyme is a protein designed by nature to make a specific chemical reaction occur rapidly and selectively.) In fact, the worldwide market for nonbiological enzymes was $1.3 billion in 1996, according to World Wide Web pages maintained by the Danish firm Novo Nordisk.

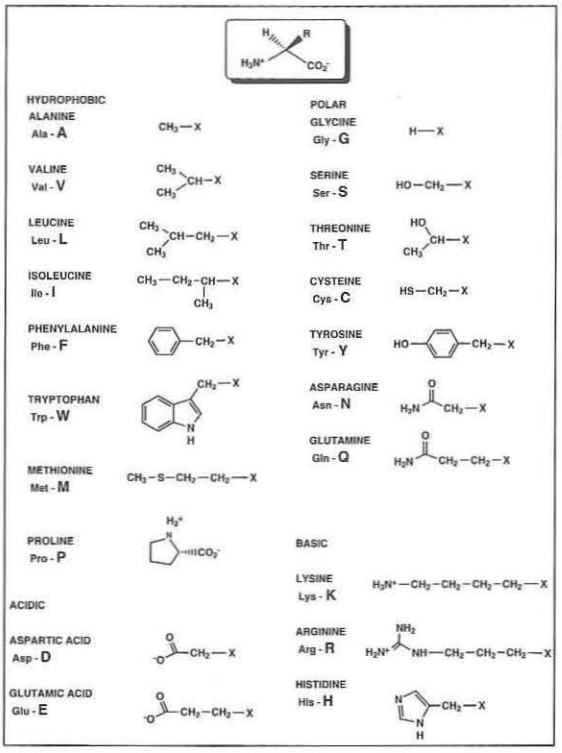
A protein is the architect's maxim of "form follows function" taken to its logical conclusion—the only thing that makes a protein work is the shape into which it folds. An enzyme has a pocket tailored to fit the reacting molecules, and grappling hooks in the right locations to entice the molecules into the pocket and hold them fast while the reaction happens. Chemical, and especially pharmaceutical, companies are getting very good at finding an enzyme that sort of does what they want, and they're starting to learn how to tinker with the structure of the enzyme until it does the right thing. But they'd really like to be able to say, "We want these two molecules to react in this manner, and we need a protein to hold them in this configuration to encourage them to do so. Computer, design me that protein!" In fact, this is one of the central challenges of modern biology. Stephen Mayo, PhD '87, assistant professor of biology at Caltech and assistant investigator with the Howard Hughes Medical Institute, has solved a simpler problem en route to that goal: designing a protein from scratch that will fold up into a predetermined shape.

Just as machines have a basic vocabulary of parts—ball bearings, springs, cotter pins, axles, washers, and what have you—proteins are made up of components called amino acids, or residues, of which 20 varieties are commonly found in nature. These amino acids are strung together in a linear sequence, like a train of railroad cars, in what is called the protein's primary structure. And just as parts become subassemblies—carburetors, for example, or distributors—sequences of amino acids can naturally assume certain shapes. These shapes, which include helices, hairpin turns, and wavy sheets, are the protein's secondary structure. (The late Linus Pauling, PhD '25, deduced these structures from crystallographic and bond-angle data at Caltech in the 1950s.) These secondary structures, in turn, come together in specific orientations, called the tertiary structure, to form the biologically active protein. But context matters, too—the tertiary structure surrounding a string of amino acids can influence the secondary structure it chooses to assume. A tertiary structure that occurs over and over again in different proteins is called a motif.

Proteins and machines differ in one crucial respect. Machines have to be built and, as any backyard mechanic knows, seemingly identical parts often aren't interchangeable, and some parts only fit one way. Steps have to be followed in order, or you'll soon find yourself removing things in order to install other things that should have gone on first. And woe betide you if you find leftover parts at the end! But a protein automatically twists its backbone around until it curls itself up into its proper shape—it's as if you could thread all the parts of a turbocharged big-block Chevy V-8 one by one onto a piece of twine, throw the twine into a tub of water, and pull out the fully assembled engine. This shape is entirely determined by the protein's primary structure: a given string of amino acids will scrunch up exactly the same way every time—it doesn't matter if the protein is being made in a cell in your spleen, or a vat

This big bag of marbles is really subtilisin, a bacterial enzyme that chews up proteins and is widely used as a stain-removing agent in laundry detergents. The protein to be cut up, a part of which is shown here in green, fits into a pocket on the enzyme's surface. Only about five percent of subtilisin's 275 amino acids go into forming the pocket; the rest are there primarily to hold that five percent in place. Carbon atoms are shown in gray, oxygens in red, nitrogens in blue; for clarity, hydrogens aren't shown at all.

Right: The 20 natural amino acids. The backbone unit is shown in the small box; the "R" stands for any of the side chains beneath. (In the side chains, the backbone is abbreviated as "X.") The amino acids designated "acidic" or "basic" are also polar. Each amino acid has a three-letter and a one-letter code, used for simplicity's sake when writing out sequences. Below: Subtilisin's primary structure (top); the colors correlate to its secondary structure (bottom)—helices are yellow and sheets are green. The protein fragment being chewed is shown in gray.



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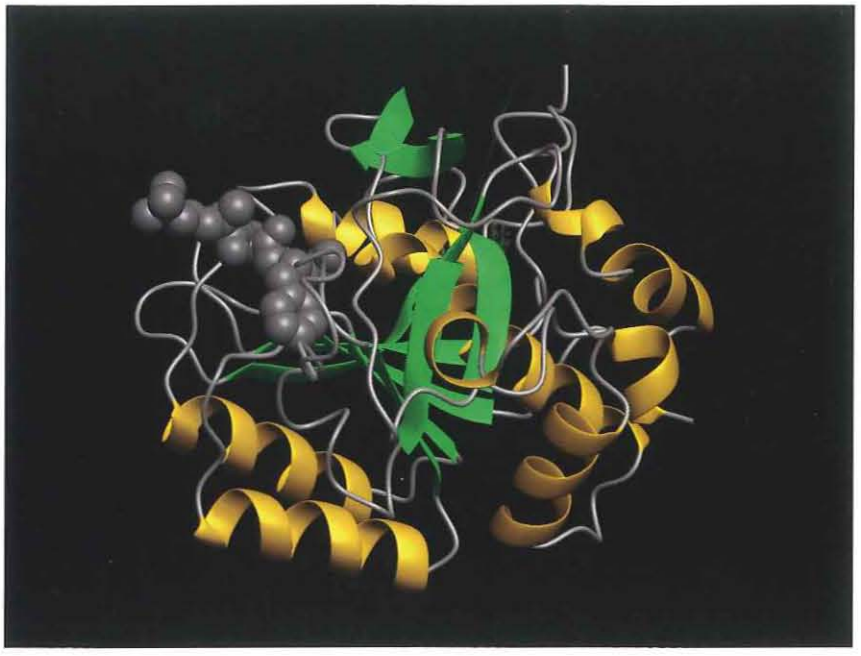
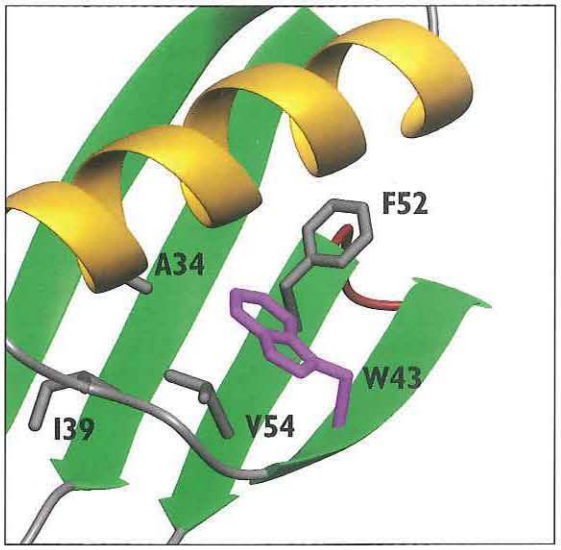


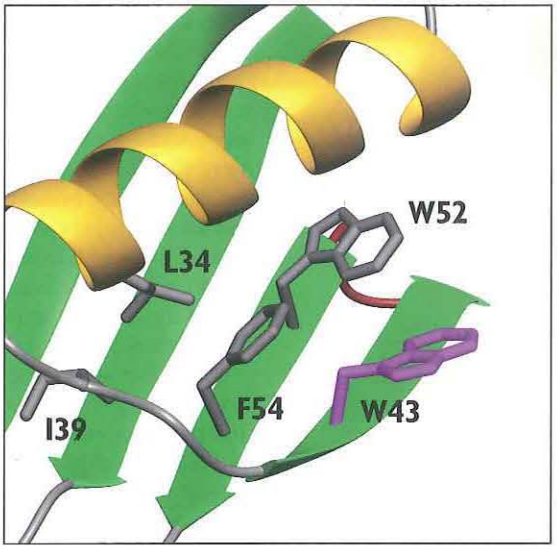
in a factory. (Assuming the protein folds at all, that is—for reasons not well understood, even a nice, stable natural protein, when synthesized outside its normal cellular environment, may lie limp or form a hopeless tangle that refuses to cooperate.)

All amino acids have the same backbone unit, enabling them to be coupled together in any order, but each amino acid has a different side chain dangling off that backbone. These side chains determine the protein's shape and all its other properties. Hydrocarbons, for example, are oily, so side chains made exclusively from carbon and hydrogen atoms mix with water like, ummm... well, oil and water. These hydrophobic side chains flee toward the protein's core, hiding as far from the surrounding water molecules as they can get. But side chains containing nitrogen or oxygen atoms are "polar"—electrically neutral overall, but with just a whiff of negative charge on the nitrogen or oxygen, and a corresponding soupçon of positive charge on an adjoining hydrogen. Water molecules are also polar, so polar side chains like to be on the protein's surface. This compulsion to embrace or avoid water is the hand that wads the protein up. But as the protein curls, the side chains have to accommodate one another. Some side chains are big and bulky and push their brethren aside to make room for themselves; others are quite compact. Some are long and floppy, like overcooked spaghetti; others are flat and stiff, like playing cards. And some form bonds of various kinds with one another. The sum of these manifold attractions and repulsions give the protein its ultimate shape.

So how do you pick the primary structure that will fold itself into the shape you want? Most people have taken one of two approaches. One way is to string amino acids together by eye, as it were—using the biochemical intuition gained through years of working with a particular motif. This has had its successes, but the knowledge gleaned about the subtleties of one motif rarely applies to another. The other method involves synthesizing as many random sequences as you have time and money to make and basically throwing them at the problem—using some sort of screening method (such as reaction-rate enhancement or binding affinity) to see if any of your sequences have the desired result. This approach rapidly gets out of hand—with 20 different amino acids to choose from at each position in the sequence, a string of 10 amino acids, which is about the upper limit for this method, gives you 20^{10} (about 10 trillion) possible primary structures. At this rate, you tend to run out of patience and raw material pretty fast. Things get worse exponentially as the string's length increases—one more position creates 20^{11}, or 205 trillion, possibilities.

Wading through these endless possibilities is clearly a job for computers rather than humans.





This is a close-up of the β1 domain of streptococcal protein G, a protein that resides on the surface of the streptococcus bacterium and is part of the molecular camouflage that allows it to sneak past the immune system. The tryptophan residue in purple (labeled W43 because it's the 43rd residue from the protein's N-terminus) is a classic transition position. In the structure at left, more than 90 percent of the surface area of the tryptophan's hydrophobic side chain is buried in the core. In the structure at right, the side chain is only 46 percent buried, leaving more than half of its surface exposed to water. The bulkier amino acids at positions 34, 52, and 54 have forced it to rotate outward.

After all, amino acids are simple molecules whose structures and properties have been studied in exhaustive detail. In recent years, people have begun to write programs—based on educated guesses as to which chemical properties to include—to generate primary structures that, when made in the lab, often fold up in manners that approximate natural motifs. But most of these programs deal exclusively with helices, sharply limiting the range of motifs that can be made. Furthermore, biological motifs are rigid, like bent coat hangers, while the man-made ones are limp, like tangled yarn. From the molecular machine standpoint, this doesn't cut it. But how to find out what interplay of properties, and in what proportions, would stiffen the tertiary structure into the one true shape?

Mayo realized that he needed a feedback loop in order to close in on the right mix of properties. To do this, he needed a target to shoot for. He decided to take the backbone of a protein whose three-dimensional structure was very precisely known, and attempt to generate a sequence from scratch whose backbone would fold up into an exact match. If the locations of all the backbone atoms matched those of the target, he figured, then the side chains would take care of themselves. (Of course, this left open the question of whether the computer would independently arrive at the original primary structure—just because every natural protein has a unique shape doesn't mean that other sequences might not also assume that same shape.) So the group would choose a set of properties, let the computer generate its best sequence, make that sequence in the lab, determine the three-dimensional structure of its backbone, and see how closely it matched the target backbone. Based on these results, they'd twiddle with the parameters, perhaps pick new ones, and try again. "This design cycle is the key," says Mayo. "Synthesizing trial sequences is a vital reality check, because the data you get from simulations are always refracted through the prism of your expectations. And you have to try sequences from all over the map, because even if you think you know where the answer is going to lie, you may have overlooked something. But this way, the experiments themselves tell us what is important."

Mayo also realized that one set of properties might not be enough—for example, the forces that make the protein's interior a safe refuge for the oily, water-hating amino acids may not hold sway at the surface. So the group broke the problem down into three parts: the core, the surface, and a transitional zone in between, where both the core and the surface properties struggle for supremacy. Finally, both to simplify the lives of the grad students who would actually have to make the stuff, and because the number of possibilities the computer has to look at gets so big so rapidly, they picked their targets from among the shortest naturally occurring primary structures that assumed reproducible shapes.

In fact, the computational swamp is deeper than you think. Side chains, as the name implies, are generally floppy, with each link in the chain free to rotate around the chemical bonds that hold them together. Even the flat, stiff side chains can rotate, like solar panels tracking the sun. So packing side chains together is not unlike working a jigsaw puzzle whose pieces are changing shape right before your eyes. (It's quite amazing, really, that such pliable stuff can hold any solid form at all.) Each amino acid has a smooth continuum of rotational shapes available to it, making the swamp, in effect, bottomless. A hint of firmer footing appeared in 1987, when J. W. Ponder and F. M. Richards of Yale University prepared tables of discrete poses, called rotamers, that each amino acid prefers to assume. Depending on the side chain's length and flexibility, the number of rotamers varies from roughly a dozen to perhaps 70, but a typical amino acid has 20 to 30 of them.

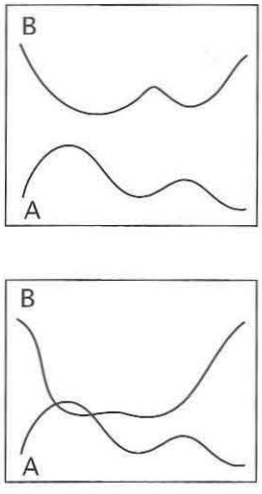
Thus a protein that's 20 residues long, with any one of the 20 naturally occurring amino acids in each position, and with each amino acid having 25 rotamers apiece, has 9.5×10^{53} possible rotamer sequences—a number that, written out, would be more than half again as wide as this column. If you examined a billion sequences per second (a feat far beyond the capacity of even Caltech's best supercomputers), it would take 10^{19}, or 10 quintillion, years to look at them all. This is a stroke of bad luck, as the universe is only about 15 billion years old—job security for the professor, perhaps, but an unwise choice for a grad student. And things get worse—nature's smallest fully functional motifs are some 30 to 40 residues long. The number of possible rotamer sequences for a typical small protein—one that's 100 residues long, say, has 7.9×10^{269} possibilities—is so staggeringly huge that your average supercomputer would gladly gnaw through its own Internet connection in order to escape having anything that big stuffed into it. According to Professor of Astronomy George Djorgovski, the best estimate of the number of protons in the entire universe is a mere 10^{80} or so.

There is a way out of this impasse. Think of the set of all possible rotamer sequences for a given primary structure as an overgrown tree of sufficient impenetrability to guard Sleeping Beauty. One string of rotamers, picked at random, is the tree's trunk. Moving any one rotamer one click to its next pose is a branch off the trunk; moving another rotamer one click as well is a branch off of that branch, and so on. In 1992, a group of Belgians (who were working on the simpler, but related, problem of trying to predict the specific rotamers that a given sequence of amino acids strung on a fixed backbone would assume) developed a procedure, called Dead-End Elimination, that prunes the rotamer tree back severely. The program lets two rotamers compete head-to-head for a single spot in the sequence, as shown in the illustrations below. If there's a clear loser, all branches in which it appears get lopped off.

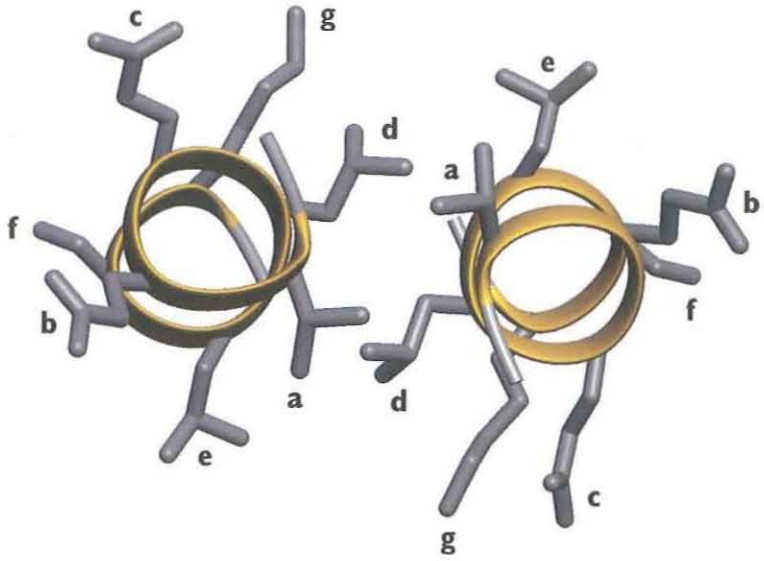
Extending the program to compare rotamers of different amino acids took considerable work, but the resulting computation is still relatively tame. It also takes a certain amount of experience to design the best pruning strategy—you want to get as close to the trunk as possible without wasting lots of time clipping the branches' tips.

So with all the tools in hand, it was time to roll up the sleeves and go to work. The group's first target backbone was the "coiled coil" motif, which consists of two identical helices wrapped around each other like strands in a rope. The fact that the helices are identical cuts the synthetic work in half—a big plus when you want to test lots of variants quickly. The coiled coil is a critical piece of a larger protein that controls DNA transcription, says grad student Bassil Dahiyat—the cell makes each helix separately, and as the two helices coil up around each other they help zip up the protein. Coiled coils are found in all cells that have nuclei, including ours—this particular motif, which rejoices in the euphonious name of GCN4-p1, comes from yeast. (The high-precision structure of GCN4-p1, which the group used as its starting point, was determined by X-ray crystallography in 1991 by T. Alber et al. at UC Berkeley.)

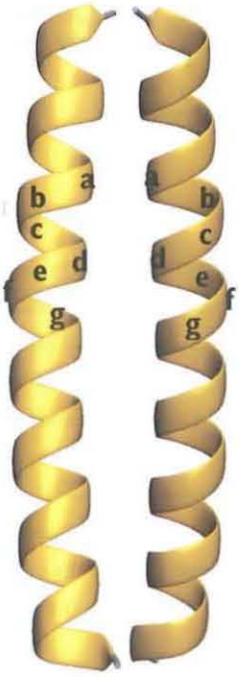
The group began with the core, whose organization largely determines how the rest of the protein arranges itself, and where the most research had already been done. The coiled-coil helix repeats itself every seven amino acids, as shown on the opposite page. If you label these amino acids **a**, **b**, **c**, **d**, **e**, **f**, and **g**, then residues **a** and **d** will always be buried in the seam where the two strands adhere to each other—in the core, in other words. Because the group was experimenting with the core, the other residues were left identical to the natural protein. All the atoms in these invariant amino acids were nailed down in their natural locations. The group studied strands 33 residues long (four repeating units plus a little extra for stability), giving eight variable amino acids per string, or 16 in all—even though the two strings' primary structures are identical, the rotamers may



Dead-End Elimination works by computing the attractive and repulsive forces between a given rotamer (here called Rotamer A) and every other atom in the protein—what's called its potential energy. The calculation is then repeated for a different rotamer (Rotamer B) in the same position in the sequence. The lower the potential energy, the more stable the structure. If one rotamer always has a higher potential energy regardless of the rest of the protein's behavior, as does Rotamer B in the upper example, then that rotamer and all its branches are pruned from the tree. But if the curves cross, then either rotamer might be favored and both must be retained.



A cross section through repeat unit of a coiled coil (above). Pairs of a's and d's alternate to form the core, as shown in the 33-residue segment (below).



still be different. The group then limited the computer's choices to the eight (reduced to seven in later work) oily, hydrophobic amino acids that one would expect to find in the core, and let the machine go to town. This much-simplified problem of 238 rotamers filling 16 positions in the sequence still gave 238^{16}, or 10^{38}, possibilities. Ouch! Fortunately, the program is very parallelizable, meaning it can be farmed out to many computers at once. A machine containing eight parallel processors took only three minutes per run to find the best sequence.

Dahiyat and Mayo eventually discovered that a combination of three parameters gives the best results. The first one, the van der Waals potential, measures how hard the rotamers' atoms are being shoved together. The side chains in the core are packed shoulder-to-shoulder, like subway commuters in a Tokyo rush hour, but you can only squeeze them so much without getting the atomic equivalent of an umbrella in the eye. The second parameter measures the amount of hydrophobic surface area that's safely buried and protected from the surrounding water molecules by other residues, and awards a stability bonus as the buried area increases. The third parameter measures the amount of polar, water-loving surface area that is similarly buried, and exacts a stability penalty as the buried area increases. This combination has correctly predicted the relative stability of all the sequences the group made and tested—an encouraging sign that they did, in fact, find the critical forces.

The stabilities were tested by comparing the temperature at which the proteins unfolded in solution—the "melting temperature." A protein that assumes a single, stable shape in solution will have a higher melting temperature than a sloppy, loosely folded protein. The floppier it is, the less energy it takes to finish unfolding it, and the lower the melting temperature.

To nobody's great surprise, the natural sequence proved to be the most stable sequence tested. However, the researchers did discover a family of only slightly less stable sequences containing different amino acids, indicating that there's room for variation, even in a structure this small. On the other hand, some of the least stable sequences were identical to the natural sequence except for a single wrong amino acid at a critical location.

Emboldened by this success, the group moved on to the surface. There are three exposed surface positions per seven-unit coil: **b**, **c**, and **f** in the lettering scheme. (Positions **e** and **g**, which snuggle up against **a** and **d** on the opposite strand, are only partially exposed and make up the transition zone between core and surface.) The group used the same general approach, but with a list of 10 polar, water-loving amino acids. This time, the dominant forces were the propensity of certain amino acids to form helices—a parameter that had been quantified in 1994 by R. L. Baldwin at Stanford, and others—counterbalanced against the number of hydrogen bonds that the overall structure could form. A hydrogen bond is a weak bond formed between a polar atom, such as oxygen, and a nearby hydrogen atom, usually from a different residue. These bonds help brace the structure.

Because the side chains in the surface positions have lots more room to flop around, it had been assumed that they didn't have much influence on the folding process. In fact, Dahiyat, fellow grad student Benjamin Gordon, and Mayo found that altering the surface amino acids caused dramatic changes in stability, as reflected in the melting temperatures. A sequence in which the surface positions were randomly filled from the list refused to curl up, and had by far the lowest melting temperature of any sequence tested. And the best computer-designed sequences were significantly more stable than the original coiled coil, having melting temperatures some 10°–12° C higher. Perhaps nature wasn't particularly interested in optimizing the coiled coil's surface for stability; in any case, it means that there's hope of improving on nature's designs if extra stability is required in the face of harsher-than-natural conditions—for example, when the protein is immersed in some oily organic solvent that's inimical to protein folding (because it won't drive oily residues to the core), but which is necessary to dissolve the chemicals you want the protein to act on.

At about the same time, the group had a go at the transition-zone residues, which they christened the boundary positions. These residues can go either way, and are likely to be swayed by subtle influences. To eliminate the possibility that the coiled coil's repeating, two-stranded structure might somehow skew the outcome, the group turned to the streptococcal G protein—a single strand that doesn't repeat. The key determinant proved to be the amount of hydrophobic surface

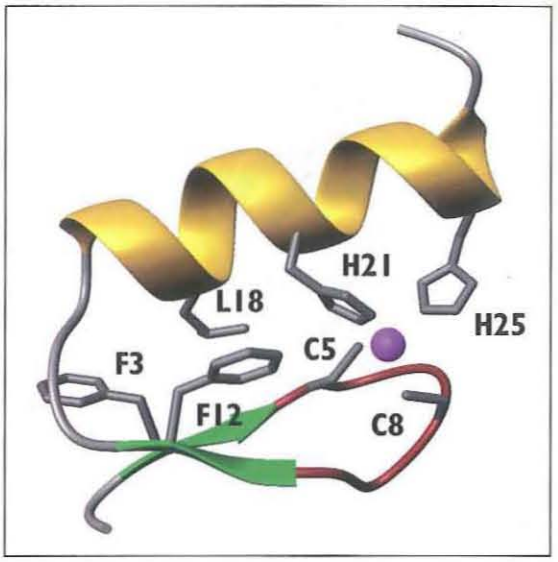
Below: The primary structure of Zif268 (left) and FSD-1 (right). The asterisks mark the zinc-binding amino acids. The orange background shows the core position, red marks the boundary positions, and blue is the surface positions. The six conserved amino acids are the white letters.

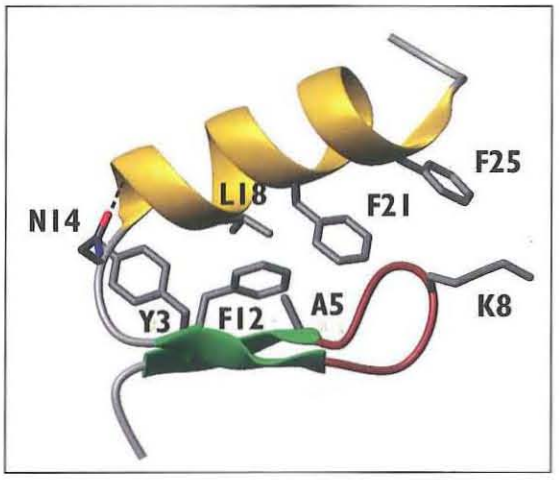
	K	Q
	P	Q
	F	Y
sheet	Q	T
	C*	A
	R	K
	I	I
	C*	K
	M	G
sheet	R	R
	N	T
	F	F
	S	R
	R	N
	S	E
	D	K
	H	E
	L	L
	T	R
	T	D
helix	H*	F
	I	I
	R	E
	T	K
	H*	F
	T	K
	G	G
	E	R

area that remained exposed to the water molecules. This exposure penalty is subtly different from the burial bonus that drove the core packing. Let's say that a small and a large hydrophobic residue are competing for the same boundary position, and that both residues have 100 square angstroms of surface area buried. This would give them identical burial bonuses. But the small residue might be almost entirely buried, while the larger one could still be sticking its head and shoulders into the water. So the exposure penalty deters large hydrophobic residues from occupying boundary positions.

Now it was time to try the core, boundary, and surface programs together, which, says Dahiyat, was "going to be a very stringent test. Basically, if you're a little bit off on any piece, it's highly unlikely that they're going to compensate and help each other." The group chose a structure called a "zinc finger," a common feature in proteins that bind to DNA and control the copying of genetic information. The particular one they used—Zif268—is a human variant, from which they selected a 28-residue motif containing a sheet, a helix, and a turn, making it a thorough test of their system. A zinc finger, as the name implies, incorporates a zinc ion to stabilize itself. (In fact, "zinc glove" might be a better name, because the zinc ion sits inside the motif like a finger in a glove.) Professor of Chemistry Barbara Imperiali's lab had demonstrated that some variants can fold up without zinc, but the catch was that these variants included two amino acids not found in nature that were custom-built to stabilize the fold.

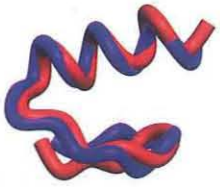
Again, the backbone was held fixed in the natural shape, but this time the identity of every amino acid in every position was left in the computer's hands. The computer decided which positions belonged to the core (only one, because of the motif's small size), the boundary (seven), and the surface (20), based on an analysis of the natural protein. The computer chose the core and surface residues from the previously developed lists of allowable amino acids; for the boundary positions, the computer was allowed to pick any amino acid from either list. All in all, the computer had 1.9×10^{27} possible primary structures to choose from. This is a quintillionfold beyond the reach of physical screening methods—had the group actually synthesized one molecule of each primary structure, the aggregate would have weighed 11.6 metric tons. Factor in the rotamer problem, and the number of possible sequences skyrocketed to 1.1×10^{62}. (This is equivalent to the number of protons in 100,000 suns, says astronomer Djorgovski—comfortably less than the number of protons in the universe, but still a hefty number.) Even so, it only took 90 hours of processing time for a 10-processor system. A nine-hour day for the computer, in other words—pretty good hours for a grad student.





The natural zinc finger (upper) and FSD-1 (lower). The zinc ion is shown in purple. With no zinc ion to cling to, FSD-1 is stabilized by the burial of hydrophobic residues and by hydrogen bonds, especially the one shown as a dashed line between N14's oxygen atom (red) and the helix's backbone. Note how boundary residues 12, 18, 21, and 25 all cluster around the core (residue 5)—even boundary residue 3 is on the interior face of its sheet. (Boundary residues 7 and 22 aren't shown for clarity, but also point toward the core.) The brown stretch of backbone is a turn.

The backbones of Zif268 (red) and FSD-1 (blue), superimposed on each other. Each tube's diameter is 0.8 angstroms, or roughly one-quarter the van der Waals diameter of a carbon atom.



The computer's winning sequence, when synthesized and its three-dimensional structure analyzed, matched the backbone atoms of the target sequence to within, in general, about half a carbon atom's diameter. (The ends were considerably floppier, because there wasn't much to hold them in place.) The computer chose nonpolar residues for all seven boundary positions, placing them in a nice, solid little packet around the one core residue. The computer also came up with well over 1,000 other primary structures that should be only slightly less stable, conserving a few critical amino acids while being tolerant of variation elsewhere. As Mayo remarked in the *Science* paper in which their results appeared, "Even if billions of sequences would successfully achieve the target fold, they would represent only a very small proportion of the 10^{27} possible [primary structures]."

Although the computer-designed backbone was right, its primary structure was completely different. The amino acids that bind the zinc ion, which are essentially invariant in all natural zinc fingers, were completely different in the computer's version, although their side chains remained pointed in the general direction of where a zinc ion would have been, had there been one. Only six of the 28 positions (21 percent, or no better than random chance) contained the same amino acid as did the original protein, and only 11 positions (39 percent) were even close. Furthermore, a search of the sequence database maintained by the National Institutes of Health's National Library of Medicine revealed that the computer's sequence (christened FSD-1, for Full Sequence Design #1) didn't look like any known zinc-finger sequence. In fact, it didn't look like any known protein, period, underlining the fact that the design program relies on chemical first principles and not some hidden biological biases.

"What's important here isn't what FSD-1's primary structure resembles, but that its tertiary structure is correct *and* stable," says Mayo. "A biological zinc finger is so tightly bound to its zinc that it has no melting temperature, but the zinc-finger sequence is so short that most people believed that it would be hopelessly unstable without the zinc to hold it together. To my knowledge, this is the shortest sequence that consists entirely of natural amino acids and assumes a stable fold with no help from metal binding, disulfide bond formation, or other assistance." As was exemplified by the subtilisin protein at the beginning of this article, the bulk of a natural protein is scaffolding: a Dr. Seussian array of props that brace other supports that hold in place the motifs that do the work. But industry can't afford to be as profligate as nature, so getting motifs to hold their shape with the absolute minimum of scaffolding is critical if a protein is to be manufactured from scratch economically.

A 30-amino-acid string is at the bottom end of the realm of functioning motifs; however, current supercomputers can easily handle sequences of 50–60 amino acids, which really gets up into the realm where useful things can be made. And if you're willing to sacrifice academic rigor and run quick-and-dirty approximations, says Dahiyat, you can manage about 100 amino acids.

Furthermore, work by grad student Alyce Su has shown that you can have a fair amount of flexibility in the target backbone, yet still come up with a sequence that will assume the correct fold. This might be the first step to having a computer wrap a backbone of its own design around the shape you want to encase—if the computer could be given some leeway with the backbone, it might simplify the task of incorporating the amino acids that actually grab the reacting molecules and hold them in place. These amino acids need to be in certain spots on the backbone in order to do their jobs, but putting them in those spots could distort the backbone. Figuring out how to put the right grappling hooks in the right spots without messing up the backbone's folding is now high on the group's agenda.

"To my knowledge, this is the shortest sequence that consists entirely of natural amino acids and assumes a stable fold with no help from metal binding, disulfide bond formation, or other assistance."

The group is now trying the system on other motifs that were not used in the development work. If the system successfully re-creates these unfamiliar backbones, it will be a good proof of its generality and a big step toward designing proteins from scratch. Mayo waxes lyrical about the potential for drug design, while Dahiyat likens the possibilities to the burgeoning of consumer plastics. "About 40 years ago, new catalysts were developed for polymer production, and now plastics are everywhere. Today, people are spending hundreds of millions of dollars a year trying to make enzymes more thermally and chemically stable. We can do that with the push of a button in a lot of cases. And by stabilizing the enzymes, you open up the applications you can use them in, and hopefully make the whole thing take off. People will be using proteins everywhere." □

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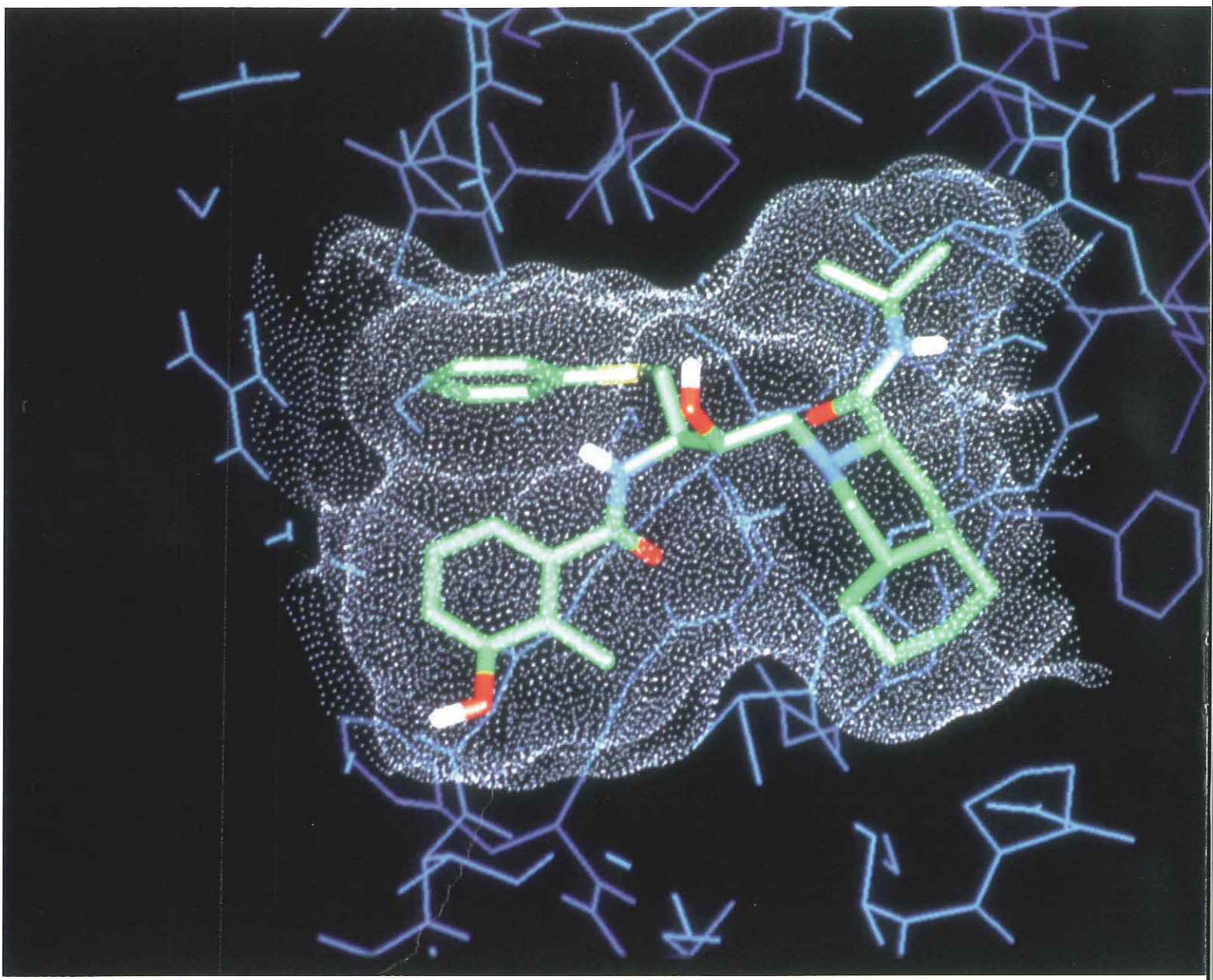
Recent research developments along several fronts permit some degree of optimism.

The fourth annual Caltech Biology Forum, on October 8, focused on the latest developments and challenges in AIDS research. This article is adapted from the remarks of three of the forum's speakers. They were joined by Brenda R. Freiberg, vice president and treasurer of the Foundation for AIDS and Immune Research and chair of the Public Policy and Planning Committee of the AIDS Service Center; and by moderator Sandra L. Thurman, director of the Office of National AIDS Policy and member of the Presidential Advisory Council on HIV/AIDS. Sponsors of the event included Glaxo Wellcome Inc., Agouron Pharmaceuticals, Inc., Huntington Hospital, the Pasadena Star-News, *and the AIDS Service Center. Videotapes of the forum may be ordered, at a cost of $29.95, by calling 626/395-4652 or 888/2-CALTECH.*



The Quest for a Cure:
AIDS Research at the Millennium

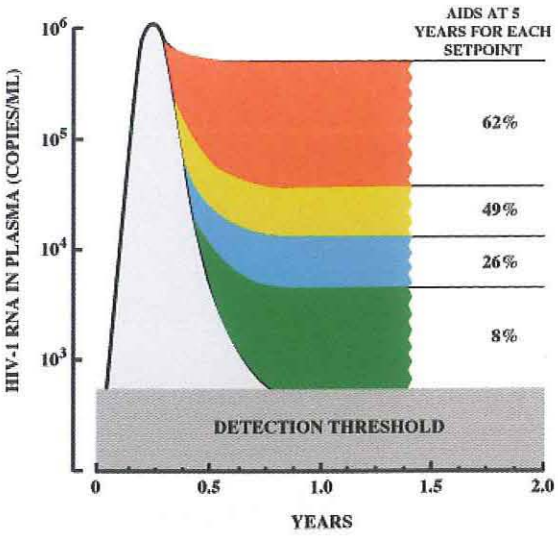
David Ho

David Ho is director of the Aaron Diamond AIDS Research Center of the Rockefeller University in New York, where he is also a professor and physician. His research using a combination of drugs to treat patients in the early stages of HIV infection brought him acclaim as Time *magazine's 1996 Man of the Year. Ho graduated from Caltech in 1974 (before going on to Harvard Medical School); he spoke at Commencement last June and this fall was named a member of Caltech's Board of Trustees.*

Left: A small molecule designed to fit exquisitely into a cavity in the protease molecule prevents the protease from carrying out its work of replicating HIV particles. Protease-inhibitor therapy, along with drugs that attack another stage of HIV replication, has dramatically slowed the progression to AIDS.

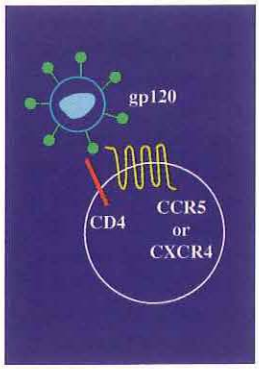
The AIDS epidemic presents a very pessimistic picture. We now have close to 30 million cases throughout the world, heavily concentrated in sub-Saharan Africa, but with a growing epidemic in southeast Asia. It's predicted that in a few more years, the Asian epidemic could surpass the African one. Each day now, there are 16,000 new infections (including 2,000 children), and 90 percent of these cases occur in developing countries, primarily in Africa and Asia. In some countries this disease is killing much of the affected population. In a particular region in Uganda, for example, AIDS now accounts for about 44 percent of deaths in the whole population and, in the 25–34 age group, for about 90 percent of deaths. HIV has become a major killer in the world, at a level comparable to tuberculosis and malaria. In the United States, too, AIDS has been creeping up as a major killer of young people between the ages of 25 and 44, surpassing even accidents and cancer since the early 1990s. Fortunately, in North America and Europe there is actually some decrease in new infections per year.

Recent research developments along several fronts permit some degree of optimism. One very important development has occurred primarily in the last 18 months. For more than a decade we have known that HIV finds its principal immune-system target cell, the CD4 T cell or CD4 lymphocyte, through a very specific recognition site, or docking site, for a molecule called CD4 that sits on the cell's surface. For about a decade, we have also known that a second docking site is required, but that receptor molecule has remained mysterious until the past year, when it was identified as a member of the family of molecules known as chemokine receptors. HIV needs to interact with the first molecule and then with the second, especially one called CCR5 and other related molecules, none of which are there to serve HIV. They're there, in fact, to bind smaller molecules—chemokines—that are released by



Right: A burst of HIV in the blood follows immediately after infection and then settles down to a plateau or set point, where it can remain for years before AIDS occurs. Just how many years is a function of the plateau level; current therapies are aimed at bringing down that set point, in hopes of stalling the onset of AIDS indefinitely.

Below: An HIV, its surface bristling with glycoproteins, infects its target cell by recognizing and docking (red line) at a surface molecule called CD4. In the past year, a second docking site (wavy yellow line), necessary for the HIV to enter the cell, has been discovered—a protein called CCR5 or CXCR4, which is a chemokine receptor. Chemokines might be employed to block this interaction.



various cells in the immune system. We might possibly be able to employ these chemokines to engage this second docking site and block this entry step for HIV, so this now becomes another therapeutic strategy. We could also specifically target this docking site via the development of other small molecules.

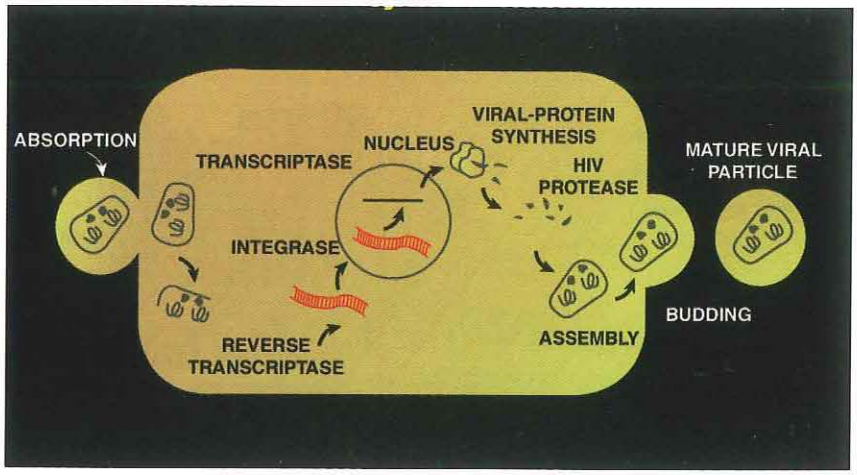
Now, as this discovery was being made, Bill Paxton, a colleague of mine at the Aaron Diamond AIDS Research Center of the Rockefeller University, was working with a number of patients who had been exposed to HIV through multiple sexual contacts and yet remained uninfected. Even in the test tube, HIV cannot infect the CD4 T cells of some of these individuals. This was distinctly unusual. With the discovery of the chemokine receptor CCR5 as an important docking mechanism for viral entry, it became logical to ask if these people had any abnormality involving the chemokine receptor molecule. And it turns out that some of these exposed uninfected individuals have a deletion of a 32-base-pair sequence in the DNA that encodes this molecule, so that, in fact, these people are missing the chemokine receptor CCR5.

This observation was followed up primarily by Dr. Huang in our group and by Steve O'Brien at the National Institutes of Health, who showed that individuals who have the CCR5 defect are principally, perhaps even exclusively, Caucasian. About 1 percent of the Caucasian population, particularly from northern Europe, has two copies of the defective gene (one from the mother and one from the father), and these people are almost, but not quite, 100 percent protected from HIV infection. People with one normal gene and one abnormal gene have a slower disease progression after HIV infection. This is an important development, because we now not only know that these chemokine receptors represent an important gateway for viral entry but also that CCR5, in particular, is dispensable, making it a rational target to go after in drug design.

Over the last couple of years, we have also learned a great deal about the levels of HIV in infected people through the work of John Mellors and others from the University of Pittsburgh. Shortly after HIV infects a person, there's a burst in the amount of virus as measured in the blood, after which the virus is brought down to a plateau, presumably by the body's immune system. But the level where the plateau is reached is quite different for different individuals. Through their work, we know now that if a person settles at a high plateau, with a high viral load, there is a great chance of progressing to AIDS in five years' time. In contrast, if the virus is brought down to a lower plateau, there is a much slower progression to AIDS. This shows in a definitive manner that the level of virus replication drives disease progression. We also now know that, once this plateau is reached, it is typically maintained for many months, even years in some patients, with the level creeping up only slowly over time. We had previously thought that HIV was quiescent during this period, but the work of several groups in the past few years has shown that HIV replication is extremely active, especially when the plateau remains high and continues mercilessly in the infected person. Infected CD4 T cells make enormous numbers of HIV particles each day. Such particles are removed very quickly by the body, although some particles go on to infect new T cells, and this cyclic process continues relentlessly. Throughout this cycle, many CD4 T cells are destroyed either directly or indirectly by the continuous replication of virus.

Now that we can begin to get a handle on the magnitude of this virus replication, it clearly has implications for how we treat HIV. We now view it as a much more active process from the very beginning, and this process destroys a lot of important immune cells in the body each day. So it doesn't really make sense, now that we have drugs available, to let this continue unchecked. In addition, once we define the magnitude of virus replication, we can calculate how many new cell infections occur daily. As HIV infects new cells, it has to take its genetic material from RNA to DNA through reverse transcription—a process that David Baltimore defined a couple of decades ago. During reverse transcription, HIV will make a lot of errors, generating many mutations. Some of these mutations will begin to confer drug resistance to HIV. So then, if we try to treat HIV with a single agent, the virus will be inhibited only for a transient period, and it will quickly rebound with a drug-resistant strain. This suggested to us, as well as to many others in the field, that we had to attack new infection by using several different drugs, trying to corner the virus so that it can't mutate sufficiently to evade several drugs at one time. This is the strategy that has generated the most promising results.

The viral life cycle is illustrated above: the



In the life cycle of HIV, the virus enters the target cell, creates a negative strand of its DNA through reverse transcription, which enters the cell's nucleus and begins to synthesize HIV components. The protease enzyme cuts these components, the viral proteins, into the smaller pieces necessary to assemble new HIV.

initial binding to the cell, entry, reverse transcription; HIV gets into the nucleus, is incorporated into the chromosome of the host cell, and then synthesizes its different components, particularly the viral proteins, which need to be chopped up into smaller pieces by an HIV enzyme called protease. Therapy these days has targeted two steps in this cycle: protease inhibitors, which began human testing in early 1994, and older drugs that target reverse transcription.

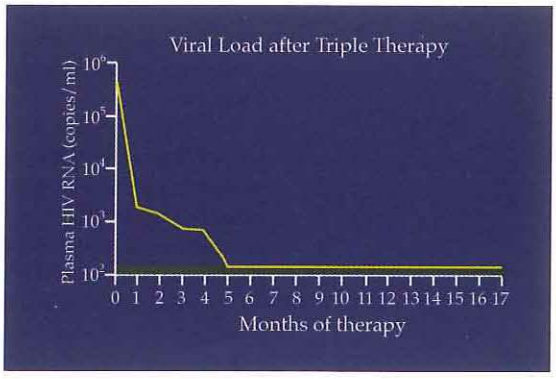
We've been able to combine many of these drugs to build a more powerful combination therapy against HIV, and the results have been truly impressive. In patients who received this therapy, the amount of virus in the blood falls by many orders of magnitude—from very high levels down to undetectable levels. Correspondingly, the lymphocyte count goes way up, although the restoration is seldom complete.

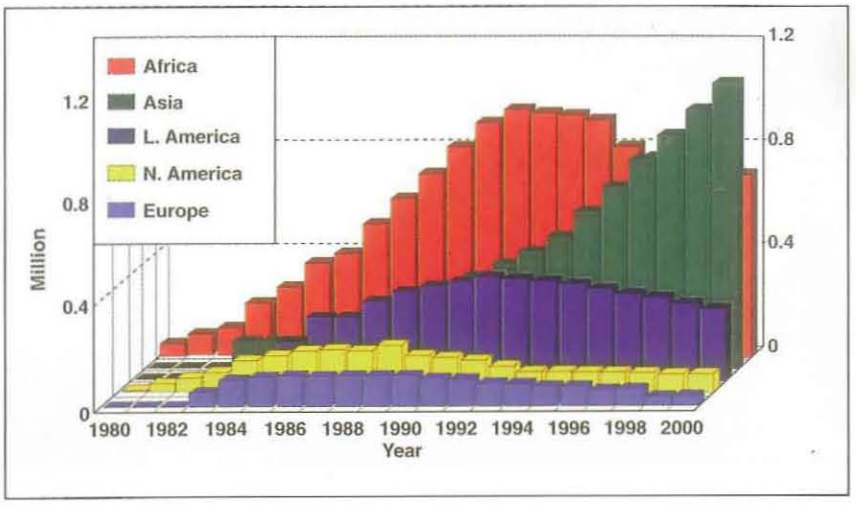
But even as HIV becomes undetectable in the blood, can it still be hiding out somewhere else? We and others have been looking in various other fluid samples (including spinal fluid and seminal fluid) from these treated patients, and our sensitive techniques have not measured any of the virus, which still doesn't mean that it has been eliminated, however. Even if HIV is not completely eliminated, the control of virus in genital secretion could potentially have a great effect on transmission of the disease.

But fluids are not the only place the virus can reside. The immune system's T cells sit primarily in lymphoid tissue—in lymph nodes, tonsils, and even such places as the gastrointestinal tract. In order to see if the virus is similarly well controlled in these lymphoid tissues, we have to take biopsies. If we take 30 tissue sections from a given treated patient, in 95 percent of them we would find no evidence of virus. In each patient who has been treated with these powerful drug combinations for from 18 to 24 months, we find that there are occasional cells that are infected and are expressing virus. The virus has not disappeared completely, but is still there in small quantities. But the number of cells that are left with HIV infection is very, very small. Our estimates lead us to think that we have lowered the viral burden 10,000 or 100,000 times, yet there is still a residual pool that we must find ways of getting rid of—burn it out somehow, or protect these individuals with immunotherapy approaches, so that if these combination therapies are withdrawn, the person would be able to fight off the infection and keep it from spreading further. We don't know at this point whether or not this is achievable.

This type of combination therapy is beginning to make an impact on the affected community, and the results are promising, although not every patient benefits from these combination therapies because of either side effects or lack of adherence to the drug regime. According to figures from the Center for Disease Control, mortality rates have decreased from 1995 to 1996, and we hope 1997 will be even better. This decrease is most prominent for Caucasian men. It's not evident at all in women and much less evident in Hispanic and black populations. This has to do with access to medication, which remains the biggest problem worldwide. As much as 90 to 95 percent of new AIDS cases occur in developing countries, where these therapies are simply not affordable. So the only way to deal with the global problem is to educate and modify behavior, which is difficult to do—or to come up with an effective vaccine.

Patients who received combination therapy involving protease inhibitors along with older drugs that target reverse transcription have seen the levels of virus in their blood drop to undetectable levels. The virus may still be hiding elsewhere in the body, but such therapy, although expensive, offers hope for controlling the disease.





David Baltimore

David Baltimore is president of Caltech, an office he assumed in October after spending most of his scientific career at MIT. He is a former president of Rockefeller University. Baltimore helped pioneer the molecular study of animal viruses and won the Nobel Prize in 1975 for his discovery of the enzyme reverse transcriptase, which permits retroviruses, such as the AIDS virus, to replicate. He is chair of the National Institutes of Health AIDS Vaccine Research Committee, a post he will continue to hold along with his Caltech duties.

As you have just heard, these exciting new drugs are too expensive to represent a global solution. Approaches to preventing HIV infection by education and behavior control involves cumbersome mechanisms that have never been more than partially effective. But we do *already* know how to prevent virus infections. We prevent virus infections by vaccination. So, in the very earliest moments of the HIV epidemic, everyone said we should be making a vaccine.

The United States government has put an increasing amount of resources behind the production of a vaccine. We are now spending more than 100 million dollars per year on AIDS vaccine research (out of a total of $1.3 billion allocated to AIDS), and as effective drugs are developed, I think that a larger fraction of that budget could now go to vaccine development, if we knew how to spend it well. But money is not enough; we need an organized program of research to find a vaccine.

In 1996, Harold Varmus, head of the National Institutes of Health, asked me to establish a committee that would oversee the AIDS vaccine development effort in the United States and make it into a coordinated program that could feed the latest information into the vaccine-development pipeline. This committee consists of molecular biologists, infectious-disease experts, AIDS-treatment specialists, researchers on the history and evolution of AIDS, and people from all aspects of the epidemic, including one member of the advocacy community. The group is small, which makes it easy to work with, but we can expand it if we need to.

Our job is solely advisory. That's an odd charge for a group that's supposed to organize a program. We are supposed to advise the vaccine research programs at NIH with regard to scientific opportunities, gaps in knowledge, and so on. It has to be advisory, because the only people who can spend money on research are Federal employees, which we are not. Over the past year we've performed our role by meeting as a committee to grapple with the issues of what the vaccine program is; by starting a new grant program; by bringing people in the immunologic and virologic communities together in workshops to talk about the issues; by generating new ideas; and, particularly, by trying to bring new people into the vaccine effort, because one of the things we saw early on was that the great strengths of the American immunology and virology communities were not totally focused on this issue.

The innovation grant program that was invented through the committee's efforts is a way of simplifying the process of getting money from Washington—making simpler grant proposals, getting them funded faster, and targeting those grants to problems that we had identified as crucial to the vaccine effort. We targeted three areas: developing better animal models; studying the protein found on the outside of the virus, which is likely to be one target of any vaccine; and finding out how to get the cellular arm of the immune system revved up to attack virus-infected cells. We were able to announce the grant program in March, have the grants come in by May, and have them funded in September—52 new grants, spending $12 million on new approaches to AIDS-vaccine development.

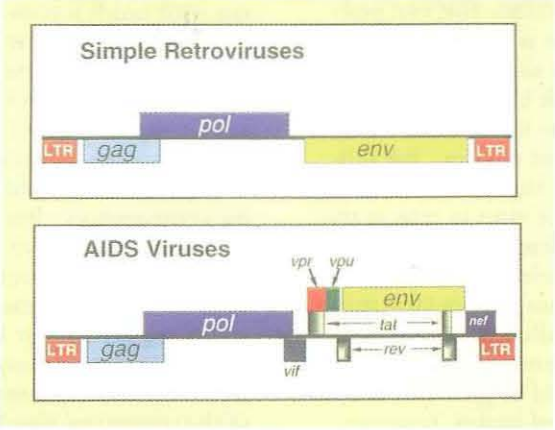
Even before I started on this committee, the first

Left: A graph of estimated annual adult HIV infections from 1980 projected forward to 2000 shows that cases will likely continue to rise dramatically in Asia at the end of the century, taking over the lead from Africa. Expensive drug therapies are unlikely to provide a solution here, underscoring the urgency of finding a vaccine.

question I asked myself was: is it possible to make a vaccine? We don't know the answer to that for sure, but I had to convince myself that there was at least a high probability of it. And I could do that because some research developments suggested that you could make a vaccine. First of all, there was work with nonhuman primates, the best model we have for HIV. A number of researchers, mainly Ron Desrosier and his colleagues at the New England Primate Center and Harvard, had found that you can protect macaque monkeys against SIV (simian immunodeficiency virus) infection with an appropriate vaccine preparation consisting of a live, attenuated virus particle. The virus is a perfectly infectious live virus, but its genes had been mutated in such a way so that, although it can grow and stimulate the immune system, it will not cause disease. The exciting thing was that it was done by mutating certain critical genes that are particular to the AIDS virus. People infected with such mutated strains of HIV have infected other human beings, and those infected people identified so far are nonprogressors, that is, the mutated virus causes a chronic infection, but the disease symptoms do not appear.

The AIDS virus is a retrovirus, but there are a lot of very simple retroviruses that don't cause AIDS, or much disease at all, unless they pick up a particular new gene, or if they integrate in a specific place in the genome (in which case they can cause cancer). To a large extent retroviruses are benign. The differences between them and HIV is a series of little genes (see illustration below), which form the heart of HIV's power to cause disease. The mutations were put into these genes, and the vaccine created from the live mutated virus. Unfortunately, even with those mutations, the virus occasionally causes disease, especially in very young monkeys, so there would certainly be a serious safety problem for human beings with this vaccine candidate. Right now, we're at a point with this vaccine concept where there is proof of principle, but we don't know how to carry that from principle into action.

Right: The difference between ordinary retroviruses, which don't cause disease, and the AIDS virus is a series of little genes—vpr, vpu, nef, rev, tat, and vif. Mutations put into these genes have produced a vaccine that works in monkeys but is still too risky for humans.

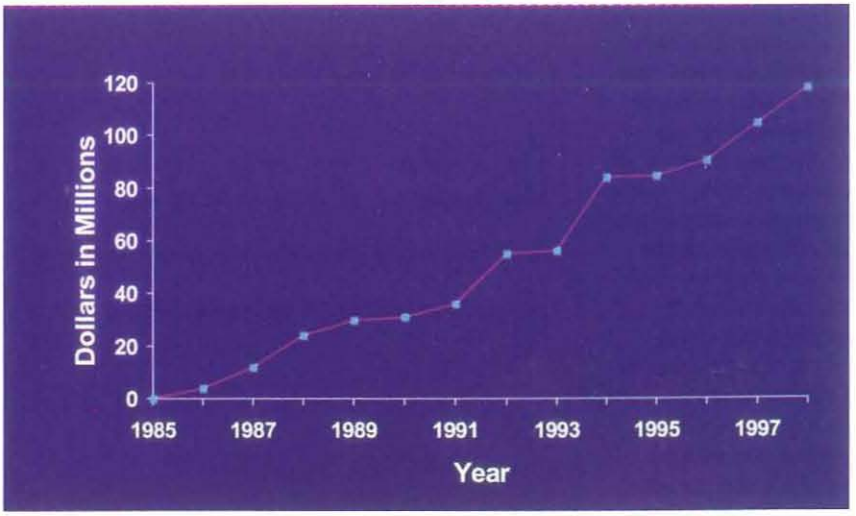


Most important, we've got to bring new creative ideas into vaccine development, or 10 years from now we may still be wringing our hands.

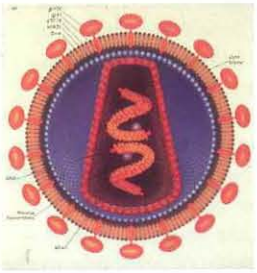
There is evidence that some kind of protection, probably of an immune nature, is possible in humans. For example, there are sex workers, particularly in Africa, who have been exposed to HIV over and over again and have not been infected. They have some kind of immunity—different from that conferred by a mutation of the chemokine receptor that David Ho talked about. It may be cellular immunity due to what are called cytotoxic T lymphocytes, the cellular arm of the immune system. Also, once a person is infected by HIV, it's very hard to infect him a second time, even after multiple exposures, suggesting that infection produces some sort of barrier against other HIV viruses coming in. If we knew how to make that barrier without the infection, we would be ahead of the game. These are the kinds of evidence that drive the vaccine program today—proof of principle, or suggestion of principle in the human cases, but no direction as to how the vaccine should be made.

Now, what does a vaccine do? We tend to think that vaccines protect us from virus infections, but they don't really. What they do is make sure that, if you are infected, your immune system reacts to that infection before any disease occurs. It's actually an abortion of the ongoing infection rather than what might be called sterilizing immunity or complete protective immunity. If we could develop an AIDS vaccine that gives sterilizing immunity, it would probably be the first virus vaccine to do that.

So what *could* it do? Well, as David Ho suggested, it could reduce the initial multiplication of HIV to reach a lower set point in the early stages of infection, to increase the time before the body loses control over the virus and AIDS occurs. In the best of cases, it might drive blood virus levels below the detection threshold so that perhaps the disease would never occur. This would involve driving down the plateau level below the point where the body can no longer control the infec-



NIH funding for research on an HIV vaccine has maintained a steady rise from 1985 to 1998.



A fanciful representation of HIV shows the Env proteins sticking up off the surface of the virus. It would make sense to use these proteins in a vaccine, but changes produced by laboratory methods of making these proteins or inactivating the virus have so far compromised their utility.

tion. That's what we imagine a vaccine can do. We're not sure this is possible, but it's certainly suggested by the work with monkeys.

What kinds of vaccines could we use? The historic vaccines that have been effective against virus diseases are of two kinds. One is the live attenuated virus like the one I described for SIV. The Sabin polio vaccine is a good example of a mutated live virus. The other kind of vaccine, such as the Salk polio vaccine, uses killed virus, in which you take a perfectly infectious virus and kill it by some chemical or physical means. It can still induce immunity, but it doesn't produce any infection. Unfortunately, HIV is a very fragile virus to any method of killing that's been found so far; it falls apart and is not really useful as a vaccine. I think it's a soluble problem, but it hasn't been solved yet.

When these problems were recognized some time ago, scientists began trying to make vaccines that consist just of the surface protein of the virus. (It's called the Env protein because it is in the virus's envelope.) The virus has on its surface little aggregates of three copies of Env protein; they have affinity for CD4 and the chemokine receptors on the surface of cells, and they use these as an entry port to infect the cell. It made a lot of sense to use Env as a potential vaccine. But two problems have emerged: the first is that the methods used to make these produce single units, not trimers, so that they don't look like they do on the virus surface. Second, in the initial work on HIV, it was necessary to make a lot of virus. For this, scientists could not just use the virus taken from people; they had to grow the virus in cells in the laboratory. We did not realize that when you grow virus in the lab, you select for changes in the structure of the virus proteins. These laboratory-adapted viruses are easily killed by the antibodies they induce, giving the impression that vaccination with these strains would be possible. People don't get infected by adapted strains, however; they get infected by field strains. The field strains are not susceptible to killing by the antibodies raised by these vaccine candidates, making their utility doubtful. It has recently been questioned whether antibodies against the Env protein of field strains can be raised at all. I think they can, but it's going to take some pretty subtle tricks to do it.

Because of these problems, the cytotoxic T lymphocyte (CTL) arm of the immune system has come to the fore as a potential way of protecting the body. When an appropriately cytotoxic T lymphocyte sees a virus-infected cell, it releases materials that cause the cell to commit suicide. Such lymphocytes exist in all of us, and their killing capacity can be stimulated by any protein made under the direction of the virus, even proteins that do not become part of the virus particle. Much of the effort today is going into inducing this kind of immunity to supplement whatever antibody immunity can be produced. The kinds of things that will do that are vectors that bring genetic material into cells—things like other viruses or naked DNA. These can be injected into the body, get into cells, and induce the synthesis of proteins that stimulate the CTL arm of the immune system. Vaccine designers today are trying to use many different techniques to induce the two kinds of immunity: peptides representing parts of proteins; vectors derived from other benign viruses to induce synthesis of proteins inside of cells; and the proteins themselves, often carried on particles that look like viruses but aren't viruses.

I've been discussing the search for a vaccine as if all of these techniques were just under development and nobody had ever tried to test a vaccine. Actually, the program is 15 years old. The day the discovery of HIV was announced in Washington, then Secretary of Health, Education, and Welfare Margaret Heckler said: "We now have the virus; in two years we'll have a vaccine." She was optimistic, but that was, in fact, the start of the vaccine program. Many vaccines, in particular some using live vectors such as the smallpox vaccine virus, have actually been tested during these 15 years. Only a little work has been done on immunization by naked DNA, but there will be a lot more. Even whole killed virus has been tried, although not with much success.

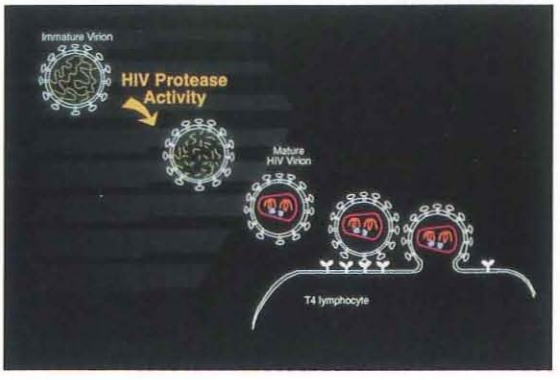
So, with all this history, why isn't there a vaccine? I think the defining moment came a few years ago, when we realized that the laboratory strains were different from field strains. Even before that, we had known that adaptation to the laboratory changed the virus, but we didn't know the consequences. But now we became aware that we were working with materials that probably would never give decent immunity. It's not certain that this is true, and these materials are still being tested, but it has forced us to go back and think about redesigning the whole program of vaccine development. This was the genesis of the committee that I represent and of the

attempts to introduce new and more innovative methodologies.

What are the main needs of the vaccine program today? First, we have to integrate into vaccine development the latest knowledge about HIV. Why did it take so long to recognize that the field strains and laboratory strains were different from one another? Partly because vaccine development was running on a track quite separate from the basic research track, and the information transfer was poor. We need to bring the latest information to the vaccine efforts and use it to modify them accordingly.

We have to introduce this information into the *human* testing process because, ultimately, we can only know that vaccines work when they've worked in human beings. More than 2,000 people have already taken vaccines in a continuing process that has been quite separate from much of the research effort. Research is mostly government-funded and takes place in universities and research institutes, while vaccines are, in the end, developed by industry. Under government direction, we need to integrate into a partnership the many different strong research institutions in the United States and elsewhere along with all the industries that will ultimately make these vaccines. Most important, we've got to bring new creative ideas into vaccine development, or 10 years from now we may still be wringing our hands. One exciting initiative that our committee has helped foster is a laboratory on the NIH campus that can carry out an integrated program of HIV vaccine research. This will help couple the vaccine development effort to advances in basic knowledge about the virus.

What should be the test of the success of our committee? Development of a safe and effective vaccine will not happen quickly. President Clinton has asked for a vaccine within a decade. I have a more modest goal. If we have exciting vaccine candidates that are safe and work well in animals within the decade, I will feel we have been successful. If we don't, I think we will have to consider the possibility that HIV has outwitted us, that a vaccine is not in the cards.



Proteases are necessary actors in HIV replication. Without their intervention, immature virus particles cannot grow to maturity and go on to infect other T4 lymphocytes.

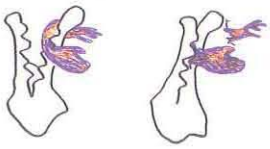
It is tremendously gratifying for us to see one of our early efforts go from molecular to design through extensive testing to actually extending the life of a patient.

Mel Simon

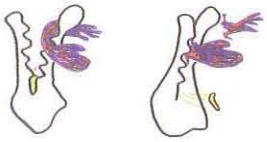
Mel Simon is chair of Caltech's Division of Biology and is the Anne P. and Benjamin F. Biaggini Professor of Biological Sciences. He came to Caltech in 1982 from UC San Diego, where he had spent most of his previous academic career. His research centers on how organisms detect and respond to chemical changes, and includes studies of the mechanisms involved in sensory cell function and investigations into the nature of the biological circuits that process information from a variety of cell surface receptors.

About 15 years ago, it became clear to me and to some of my colleagues that we were in the midst of a technical revolution in biology and biochemistry that could provide novel strategies for dealing with infectious disease. The dream was that, using molecular biology, we could identify the molecules intimately involved in the mechanisms of infection, and then characterize them in atomic detail, and design inhibitors that would bind only to those target molecules and inactivate them. The notion of specifically designing drugs atom by atom was different from previous approaches to drug discovery. Many of the drugs that were used to fight infection by microorganisms were natural products that were derived or extracted from plants or other organisms. In fact, there were very few drugs that could cope with viral infection.

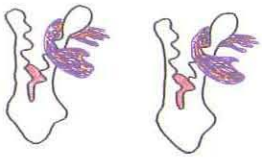
By the early '80s, molecular biology had developed enormously, enabling much of this dream to become possible. First, we can, in fact, identify target proteins required to initiate and propagate disease—in the case of HIV, the reverse transcriptase, the protease, and the integrase. These proteins are part of the process of building the virus, and they are absolutely necessary for propagation of infection. Second, in order to wage this war at the atomic level, we have to know the atomic structure of the target molecules. This requires knowing the position of every atom in the target molecule. This picture of the target molecule also tells us a bit about how the molecule works. You can't see the virus with a light microscope; you can just make out gross viral structure with an electron microscope. To actually determine the atomic structure of components of the virus, we need x-ray crystallography, a technique



The protease recognizes part of the virus precursor (the purple thing) that needs to be cleaved, and chops at a jawlike site formed between protease subunits.



A small molecule (yellow) could be designed to sit in the crevice between the two units and keep them from clamping shut. If the fit isn't precisely right, it can fall out, and the protease can continue its work.



But a snugly fitting molecule (pink) will lock the "jaws" open and inhibit the protease from recognizing the virus precursor and carrying out its chopping operation, thus preventing the production of new HIV particles.

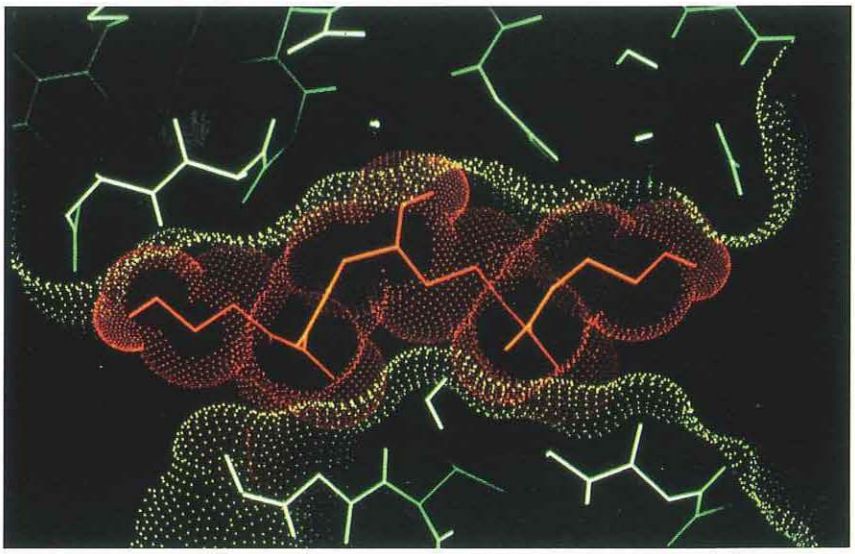
that has been around for almost 100 years but whose development has really accelerated in the last 20 years. The great advances in computational techniques, computers, and software for computational chemistry have greatly facilitated protein crystallography. Sophisticated computer displays are available to help us visualize these molecules in three dimensions, to stimulate their interactions with other molecules, and to try to understand how molecules recognize each other. Advances in organic chemical synthesis permit us to optimize molecular designs, and to build molecules that can interact with each other in a very specific way.

Bringing all of these elements together involved uniting a variety of different sciences. A group of us at the University of California at San Diego decided to form a company to do just that. The corporate structure is in many ways ideal for blending cultures and approaches and for focusing the efforts of diverse people on a specific goal or product. It was at this time that we also became aware of the proportions of the AIDS epidemic, the grief that it was causing, and the discouragement that had been experienced in attempts to develop methods for dealing with it. We realized that we could very quickly describe the proteins that make up HIV and that are essential for its replication—the reverse transcriptase, the integrase, and about 12 other proteins, including the HIV protease, whose function is to tailor the viral proteins into smaller pieces. These targets were relatively easily available and provided an excellent model to test the notion of drug design.

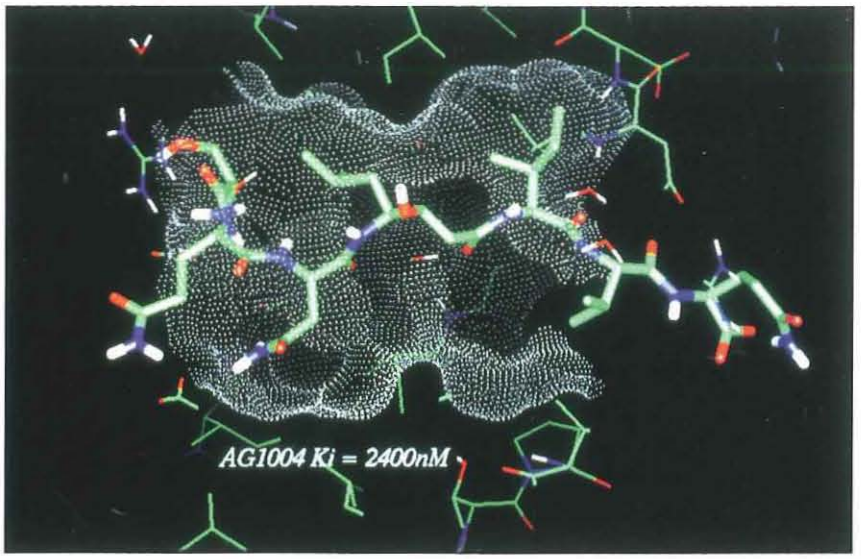
Our company, Agouron Pharmaceuticals, used the techniques of molecular biology to isolate large amounts of these proteins, to determine their crystal structures, and to try to design drugs that would block their function. Thus, for example, the HIV protease has to digest a larger protein at specific places, in order for the virus to make an effective "coat." If you block protease from acting, then you don't get a mature virus particle, and the particle that is generated cannot infect cells. First, we and a number of other companies worked out the atomic structure of the protease using x-ray crystallography. The HIV protease is made up of two subunits, which cleave a protein substrate that specifically fits between them. In the close-up (below) of the heart of the molecule, you can see the surface of the protease and the substrate of the virus that it is going to have to chop. (The scale here is in angstroms and fractions of angstroms.) This is a static picture, but these parts are actually all wiggling around, and you can see that the fit is exquisitely perfect. This atomic fit is the source of the protease's ability to recognize a specific substrate.

The protease sees a very specific part of the virus and cuts it. What we wanted to do was to design a small molecule that would sit in the cavity between the two subunits and fit so well that it will not allow the usual substrate protein to work. This small molecule would go into all the viruses, get into the middle of all the proteases, and block them from working. You need perfect molecular recognition at the atomic level for this strategy to work. At right is one of the first molecules that our company made. You can see that it didn't fit snugly, and it fell out of the "active site"; the protease was therefore still able to "do its thing." In other words, you would see very weak inhibition of protease activity. So the designers had to go back to the drawing boards. Each time around, they take an "x-ray snapshot" of the molecule, that is, they generate a co-crystal of the "target" and determine exactly how the putative inhibitor sits in the active site. They see what parts still have to fit; and then they redesign the small molecule inhibitor.

In the case of the protease inhibitor, this design process involved more than 40 iterations. Different small molecules were built and inserted into the active site; the complex was crystallized and its



The two parts of the protease are shown here in green, with the viral protein (orange) that they are supposed to cleave between them. The scale is in angstroms. All the parts are actually in constant motion, but the fit has to be perfect.



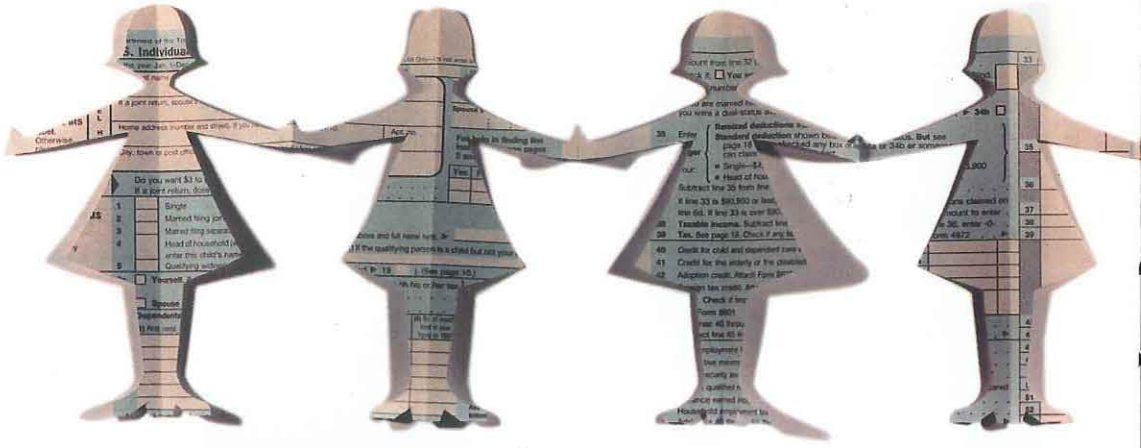
One of Agouron's first designs for a protease inhibitor clearly doesn't fit the site (whose surface contours are represented by the small dot pattern) very well—hanging out on both ends and leaving spaces unfilled. Many redesign attempts finally arrived at the successful molecule illustrated on page 24.

structure determined. In this way the small molecules were tested to find the inhibitor that fit the site best. You can see how much better this final small molecule on page 24 fits the site. Electronic calculations indicate that it recognizes the active site of the protease with great specificity. When it gets into that crevice, it binds to the protease extremely tightly, and prevents it from acting. The putative inhibitor had to then be tested in a variety of ways to see if, in fact, it blocked virus replication. Then we had to determine if it was harmful to people or if it had side effects that were deleterious to living organisms. Finally it needed to be tested for efficiency in clinical studies. It became clear that the protease inhibitors represented one part of a strategy that David Ho was instrumental in inventing and pursuing—that is, the notion of using multiple inhibitors of viral replication—which lowers the amount of virus in the blood and keeps it down for an extended period of time. The idea that is essential to this treatment was presented by Dr. Ho. Since the virus replicates very rapidly and mutates rapidly, the application of multiple inhibitors that block different steps in replication lowers the number of replicating viral particles, and at the same time requires multiple simultaneous mutations in order to bypass the inhibitors. This lowers the probability that effective resistant viral particles will arise.

Unfortunately, it doesn't work for everybody, but for a large fraction of the patients (more than 80 percent) the cocktail of protease inhibitors and multiple reverse transcriptase inhibitors does have a dramatic effect. Many people who have been taking this combination for over a year have improved in various ways. It is tremendously gratifying for us to see one of our early efforts go from molecular to design through extensive testing to actually extending the life of a patient. The effect that biologists hope to see in their work has been realized in this case—to use our understanding of nature and the tools of molecular biology to improve, or even save, lives.

We know that HIV replicates at an enormous rate. Because it can replicate so prodigiously, and because it can mutate at a high rate, the virus is able to evolve rapidly. Thus, the probability of a mutation that can bypass the drug or cause resistance to the drug is high. The use of multiple drugs raises the barrier to complete resistance, but nonetheless, resistances arises. How does it happen? One of the things that Agouron has found in patients and in tests in the laboratory is that the virus can sustain a particular mutation that will change the protease at one particular position. This change breaks one of the bonds that holds the molecule in the active site, but still allows the protease to function. The same inhibitor no longer fits as perfectly as it did before, and the protease can bypass the inhibitor and work again.

By using multiple drugs and prescribing them early in the course of the disease (along with high compliance by patients), we can lower the probability that these kinds of mutations and this kind of resistance will occur. A variety of drugs is now available, and combinations of these drugs are being used and shown to be effective in averting resistance. Many companies are working on other drugs. Some of these might fit the active site differently and thus augment current treatment. A tremendous amount of research is currently going on to try to perfect this method of recognizing the targets and designing specific drugs fitted to them. Agouron and other companies are working on the HIV-Integrase, the HIV-RNAase H, and other proteins that are necessary for viral replication. It may eventually be possible to design inhibitors that are so clever that they can actually minimize the effects of mutations to resistance. This is clearly an enormous problem, but one that is being pursued at different levels and that will lead to a new generation of antiviral therapies. □



Taxing Women

by Edward J. McCaffery

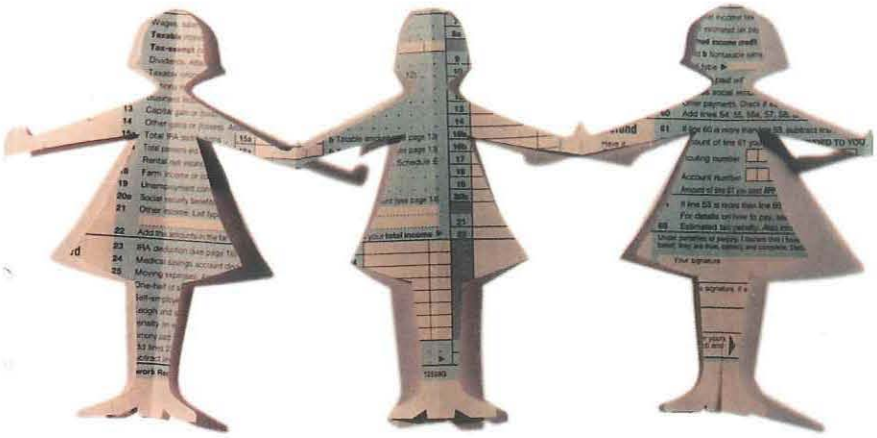
This article was adapted from Ed McCaffery's Watson Lecture last May, the month after his book, Taxing Women, *was published by the University of Chicago Press.*

McCaffery, who has taught law as a visiting associate professor in the Division of the Humanities and Social Sciences at Caltech since 1995, is also professor of law at the University of Southern California Law School, where he has taught since 1989. He studied Latin and philosophy as an undergraduate at Yale (BA 1980), earned his JD from Harvard in 1985, and received an MA in economics from USC in 1994. His research approaches the law from the vantage points of public finance and feminist theory, among other perspectives.

Our tax system has a strong bias against two-earner, married families. This bias came to be because of a series of decisions made in the 1930s, 1940s, and 1950s, and hardly reexamined since. The bias cuts differently at different income levels. Among the poorest Americans, a bias against two-earner marriages is a bias against marriage itself. Thus it is no surprise, although I think it's a stunning disappointment, that one out of four American children live in single-parent, female-headed households, and that more than half of them are below the official poverty line. It's also not all that surprising that the traditional image of the family, in which the husband/father works full time outside the home and the wife/mother works full time inside it, continues to predominate among the very wealthiest Americans. Eighty percent of CEOs of Fortune 500 companies are married men with stay-at-home wives. Finally, the same bias creates stress in the vast middle classes, where married mothers face difficult choices between staying home full time—thereby sacrificing an important labor-market presence and harming themselves in the increasingly likely possibility of a later divorce—and working full time inside *and* outside the home, juggling two domains of work for little, if any, take-home dollars. All the while, men face little pressure to change their ways, and the workplace continues to favor a dominant model of full-time, full-commitment work.

That's the basic story of my book, *Taxing Women*—how a large and coercive instrument of state control, the tax system, was set up in one set of circumstances to favor one kind of family, and how it continues to exert pressures today under very different circumstances.

I'd like to view this basic story from different perspectives by telling four tales, with apologies to Chaucer: the Accountant's Tale, the Historian's Tale, the Economist's Tale, and the Social Theorist's Tale. I'll cut right to the bottom line and



begin with the most basic and down-to-earth perspective.

The Accountant's Tale

Let's start with some basic facts of life in America today. Almost all married men work, and almost all married men who do so work full time, well over 95 percent. They usually earn much more than their wives do when the wives also work. Conversely, about 40 percent of married mothers of young children stay home. In most single-earner families, it's the husband who works. A single-earner family where it is the wife who works is likely to be a lower-income family with an unemployed or unemployable husband. So, for various reasons, the man's salary is fixed as the primary one. It's not just because he earns more, but also because it's taken for granted that he works. Men's work comes first, and once we take that as a fixed fact of life—it doesn't have to be, but it is for most Americans—the following things start to happen.

Let's consider the situation of a husband and wife with two children. Let's say the man is earning $60,000, and the wife is offered a job paying $30,000. That roughly captures the ratio of working wives' salaries to their husbands'. On average, a working wife earns about two-thirds of what her husband earns, but to make it easy here we're saying she's offered a job paying $30,000. But she's not going to take that home. The first thing we do is factor in taxes, and taxes are going to cut her salary in half. She's going to lose about $15,000 to a combination of income tax, Social Security, and state and local taxes. The income tax starts at a high rate because of joint filing; this is something I'll talk about more in the Historian's Tale, but basically joint filing means that *her* first dollar is taxed at a rate dictated by *his* salary. So she doesn't have a zero bracket like he does when he entered the work force. Her very first dollar, in this particular example, is in the 28-percent income tax bracket. And over the range in which they are earning together, she'll enter into even higher brackets of 31–32 percent.

She also starts, on her very first dollar, paying Social Security taxes of about 7.65 percent out of her paycheck; her employer has to chip in an equal amount. Working wives are already benefited under the Social Security system by virtue of a stay-at-home spousal share, so she's paying a pure tax here with no benefit. When we add the 7.65 percent to 30 percent, and then add in state and local taxes, an increasingly significant phenomenon in America, it's pretty easy to get up to a 50 percent marginal tax rate.

Taxes are not, however, the only expenses that come out of her salary. If she goes to work, the family is going to have to do something about child care. The most common way of dealing with the situation of child care is to use some unpaid option—to take advantage of relatives or friends. But if you have to pay, you have to pay dearly. So let's say the family has to pay $200 per week to care for two children. (Surveys and statistics suggest that this is not an unrealistic figure. It's higher than the average, but the average numbers tend to be pulled down by unpaid options.) The sum of $200 per week adds up to roughly $10,000 per working year. The biggest benefit she could get back on her tax forms, in terms of a child-care credit, would be less than $1,000. Since she's unlikely to get even that, for a variety of reasons that I won't go into here, I've simplified the situation and left it out of the equation.

Child care is not the only additional expense the family will face. Two-earner couples face a myriad of costs over and above one-earner couples. They see expenses from the loss of the services that a stay-at-home wife would be providing—dry cleaning, housekeeping, restaurant meals, more expensive in-home food, commuting. If all these extra expenses average $100 a week—and that seems to be a conservative estimate from consumer surveys—that's another $5,000 per year with no tax break. If you've been doing the arithmetic, you can quickly see that the bottom line is zero. Her $30,000 job brings home nothing. This is not an unrealistic story. The average working wife sees two-thirds of her salary lost to taxes and work-related expenses, and some women actually lose money by working.

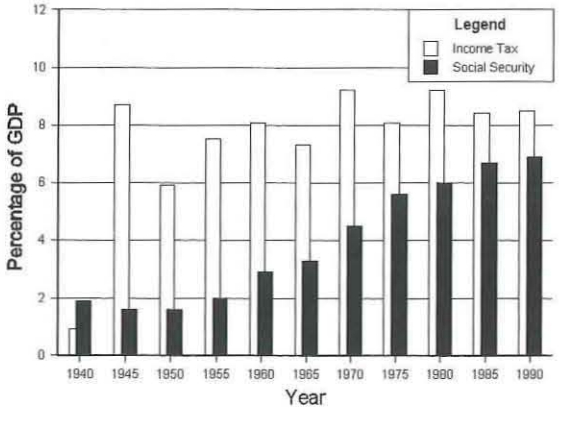
Now let's talk about the primary earner. In an example I work out in my book, I show how a $2,000 raise can more than match the $30,000 job offer for the wife. Of course, in this example, with that bottom line of zero, even a $1 raise is better. He might even be in a lower marginal tax bracket than his wife, if he has passed the Social Security ceiling of approximately $60,000. His additional work doesn't open up the need for child care or generate many, if any, of those additional work-related expenses. This incentive structure favors and rewards traditional one-earner families. If a

woman wants to work, there are plenty of incentives not to be married or not to have children in the first place. Children provide an incentive for one person to stay at home—almost always the wife—and for the other person to work more. When we look across America, we see that pattern playing itself out.

To summarize more systematically, six factors underlie the Accountant's Tale: 1) joint filing; 2) the structure of Social Security; 3) nontaxation of the imputed income from self-supplied child care and other home production (i.e., if you stay at home, you're providing valuable services, but you're not paying any tax on the value of these benefits); 4) inadequate deductions for child care and other work-related expenses of the secondary-earning spouse; 5) the fringe benefits system, which rewards you through the tax system if you're a single-earner family and often forces the second earner to take fringe benefits the family doesn't need; and 6) state and local taxes that are parasitic on the federal tax structure. Now, I'm not going to turn this into a Lawyer's Tale; nobody wants to hear that. But I want to underscore an important idea—that the way things are is political and that the system was set up to entrench and reward one particular model of the family, to the exclusion of other possible models. The kinds of changes that I think are possible and that I'm advocating are in the direction of a more flexible, more just, and more neutral set of rules. To make this clear, I would like to discuss these six factors in their historical context.

The Historian's Tale

I'll deal with them chronologically, starting with Social Security. Social Security is a big tax. When it began in the 1930s Social Security was a bigger tax than the income tax. The income tax progressed from a small tax on the wealthy to a mass tax during World War II, with the brilliant invention of wage withholding. By 1945, the income tax had leaped up to a level of 9 percent of gross domestic product, where it has pretty much stuck. (When it gets too high, a Democrat usually gets thrown out of office and some Republican comes in and cuts taxes.) It's a different story with payroll taxes such as Social Security, which have shown a steady increase, so that by 1990 the payroll tax system accounted for 85 percent as much revenue as the income tax did. This may be a surprising fact, but more than 90 percent of Americans pay more in payroll taxes than they pay in income taxes. You don't see it; you don't fill out a form; it's not associated with the IRS; politicians don't talk about it. But when you combine the 7.65 percent that comes out of each employee's pocket with the equal share that comes out of the employer's—but which all economists agree is in effect paid by the worker—you see that Social Security is pretty much a flat 15.3 percent tax on wages, with no zero bracket or other adjustment for family size, etc. That is a steep tax.



After a big jump in 1945 to 9 percent of gross domestic product, the income tax has remained quite level. Social Security, however, has seen a steady rise, and in 1990 brought the government almost as much revenue as the income tax.

Social Security also has a very strong gender bias built into it because of decisions made in 1937 and 1939. Social Security was first put in place in 1935 by the Roosevelt administration. It was intended to be an actuarially funded system, so in the early years it was building up a reserve. But in 1936, things changed. John Maynard Keynes's *General Theory of Employment, Interest and Money* was published, which suggested that maybe it wasn't a good idea for a government to be building up a surplus in the Depression. The government decided to spend the reserve money, so a Social Security Advisory Commission was set up in 1937 to decide how to spend it. There were two candidates for extending benefits: one was to give some benefits to domestic and agricultural workers, who were largely African American; that idea didn't go very far. The second recommendation was to extend benefits to stay-at-home wives by creating a spousal share. This idea was widely popular. When the modification was first put into place, it was even explicitly sexist: wives who didn't work got a benefit.

But there was a little glitch that the reformers didn't think of—or did think of, actually. What about the working wife? This wife would get benefits anyway as a spouse. What were we going to do with her when she entered the work force? And the answer was: tax her anyway by not giving her any exemption level over the range in which her work did not generate any additional Social Security benefits. The commission noticed this in 1937, but they thought it was a *good* thing, for the explicit reason that married women ought not to be competing with single women. (In other words, they accepted that there was a segregated work force—there were male jobs and female jobs. They were simply trying to protect single women by keeping married women from working.) That might have been all right then, when Social Security was a flat two percent tax. But right now Social Security is at 15.3 percent, when we factor in the employer's share along with the employee's. It's a big tax, and working wives are getting little if any benefit from what they're paying into that

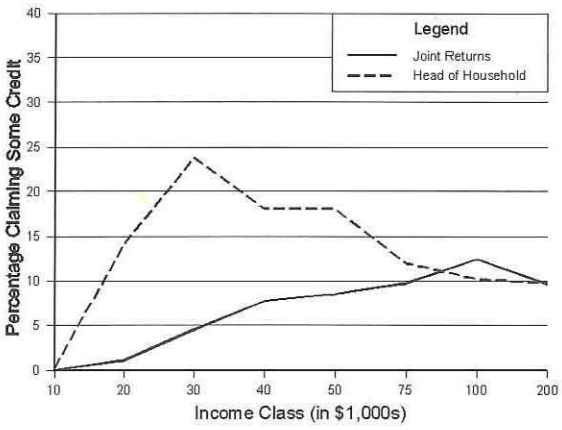
More than 97 percent of married couples file jointly, and, as far as I can tell, the other 3 percent consists mainly of estranged but still married couples who won't sign the same form.

system. (There are possible exceptions, as when a secondary-earning spouse divorces before 10 years of marriage. Social Security is a complex matter. But these details don't change the basic fact that many women are paying a pure tax under Social Security.)

Joint filing under the income tax also has an interesting and complex history. It was instituted in 1948. Husbands and wives were defined as a single taxable unit. This, by itself, did not create a "marriage penalty"; during the period from 1948 to 1969, you could see your taxes go down on getting married but never go up. But joint filing created a big factor in the Accountant's Tale—the secondary-earner bias. By calling husbands and wives a single taxpayer unit, the government created an incentive to think of whose salary came first. Who got to take advantage of the zero bracket? Who was it who might *not* work? This is a perfectly appropriate way to think in accounting and economics. Once you think that way, it's overwhelmingly likely that the wife's work is going to come second, pushing her into a tax bracket dictated by her husband's salary. The secondary-earner bias is rarely talked about, but it's a big problem. Most countries that have a comprehensive tax system have now moved away from joint filing. Italy reverted from joint to separate filing in 1979, and England did so in 1990. We do have a possibility of filing separately even though married, but it's not the same thing as separate filing, and most married couples would pay more tax if they did this. More than 97 percent of married couples file jointly, and, as far as I can tell, the other 3 percent consists mainly of estranged but still married couples who won't sign the same form.

The third and fourth factors from the Accountant's Tale concern child-care deductions. A court decision in 1939 ruled that child-care expenses of two-earner couples were not business expenses; they were personal expenses, attributable to the fact of having children. This set a baseline in which we view child-care relief as exceptional or aberrational—as somehow subsidizing a personal decision to have children, rather than a legitimate work-related expense of two-earner families. Starting in 1954, the government did some very small and grudging things: they instituted a maximum $600 per family deduction, which stayed at $600 into the 1970s, not indexed for inflation. This just applied to couples earning less than $20,000 in 1954, a number that did go up a bit. The idea was to target some small relief to lower- and middle-income families, on the theory that rich wives shouldn't be working anyway. The law viewed working wives as some kind of exception to a general rule.

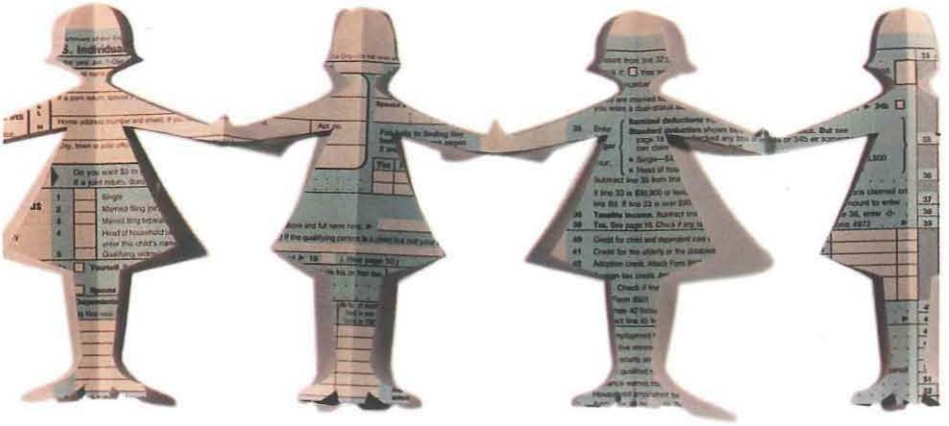
Today we have a child-care credit. It's more generous than the 1954 deduction, but the fact of the matter is that it is still grudging, and not very many people bother to take advantage of it. Among married couples earning less than $10,000, no one gets a child-care credit; couples earning $10,000 to $20,000 get an annual average of $250, or about $5 a week. By the time you're making $200,000 a year, the federal government might give you $500 back, or $10 a week, for



Not very many families even bother to claim the stingy child-care credit currently allowed them on their income tax. For joint filers (solid line), it makes sense only at higher incomes, while middle-income single parents (represented by the dashed "head of household" line) find it somewhat more worth their while.

child care. The numbers are a little better for single parents, but not much. You can see that an incredibly small percentage of married couples even bother to claim the credit (the dark line in the graph is the joint returns). That continues to be true until you get into pretty high income ranges.

The particular problem among lower-income families is that the child-care credit is nonrefundable. That means that you don't get any benefit from it unless you're paying positive income taxes. (A negative income tax means you get a net payment from the government.) Since the poorest one-third or so of American households do not pay positive income taxes, they get no benefit from a nonrefundable credit. Those households are paying taxes, but they're paying taxes in the form of losing the benefit of the earned-income tax credit. The people who set up this system were



aware of what they were doing. Legislators would stand up on the floor of Congress and say, in essence: "We don't want to help two-earner families; we don't want to help working wives; we don't think women should be working." Lately, in the 1980s and 1990s, people use code language about how important it is to have stay-at-home parents, but they are still opposing working wives.

The secondary-earner deduction also has an interesting history. As I mentioned earlier, a typical second earner enters the work force at a 50 percent tax rate. She doesn't have the benefit that a primary earner has of going through a range in which she's not paying positive taxes. An obvious thing to do would be to give her some deduction to account for various work-related expenses and to replicate the effect of having her own zero bracket. In 1981, the first great Reagan tax reform was put

The conservatives had convinced themselves that women were working only in order to pay taxes. If, in fact, women want to work, the logic would run exactly in the opposite direction.

in place, which allowed a relatively small secondary-earner deduction: 10 percent of the lesser-earning spouse's salary up to $30,000, thus a maximum deduction of $3,000. That looks like it was a nod to help working women, but in 1981, there were also serious proposals for separate filing and other things that would have helped two-earner couples a lot more. This limited second-earner deduction was the cheapest option on the table, and they went with it. The end of the story comes five years later, when they repealed it.

Many of you may still remember the Tax Reform Act of 1986—it featured a quite radical simplification of the tax laws and a dramatic lowering of the tax rates. In the decades before that, the highest rate bracket was very high for the wealthiest Americans—for a brief while during World War II it was 94 percent. Since then we've had two great tax-reducing presidents. The first was John F. Kennedy, who lowered the top rate to 70 percent in 1963. It stayed there until 1981, when Reagan lowered it to 50 percent. Five years later, he lowered it again to 28 percent.

By 1986, a conservative idea had been floating around for many years, namely that women were working in order to pay taxes. Conservatives believed that the reason we were seeing more two-earner families was that taxes had increased so that the husband's salary alone was no longer sufficient to support the household, and the wife had to work to generate enough cash to pay the taxes. The conservatives thus thought that the way to get women back into the home was to lower taxes. Reagan, in his brief statement in signing the Tax Reform Act of 1986, singled out its incentives to get women to stay home as one of the principal advantages of the law. He said it would make it economic to raise children again.

This turned out to be exactly wrong. The conservatives had convinced themselves that women were working only in order to pay taxes. If, in fact, women want to work, the logic would run exactly in the opposite direction. High taxes deter work effort; hence, lowering taxes would increase married women's work. Guess which answer won? Women *want* to work. After the 1986 tax reduction, there was a 25 percent surge in married women entering the work force.

Bringing the Historian's Tale up to the present, conservatives now face the question: how can they lower taxes, which they always want to do, without helping married working women, which they never want to do? We learned in 1986 that lowering tax rates across the board was the wrong answer. What's the right answer? A per-child (not child-care) credit. In his 1996 campaign, Bob Dole came up with the witty slogan of "15-15-15"—a 15 percent across-the-board rate cut. Conservatives didn't want him to do that, because a general rate reduction, as in 1986, would help working wives. The *Contract with America*, written in 1994, called for a per-child credit instead of any general rate reduction; the Christian Coalition published a parallel tract, also supporting the idea. It was by far the most expensive element of tax reduction in the *Contract with America*, accounting for $162 billion out of a $300 billion total tax reduction—much more expensive than capital gains reduction or anything else. Bill Clinton signed onto this, and this is the proposal in the budget act—a per-child (not child-care) credit. [The Taxpayer Relief Act of 1997, signed into law in August, incorporated this change.]

There are two problems with a per-child credit from the point of view of taxing women. First of all, it's nonrefundable. Forty percent of American families will get no benefit from the per-child

credit, and, of course, they're the poorest, neediest 40 percent. Second, it maintains the high tax rates facing working wives. It's a form of tax reduction that doesn't change the Accountant's Tale at all, except to give families more money in the first place, whether the wife works or not. This would presumably cut against her incentive to work, and if you look at the language of the *Contract with America*, you can see quite clearly that it's deliberate.

The history of tax in America, from at least the 1930s down to the present day, shows time and again the rules being set in a way rigged against working wives and mothers. The work force itself has changed, but tax hasn't.

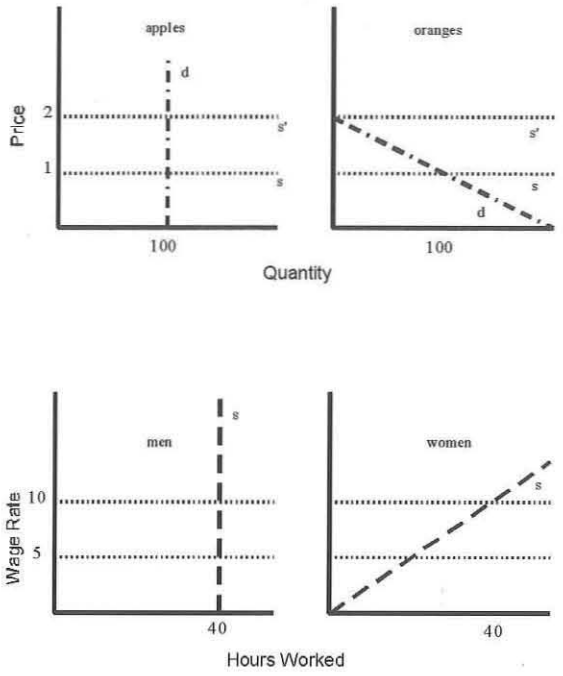
The Economist's Tale

Now we get to the fun stuff. I'd like to sketch out some of the basics of the theory of optimal tax, originated by Frank Ramsey in 1927, and show how it applies to the problems of taxing women. I'm also looking ahead to the ultimate connection to the Social Theorist's Tale.

To learn about the idea of optimal tax, I invite you to imagine that you're on an island that has only two commodities—apples and oranges, each selling for $1. (Someone told me that at Caltech that should be Apples and IBMs, but I'm a lawyer; I'll stick with the fruit.) Because the market is perfectly competitive, an individual seller raising the price would be undersold; anybody selling for less would go bankrupt. In this simple story, let's imagine that 100 apples and 100 oranges are purchased.

Now the government comes onto the scene and decides that it needs $100. How is the government going to raise the $100? The easy answer is to put a 50 percent tax on both apples and oranges. But nothing is too easy in economics, because things change in the face of tax. First, the price of apples and oranges would each go up to $2 because the seller still needs to net $1. If he sells for $2, he will give 50 percent, or $1, to the government, keeping the $1 he needs, by definition, to break even. This price change means changes in the demand for apples and oranges. But what, exactly, happens next? Because we're in the Economist's Tale, the answer is: it depends. More specifically, it depends on the nature of the demand side.

Under this stylized example, let's assume that the demand for apples is what we call completely inelastic. Because people have to have an apple a day to keep the doctor away, they'll pay any price for that apple. So that means the 100 apples will still be purchased, even at the price of $2. On the other hand, let's say the demand for oranges is relatively elastic, or flexible in the face of changing prices. When the price goes up, people decide that oranges aren't worth it. If we insisted on taxing oranges, no one would buy them, and the market in oranges would shut down completely.



If the demand for apples is inelastic (people will buy them no matter how high the tax) and the demand for oranges elastic, you get the demand curves shown at top right. When it comes to taxing the labor supply, men look like apple eaters and women like orange eaters (the line slopes the other way because these are supply curves).

Economists view this as silly, or inefficient, and this is why Ramsey developed his theory of ideal taxation. Applying the optimal tax solution, you should only tax inelastically demanded goods. So, we should be taxing apples—and things like cigarettes, alcohol, and gasoline—and not oranges. Let's go back to the island example. If we imposed a 50 percent tax on apples alone and no tax at all on oranges, the government would still get its $100. Apple sellers wouldn't care because they would still be selling their 100 apples for a net, after-tax price of $1. But orange sellers and orange buyers would now be satisfied. We could repeal the silly and unproductive 50 percent tax on oranges.

Now you may be asking: What does this have to do with taxing women? It turns out that women are like orange eaters and men are like apple eaters. Men are inelastic suppliers of labor; that is, they work full time, all the time. They don't know what else to do. They work at least 40 hours a week; it doesn't matter what you pay them. Women, on the other hand, are very sensitive to the wage rate. This is what we learned in 1986, a fact that anybody who had been paying attention would have already known (but that anybody didn't include Ronald Reagan or the other conservative advocates of the Tax Reform

Act of 1986). When we effectively raised the take-home pay of women by lowering taxes, women worked more.

Michael Boskin, a conservative economist at Stanford University and the Hoover Institute, and chairman of President Bush's Council of Economic Advisers, coauthored a paper in 1983 that suggested taxing married men twice as much as married women. That's the optimal thing to do. Men are like apple eaters; we should tax them. Women are like orange eaters; we should leave them (relatively) alone. So that's the Economist's Tale.

Between the Economist's Tale and the Social Theorist's Tale lies an academic divide that I'd like to try to bridge here. It is especially noticeable in the legal academy, where I spend much of my time. On the one hand, the law and economics movement has been pursuing a wealth and utility maximizing project, drawing on all of the tools of modern economic theory, including finance, game theory, and welfare economics. On the other hand, many other scholars, operating out of a classical liberal, social contractarian, or communitarian perspective, have recoiled from what they see as the quasi-science of the utilitarian camp—or what they take as an obsession with markets and money, to the exclusion of other more important and fundamental values. The two broad camps generally fail, and sometimes even refuse to attempt, to communicate with each other. I take this to be an unfortunate state of affairs. Life lies in the middle of such academic divides. Real people care about money and markets, for one obvious thing, but there is also much that a social theory, not wholly utilitarian, can learn from social science and vice versa.

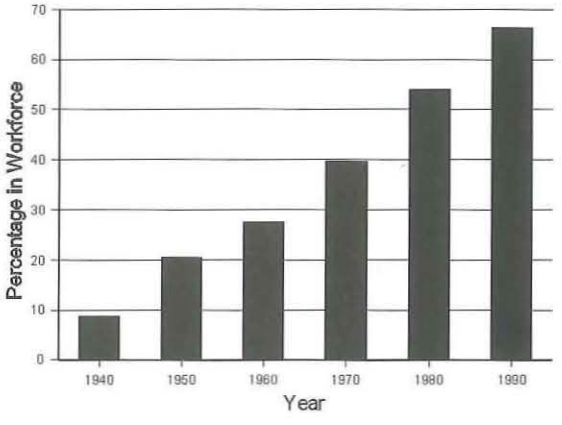
In the half century between 1940 and 1990, the percentage of married women who were working rose steadily from 8.6 percent to 64 percent.

Matters such as tax are first, foremost, and finally matters of politics. But we can no longer afford to dismiss politics as "mere politics," as if reason and logic can play no role in advising our communal political decisions. It's an unavoidable fact that we have only our common sense, our collective reason, to appeal to in deciding what is just or fair in matters such as tax. But there is no very good reason for our collective community not to look at the teachings of social science and to interpret them as it sees fit—all in the interest of a deeper and richer conception of what is fair or just.

It's pretty easy to see that we would not want simply to go down the route of optimal taxation—taxing only the apples. For example, on the commodity side, optimal taxation would suggest taxing life-sustaining drugs, such as insulin, at particularly high rates, and trivial commodities, such as candy bars, at low rates. But that would interfere with our settled intuitions of fairness and justice. On the labor-supply side, optimal income taxation might suggest finding ways to single out people with a relatively committed work ethic—recent immigrants perhaps—and tax them at high rates, while leaving lazy people more or less alone. We wouldn't want to do that either. But the Economist's Tale *is* relevant nonetheless, even if it is not decisive, and it is particularly relevant to gender justice in America today. The Economist's Tale showed us that women are conflicted; they face difficult choices. The Accountant's Tale told us that we have piled a tax burden on what was already an overstressed and overburdened group of people. And the Historian's Tale told us that we did it on purpose. There's something wrong there. With the Social Theorist's Tale, I come to what I consider the biggest payoff for work in tax. It doesn't necessarily have anything to do with tax, but rather with the broader ideals of fairness.

The Social Theorist's Tale

There are many objective measures of women's equality—such as labor-market participation rates and wage levels, which are improving—but there are also subjective indications of their distress. Women of all sorts, but particularly married mothers, appear to be unhappy. How can this be? How can the objective signs of success or equality coexist with subjective signs of despair? In 1940, 8.6 percent of married mothers with children under the age of six worked. By 1990, this was 64 percent—a pretty steady increase of about 10 percent per decade. That's an astonishing demographic change, and you would expect it to be accompanied by changing models of work or the family. Unfortunately, this didn't happen. Meanwhile, the gender wage gap, after sticking at 59 percent throughout the 1960s and 1970s, has dramatically declined since about 1980. The gender gap is gone altogether in some subsectors. We are now getting calls to repeal affirmative action. It looks to many as though we've arrived in the Promised Land.



But we know better than that because the Accountant's Tale, the Historian's Tale, and the Economist's Tale have raised objectively grounded doubts. We can support these doubts with an

The gender wage gap, most remarkable during the sixties and seventies, when women earned less than 60 percent of male salaries, now appears to be on its way out.

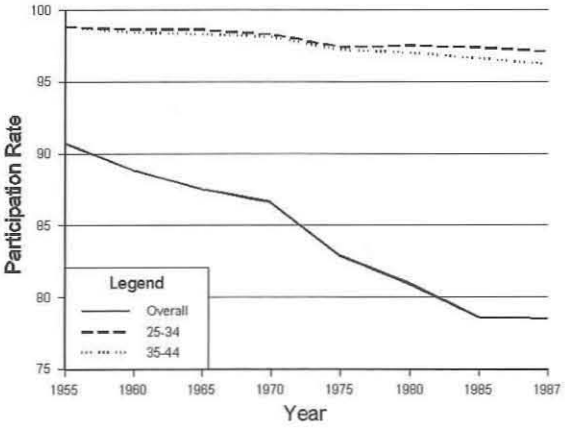
empathetic common sense that tells us that something is wrong when it comes to gender and justice in America today. So let's look a little more closely, and also more broadly, at labor markets in America.

Because of that stunning demographic change involving married women working, we would expect either the workplace or male behavior to have changed. But we're not going to find either one of those trends. First, let's look at part-time work. A lot of people think that more married women are working part time. That's wrong. There are fewer married women working part time today than there were, as a percentage, in 1959. About 25 percent of women who work do so part time. This figure is often cited to suggest that part-time work is a helpful answer to the dilemmas facing married mothers today. It's not. In tracking the situation of women, the Census Bureau lumps them into four age categories: 16–19 (teenagers), 20–24 (often students), 25–54 (most women doing most things), and 55 and older. The 25–54 age group is the *least* likely to feature part-time workers. The overall average that makes up that 25 percent is fueled by older women and teenagers, so it's clear that part-time work hasn't offered much of a solution to the problems facing married mothers. Part-time work is usually low in pay, low in prestige, and unlikely to survive the Accountant's Tale's rigid calculus.

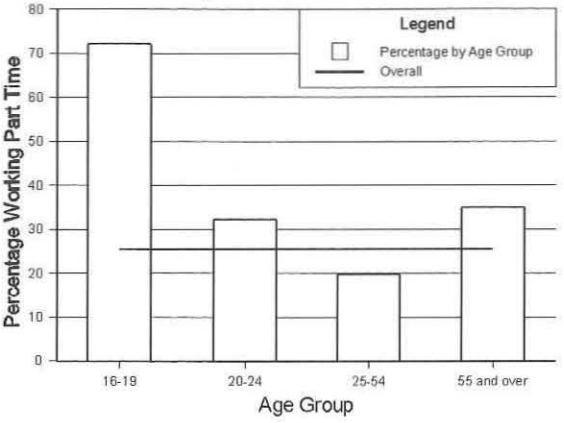
Across the board, there's some movement in part-time and flexible-time work, but it's more on the demand side of firms, rather than on the supply side of individual workers. It's motivated by firms that are looking to circumvent fringe-benefit laws, firms that want a more flexible work force that they can hire and fire in the event of business-cycle contractions and so forth. We can also pull out of the part-time labor numbers that percentage of people working part time who are doing so involuntarily. This turns out to be a very high percentage.

Now, what's been happening to the male side of the picture? Occasionally people say that part of the narrowing gender wage gap is due to changing male behavior, and they can point to some statistics to show this. In 1955, 91 percent of all married men were in the work force; by 1987, the number had dropped to about 78 percent. That's not as big a change as on the women's side, but it still looks like a significant shift. But there's reason to be suspicious that it is really much of a change at all. If we break that into the subcategories of men 25 to 34 and 35 to 44, men in their prime parenting years, we find that nothing much has happened. More than 97 percent of married men between 25 and 44 were in the work force in 1955, and by 1987 the number had dropped to about 96 percent. Why is it, then, that male labor force participation appears to be declining? The entire effect is driven by what is happening to men older than 55. They're retiring earlier and living longer; they're a bigger percentage of the population.

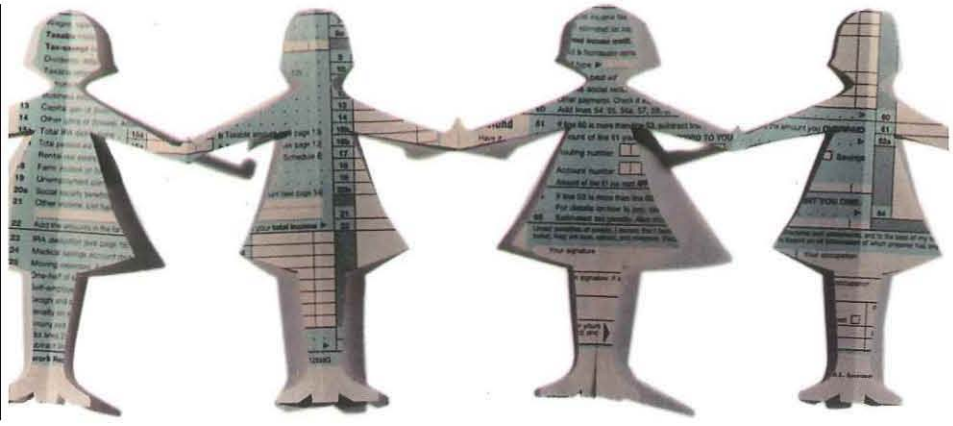
So, what *has* happened since 1940? It turns out that, although the workplace didn't change and men didn't change, women did. Women now face a choice between staying home full time or working full time, and those women who work are now working like men have always worked—full time and with full commitment. Since they haven't



Far right: Although men's rate of participation in the labor force appears to have declined sharply over the last 40 years (solid line), the percentage of men 25-44 working has consistently remained over 95 percent. The drop is due to the fact that men are retiring earlier and living longer.



Right: About 25 percent of working women work part time, but this figure is mainly driven by women under 25 and over 55. Part-time work has not offered a good option for women in their prime earning years.



been given good part-time opportunities, and since there's been relatively little change on the male side in regard to men helping out at home or working less than full time, women have started to act like men. A variety of statistics back this up. Like men, women are now marrying later; for example, over the last 20 years, the percentage of

If we had better tax provisions for child care, it might give more money to the important sector of our economy that cares for children, while giving women more choices.

women age 25 who are married has dropped from 67 to 33 percent. Women are having fewer children and are having them later in life. And working women are educating themselves more, signaling that they're serious about work. Women are also staying in their jobs longer.

This whole scenario could have been predicted from the biases I mentioned at the beginning. Remember, the basic story of taxing women is the bias against two-earner families. Among the poorer classes, that's a bias against marriage. This means more single parents and more single parents working full time. Among the wealthier classes, it's a bias for stay-at-home wives, while the men continue to work full time. And in the middle, it means stress and an all-or-nothing effect for women—stay home or work full time inside *and* outside the home. A small group of women is looking for flexible options, but they're not going to find them, by and large. They are going to have to work full time and with full commitment, too.

Conclusion: Hopes for Change

There is some good news. There are lots of relatively easy things we can do to fix the problems of taxing women. The first one is to return to separate filing under the income tax. We had separate filing in America before 1948, and, as I mentioned earlier, most countries that have a comprehensive tax system have now moved to separate filing. This would treat husbands and wives as individuals, and would mean that a secondary-earning wife would have her own zero bracket; she would not be in an income tax bracket dictated by her husband's salary. Another feature of it is that it picks up the optimal tax solution, because it is, in effect, an increase in the tax on men and a decrease in the tax on women. It also gives an inducement and encouragement for men to cut back on their labor-market participation and provides an incentive to families and firms to think of more creative part-time and flexible-time work.

Another fairly easy thing to do is to allow a secondary-earner exemption under Social Security. It's a pure tax on working wives designed to subsidize single earners and other families. It's pretty easy to give a secondary-earner exemption, which would give two-earner families money that they could use for work-related expenses, such as child care.

That's yet another easy thing to do: better child-care provisions. There are many reasons to consider child care a legitimate business expense. It's occasioned by the decision of the family to have two earners. It's the work and not the kids that is the proximate cause of the expense. If we had better tax provisions for child care, it might give more money to the important sector of our economy that cares for children, while giving women more choices.

Then there's fringe-benefit reform, which looks a lot like the secondary-earner exemption. Lots of families are forced to take duplicate fringe benefits that have already been extended to the whole family under the primary earner's salary. To keep things neutral and fair, we should let women opt out of these benefits that they simply don't need, and take cash instead. This should be tax-free cash, since fringe benefits are tax free. And we should simply get rid altogether of marriage penalties on lower-income families.

All of these proposals are relatively easy to implement, and they all have precedents in what other countries have done or what we ourselves have done in times past. They can be justified on the basis of social fairness and neutrality. And they are all supported by the "utilitarian" teachings of social science. But we need both the will and the understanding in order to do them. I hope that my work helps, at least with the latter. □

Republicans in Congress, partly inspired by McCaffery's book, have recently proposed a Marriage-Tax Elimination Act that would give married couples the option of filing separately.

Honors and Awards

Peter Dervan, Bren Professor of Chemistry and chair of the Division of Chemistry and Chemical Engineering, has been elected Scientist of the Year by the Achievement Rewards for College Scientists (ARCS).

Postdoctoral scholar Mary E. Dickinson has been selected by the Cancer Research Fund of the Damon Runyon–Walter Winchell Foundation to receive one of 20 postdoctoral fellowships awarded in 1997. Dickinson, a postdoc in the laboratory of Rosen Professor of Biology Scott Fraser, studies the neurogenesis of the spinal cord in zebra fish. Anthony P. West, Jr., a graduate student in chemistry, has also been selected to receive a Cancer Research Fund fellowship for a postdoctoral project beginning in July.

Associate Professor of Geochemistry Kenneth Farley has been selected by the European Association of Geochemistry to receive the Houterman Award, which is given to an outstanding young geochemist. Farley's research involves the application of noble gases to the study of several earth science problems, including mantle/atmosphere evolution and extraterrestrial debris on Earth.

Beckman Professor of Chemistry and Director of the Beckman Institute Harry Gray has been elected a foreign member of the Royal Swedish Academy of Sciences. The journal *Inorganica Chimica Acta* has also presented Gray with the Sigillum Magnum; the award is conferred on individuals judged to have made the greatest contribution to inorganic chemistry during the last 30 years.

Philip Hoffman, professor of history and social science, has been selected by the Economic History Association to receive the 1997 Gyorgy Ranki Prize for *Growth in a Traditional Society: the French Countryside, 1450–1815,* which the association judged the outstanding book published between 1994 and 1996 on European economic history. The book also garnered Hoffman the Allen Sharlin Memorial Award from the Social Science History Association.

Assistant Professor of Political Science Jonathan Katz has been selected to receive the Pi Sigma Alpha Award for Best Paper at the Midwest Political Science Association Convention, for his paper, coauthored with Gary King, "A Statistical Model of Multiparty Electoral Data."

Professor of Political Science D. Roderick Kiewiet has been named an executive council member of the Midwest Political Science Association.

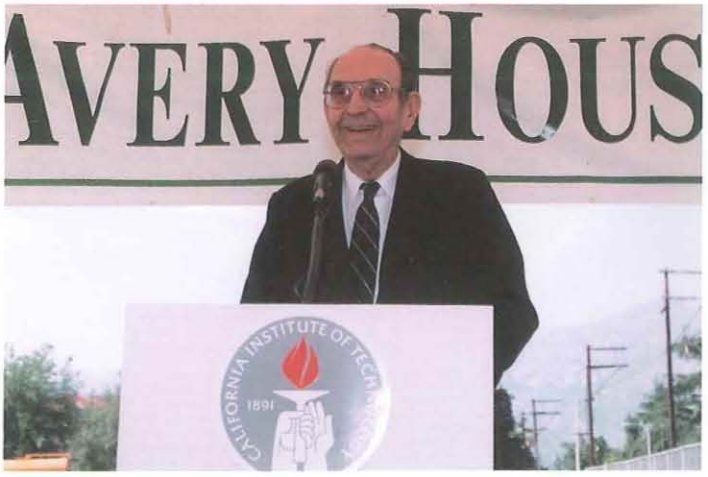
Steven Mayo, assistant professor of biology and assistant investigator, Howard Hughes Medical Institute, has won the 1997 Johnson Foundation Prize from the University of Pennsylvania's Johnson Research Foundation.

James Morgan, Goldberger Professor of Environmental Engineering Science, has been awarded the 1997 Simon W. Freese Environmental Engineering Award and Lecture by the American Society of Civil Engineers, for research that has brought "fundamental aquatic chemistry to bear on issues of environmental engineering practice."

Associate Professor of Astronomy Charles Steidel has been awarded a $500,000 David and Lucile Packard Foundation fellowship. Steidel's research area is the formation and evolution of galaxies; he will use the award largely for instruments to be fitted onto Palomar's 200-inch telescope, to aid him in his search for galaxies as they appeared when the universe was less than 15 percent of its current age.

David Stevenson, Van Osdol Professor of Planetary Science, will receive the 1998 Harry H. Hess Medal of the American Geophysical Union, to be awarded at its spring meeting. The award honors outstanding achievements in research on the constitution and evolution of Earth and sister planets.

Ahmed Zewail, Pauling Professor of Chemical Physics and professor of physics, has been selected by the American Chemical Society to receive the 1997 E. Bright Wilson Award in Spectroscopy. Zewail pioneered the field of femtochemistry, which uses lasers to observe nearly instantaneous chemical reactions in real time. □



Stan Avery speaking at the Avery House groundbreaking ceremony.

R. Stanton Avery 1907–1997

R. Stanton "Stan" Avery, maverick inventor and longtime Caltech trustee and benefactor, died early Friday, December 12, at Huntington Hospital in Pasadena.

Long a household name because of the Avery labels he created and marketed, Avery had a strong association for many years with the Institute. He became a member of the Board of Trustees in 1971, and served as chair between 1974 and 1985. At the time of his death he was a Life Trustee and trustee chair emeritus.

"The whole Caltech community is deeply saddened by the passing of Stan Avery," said Dr. Gordon E. Moore, chair of the Caltech Board of Trustees and chairman emeritus and cofounder of the Intel Corporation. "He was a great supporter of the Institute, and he will be fondly remembered by the trustees, the faculty, and the students and staff."

Avery's most recent major gift to Caltech was Avery House, an 80,000-square-foot dormitory on the northeast side of campus, that was completed in September 1996. True to his entrepreneurial spirit, he provided the resources to make Avery House one of the most innovative dormitories in America—to wit, a dorm with an entrepreneurial focus that both celebrates and supports the spirit of innovation and invention. The dorm has space for graduate and undergraduate students as well as faculty families and visiting entrepreneurs and other special guests.

In October, Avery participated in an Avery House event for inner-city teenagers. The event, "Tomorrow's Entrepreneurs Today," drew a number of 13- to 17-year-olds with an interest in entrepreneurship who heard the 90-year-old Avery tell the story of his career.

An Oklahoma native, Avery came to Southern California after high school and earned his bachelor's degree from Pomona College. He initially thought about entering the import-export business after spending an entire college year in China, but the grim business climate of the Depression eventually led him into the circumstances that would ultimately make his fortune as an entrepreneur and inventor. In 1932, with a $100 loan from his bride-to-be and some machine-design experience with a company that had gone out of business, he cobbled together a prototype labeling machine from various mechanical parts. This machine was to make possible the first commercially successful self-adhesive labels, and is the ancestor of the Avery label enterprise as it exists today.

From that modest beginning, the company grew into the multi-billion-dollar international Avery Dennison Corporation.

In addition to his Caltech affiliation, Avery through the years was also a member of the Huntington Library board of trustees, director of the Los Angeles World Affairs Council, president of United Way, trustee of the Los Angeles County Museum of Art, member of the Claremont University Center board of fellows, and vice chairman of the Performing Arts Council of the Music Center board of governors.

Avery is also widely known to college students and faculty in Southern California for the Durfee Foundation, which was created in 1960 by Avery and his first wife, the late Dorothy Durfee Avery, to promote a number of individual efforts that are one-of-a-kind ventures that are unlikely to receive support from any other source. Since 1985, the Durfee Foundation has sponsored the American/Chinese Adventure Capital Program to nurture creative interaction between Americans and the people of mainland China. □—*RT*

A portrait of President Emeritus Tom Everhart was unveiled at a January 13 luncheon attended by members of the Board of Trustees and Tom and Doris Everhart. Painted by Los Angeles artist Brent Benger, Everhart's likeness will join those of his predecessors—Millikan, Brown, and Goldberger—on the Athenaeum walls, just outside the lounge. The Everharts were also presented with memorabilia of their leavetaking last June, as well as the framed charcoal study for the portrait.