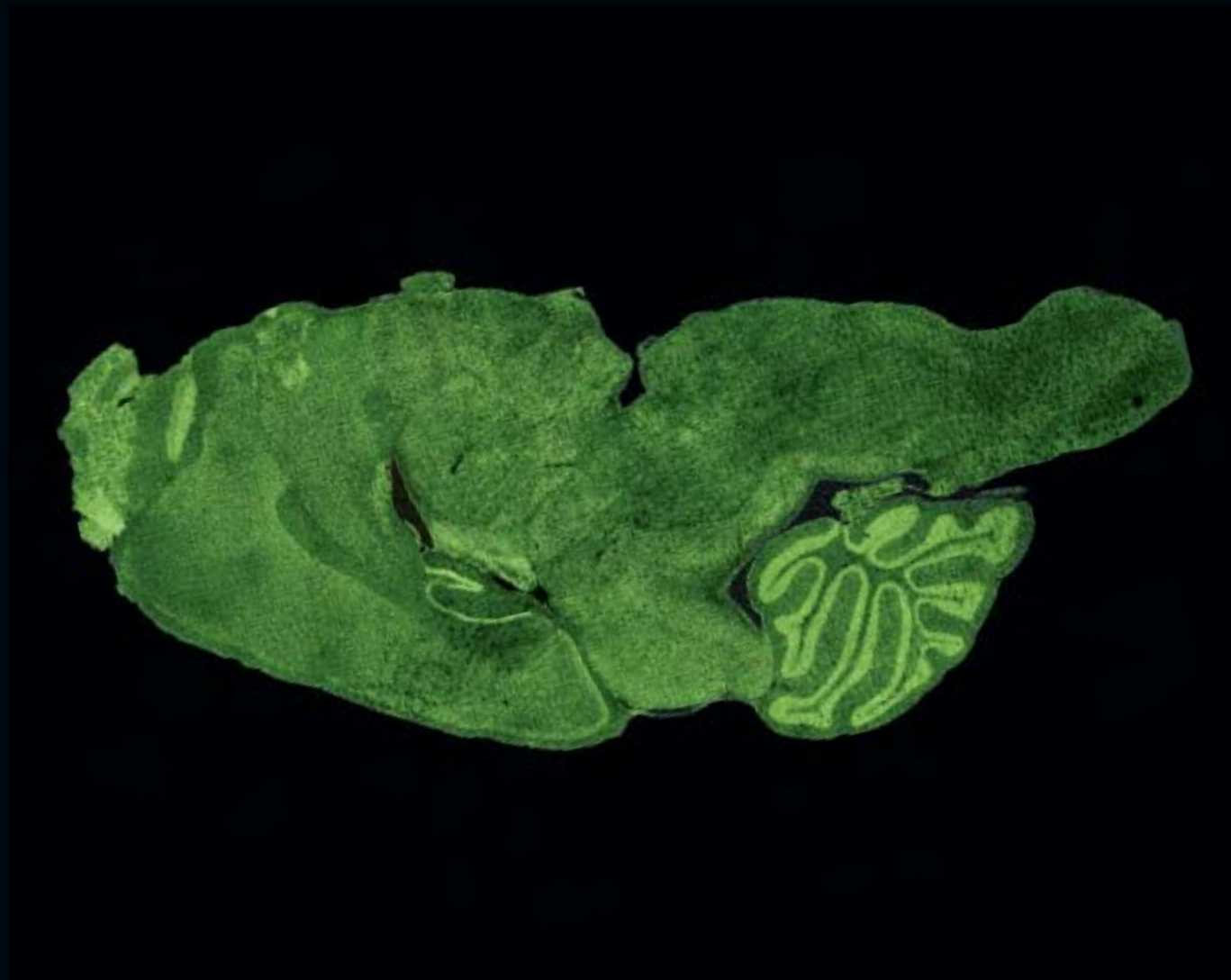


HHMI BULLETIN

Howard Hughes Medical Institute

www.hhmi.org



Juan Young / Zoghbi lab

Communication Breakdown

DEFECTS IN A SINGLE GENE CAUSE RETT SYNDROME, A RARE NEUROLOGICAL "AUTISM SPECTRUM" DISORDER THAT DESTROYS MOTOR COORDINATION AND COMMUNICATION SKILLS OF GIRLS WITHIN THE FIRST YEAR OR SO OF LIFE. IN 1999, HUDA ZOGHBI IDENTIFIED THE CULPRIT MUTATIONS IN THE GENE CALLED *MECP2*, AND BY STUDYING THE DISORDER IN MICE HAS LEARNED THAT THE MECP2 PROTEIN (GREEN) IS EXPRESSED IN EVERY MATURE NEURON IN THE BRAIN AND REGULATES THE ACTIVITY OF OTHER GENES. IT'S NO WONDER, SHE SAYS, THAT A DEFECT IN THE GENE WREAKS SUCH HAVOC. NOW, ZOGHBI'S GROUP HAS DISCOVERED A ROLE FOR MECP2 AT THE SYNAPTIC CONNECTION BETWEEN NEURONS—AN AREA OTHER AUTISM RESEARCHERS ARE FOCUSING ON AS WELL (SEE PAGE 20).

HHMI
HOWARD HUGHES MEDICAL INSTITUTE

4000 Jones Bridge Road
Chevy Chase, Maryland 20815-6789
www.hhmi.org



MAKING MATH COUNT Real-world math problems may make students better biologists.
IN THIS ISSUE Autism's Invisible Barriers / X Inactivation / Fidgeting Enzymes

Studying X chromosome inactivation in several cultured human embryonic stem cell lines, researchers in Jeannie Lee's Harvard lab found that, after one X was inactivated, the cells in some lines stopped producing the molecule responsible for the silencing. The finding raises questions about the possibility of genes being "reawakened" in embryonic stem cells after normal genetic silencing.

OBSERVATIONS



EMERGING STRANGERS

When psychiatrist Leo Kanner observed 11 children in the 1930s and 1940s who shared certain unmistakable characteristics, he coined the term "infantile autism." From their earliest days the children's behavior, he says, was "governed rigidly and consistently by the powerful desire for aloneness and sameness."

According to his mother, [Herbert] was "always slow and quiet." For a time he was believed to be deaf because "he did not register any change of expression when spoken to or when in the presence of other people; also, he made no attempt to speak or to form words."...

Herbert, when examined on his first visit [at 3 years, 2 months of age], showed a remarkable intelligent physiognomy and good motor coordination. Within certain limits, he displayed astounding purposefulness in the pursuit of self-selected goals. Among a group of blocks, he instantly recognized those that were glued to a board and those that were detachable. He could build a tower of blocks as skillfully and as high as any child of his age or even older. He could not be diverted from his self-chosen occupations. He was annoyed by any interference, shoving intruders away (without ever looking at them), or screaming when the shoving had no effect.

He was seen again at 4 years, 7 months, and again at 5 years, 2 months of age. He still did not speak. Both times he entered the office without paying the slightest attention to the people present. He went after the Seguin form board and instantly busied himself

putting the figures into their proper spaces and taking them out again adroitly and quickly. When interfered with he whined impatiently. ... He did not respond to being called or to any other words addressed to him. He was completely absorbed in whatever he did. He never smiled. He sometimes uttered inarticulate sounds in a monotonous singsong manner. At one time he gently stroked his mother's leg and touched it with his lips. He very frequently brought blocks and other objects to his lips. There was an almost photographic likeness of his behavior during the two visits, with the main exception that at 4 years he showed apprehension and shrank back when a match was lighted, while at 5 years he reacted by jumping up and down ecstatically. ...

Five of our children have by now reached ages between 9 and 11 years. ... The basic desire for aloneness and sameness has remained essentially unchanged, but there has been a varying degree of emergence from solitude, an acceptance of at least some people as being within the child's sphere of consideration, and a sufficient increase in the number of experienced patterns to refute the earlier impression of extreme limitation of the child's ideational content. One might perhaps put it this way: ... our children gradually compromise by extending cautious feelers into a world in which they have been total strangers from the beginning.

From the paper "Autistic Disturbances of Affective Contact," Leo Kanner, Nervous Child 2, 217-250 (1943).

VOL. 21 MAY '08 NO. 02

DEPARTMENTS

PRESIDENT'S LETTER

03 A Season of Change

CENTRIFUGE

04 Men Overboard

05 The Sweet Smell of Exhaust

06 A Baroque Biochemist

UPFRONT

08 Know When to Fold 'Em

10 Freeze-Framing a Fidgety Molecule

12 Baring HIV's Dependencies

PERSPECTIVES AND OPINIONS

38 Stuart L. Schreiber

40 Claudia M. Neuhauser

42 Q&A – How does chemistry affect the average person?

CHRONICLE

Science Education 44 Cue the Crickets

46 Strategizing to Diversify Science

Institute News 47 In Memoriam: Richard G. Darman

47 HHMI Offers Boost to Early-Career Scientists

Lab Book 48 Of Fish and Men

49 Fixing Fragile X

50 A New Clarity

Ask a Scientist 51 Do only the “long bones” of the arms and legs contain growth plates?
And if so, how do all of the body's bones stop growing at maturity?

Up Close 52 Hearing Through the Din

Nota Bene 54 News of recent awards and other notable achievements

OBSERVATIONS

Emerging Strangers

FEATURES

14



Teaching Young Biologists New Tricks

Long the science where math mattered less, biology increasingly demands powerful quantitative skills. Teaching students the math they'll need, though, is more than just $1+1=2$.

[COVER STORY]

20



Invisible Barriers

Children with the subtlest form of autism suffer social isolation; those with more severe disease face a much tougher road. New genetic clues put the spotlight on the communication hubs of the brain's neurons—the synapses.

26



Genetic Balancing Act

Animals have evolved intricate ways to ensure that gene activity is the same in males and females despite the inherent imbalance of X chromosomes.

32



Sources of Renewal

As scientists learn more about how to produce and manipulate stem cells—amid high expectations and close scrutiny—no one is ready to choose any one approach over another.

A fan of deserts, writer **MITCH LESLIE** somehow ended up in drizzly Portland, Oregon, where he covers cell biology and immunology for *Science* magazine. Between sets of tennis, he has also written for *Cell*, *Smithsonian*, and *New Scientist*.

Despite an online quiz naming her ideal career as ice cream tasting, **SARAH C.P. WILLIAMS** decided to become a science writer. A graduate of the science writing program at the University of California, Santa Cruz, she has written for *ScienceNOW*, *Yale Medicine*, and *Stanford Medicine*, and is now assistant editor of the *HHMI Bulletin*. Williams lives in Washington, D.C., with her Great Dane and a freezer well-stocked with ice cream.

Cut out bits of paper, fragments of hand-drawn figures, and colorful patterns are just some of the elements that make up the illustration work of **LUKE BEST**. His diverse output includes video and animation, window displays, art direction, fashion, and homemade spacesuits. Best lives in London, where he works as part of an artistic collective known as Peepshow.

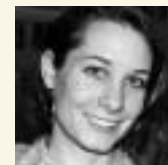
LEAH FASTEN says she loves the way science looks, admitting that it probably comes from too much time spent hanging around her husband's biochemistry lab. Leah left a career in airline finance to pursue photography full time. Since launching her Boston studio in 2003, she has photographed scientists for the Massachusetts Institute of Technology, Harvard University, *The Scientist*, and *Make* magazine, among others.



(1)



(3)



(2)



(4)

HHMI TRUSTEES

James A. Baker, III, Esq.
Senior Partner / Baker & Botts

Ambassador Charlene Barshefsky
*Senior International Partner
WilmerHale*

Richard G. Darman
*Partner / The Carlyle Group
Chairman of the Board / AES Corp.
(Through January 25, 2008)*

Joseph L. Goldstein, M.D.
*Regental Professor & Chairman, Department of Molecular Genetics
University of Texas Southwestern Medical Center at Dallas*

Hanna H. Gray, Ph.D., *Chairman*
*President Emeritus & Harry Pratt Judson
Distinguished Service Professor of History
The University of Chicago*

Garnett L. Keith
*Chairman / SeaBridge Investment Advisors, L.L.C.
Former Vice Chairman & Chief Investment Officer
The Prudential Insurance Company of America*

Jeremy R. Knowles, D.Phil.
*Amory Houghton Professor of Chemistry and Biochemistry
Harvard University
(Through April 3, 2008)*

Paul Nurse, F.R.S.
President / The Rockefeller University

Kurt L. Schmoke, Esq.
Dean / Howard University School of Law

Anne M. Tatlock
*Director, Retired Chairman & CEO
Fiduciary Trust Company International*

HHMI OFFICERS & SENIOR ADVISORS

Thomas R. Cech, Ph.D. / *President*
Craig A. Alexander / *V.P. & General Counsel*
Peter J. Bruns, Ph.D. / *V.P. for Grants & Special Programs*
David A. Clayton, Ph.D. / *V.P. for Research Operations*
Joseph D. Collins / *V.P. for Information Technology*
Jack E. Dixon, Ph.D. / *V.P. & Chief Scientific Officer*
Joan S. Leonard, Esq. / *Senior Counsel to the President*
Avice A. Meehan / *V.P. for Communications & Public Affairs*
Edward J. Palmerino / *V.P. for Finance & Treasurer*
Gerald M. Rubin, Ph.D. / *V.P. & Director, Janelia Farm Research Campus*
Landis Zimmerman / *V.P. & Chief Investment Officer*

HHMI BULLETIN STAFF

Mary Beth Gardiner / *Editor*
Cori Vanchieri / *Story Editor*
Jim Keeley / *Science Editor*
Patricia Foster / *Associate Director of Communications
for Web & Special Projects*
Sarah C.P. Williams / *Assistant Editor*
Dean Trackman, Maya Pines / *Contributing Editors*

ADDITIONAL CONTRIBUTORS

Cay Butler, Michelle Cissell, Mark Farrell,
Nicole Kresge, Steven Marcus, Heather McDonald
VSA Partners, NYC / *Concept & Design*

HHMI
HOWARD HUGHES MEDICAL INSTITUTE

Telephone (301) 215.8855 • Fax (301) 215.8863 • www.hhmi.org
©2007 Howard Hughes Medical Institute

The opinions, beliefs, and viewpoints expressed by authors in the *HHMI Bulletin* do not necessarily reflect the opinions, beliefs, viewpoints, or official policies of the Howard Hughes Medical Institute.

A Season of Change

EVEN THE DAMP, GRAY DAYS SO PERVASIVE THIS SPRING CAN'T diminish the extraordinary magic with which the season unfurls outside my office here at HHMI's headquarters in Maryland. Arrayed to the right, in all their diaphanous glory, the pale pink blossoms of cherry trees rim the oval pond. Out another window, I glimpse the spreading forms of flowering crab apples with blooms of deep rose. It's a season of change here at HHMI, and that change is not restricted to the surrounding landscape.

For example, the Institute has just announced a major new program for highly talented, early career scientists. It's an initiative we hope will inject some much-needed optimism into a research community dispirited by the dim prospects of being funded by the National Institutes of Health. The scientists we are targeting—those within two to six years of their first appointment as an assistant professor or equivalent position—are often at a high point of their creativity but face daunting odds in winning stable funding for their research. As we move into year five of flat budgets at the NIH, our colleagues there are concerned about the issue and are trying to address it but lack the flexibility we enjoy.

The HHMI initiative comes at a critical moment for the nation, and we're fortunate to be able to respond in a meaningful way. Having said that, we're mindful that nonprofit organizations face a surfeit of opportunities to respond to gaps in federal funding. They must exercise care in deciding when to intervene, or they risk dissipating that flexibility. We balanced that appropriate caution against the views of scientists who believe the situation is dire—in other words, if someone seems to be drowning, you throw that person a life preserver and debate the finer points about whether the sea is rising due to global warming at a later date.

The opportunity to act decisively to stimulate biomedical research and science education makes being president of HHMI a unique position in scientific leadership. Yet, as many readers of this column already know, I will step down from this extraordinary position a year from now to return to my laboratory at the University of Colorado on a full-time basis. This decision reflects my desire to be fully engaged in research and teaching but also the realities of charting new strategic directions for HHMI.

The Institute is beginning to plan its next scientific initiatives, and I think new leadership should be in place before those are launched. As I have shared with HHMI staff, continuity of leadership was very important for building the Janelia Farm Research Campus. The same leadership team was involved in the entire process—beginning with the early vision that developed from a conversation I had with Gerry Rubin, now Janelia Farm's director, and David Clayton, now vice president for research operations—through to program planning, architectural design, construction, staffing, and the emergence of a lively scientific community. The “next great thing” deserves that same leadership commitment over an extended period of time.



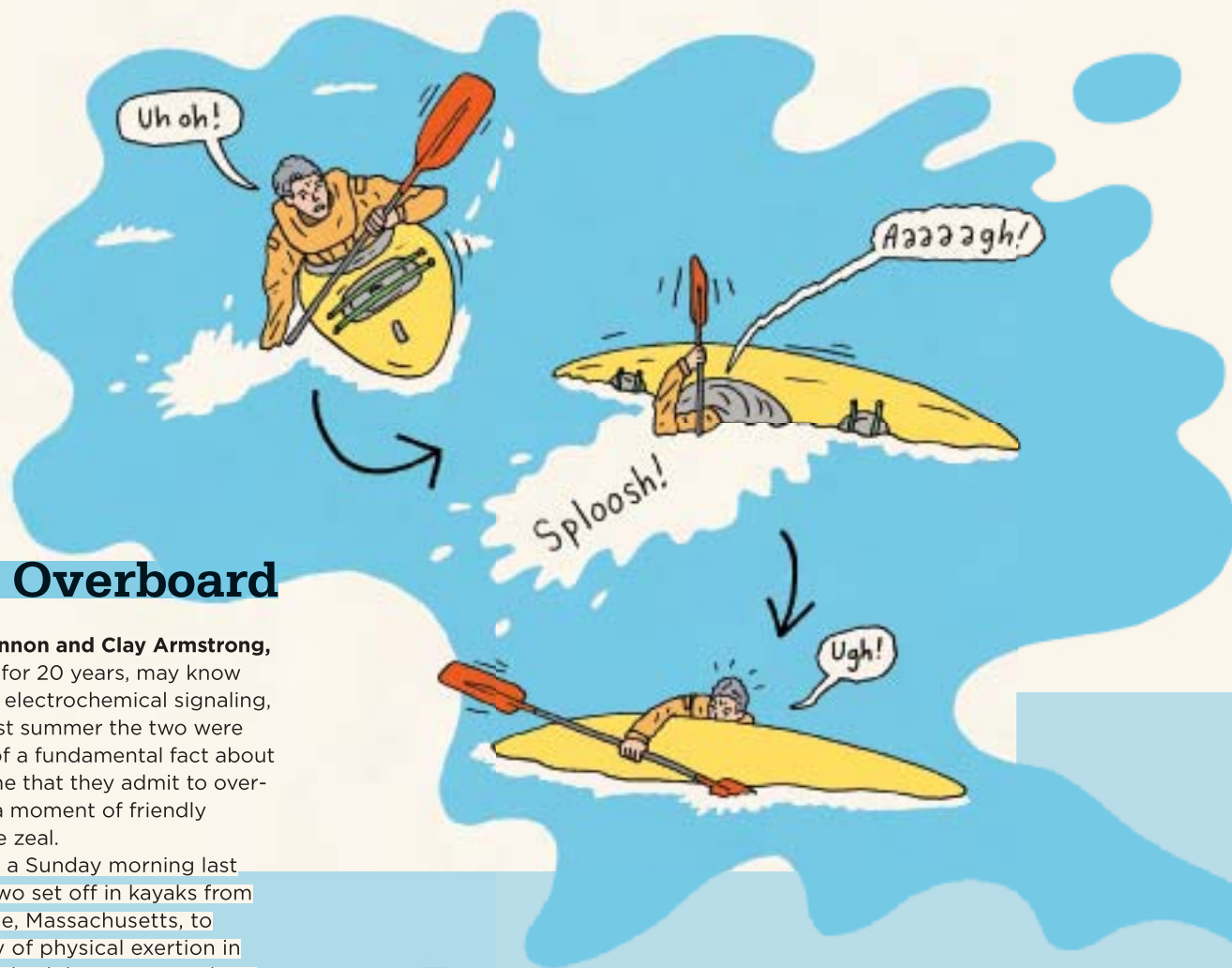
“This is an exciting moment in HHMI's history, and I look forward to the outcomes of several initiatives now under way.”

THOMAS CECHE

This is an exciting moment in HHMI's history, and I look forward to the outcomes of several initiatives now under way. For example, we're in the final stages of selecting a new group of investigators, the result of our first general competition in which scientists applied directly to HHMI. The process of soliciting institutional nominations worked well in the past, but direct applications are bringing us a broader and deeper pool of candidates. The open application process is stimulating our long-term efforts to expand the definition of biomedicine to embrace interdisciplinary work involving chemists, physicists, engineers, and computer scientists.

Under the leadership of Jack Dixon, HHMI's chief scientific officer, we're undertaking another initiative called the collaborative innovator awards to further interdisciplinary research and extend HHMI's support into the wider scientific community. For the pilot round, we asked our investigators to propose particularly challenging and potentially transformative research opportunities that involve collaborators outside the HHMI community and to devise plans for tackling them. We hope to select the first recipients shortly; as the effort proceeds, we will consider expanding the program to a larger group of scientists.

The work of HHMI will continue to unfold over the next year as the Trustees seek my successor, who will have the responsibility and joy of planting new ideas and watching them flower. I will have the privilege of returning to a cherished role, that of an HHMI investigator, and the joy of discovery.



Men Overboard

Rod MacKinnon and Clay Armstrong, colleagues for 20 years, may know a lot about electrochemical signaling, but this past summer the two were reminded of a fundamental fact about the sea—one that they admit to overlooking in a moment of friendly competitive zeal.

Early on a Sunday morning last June, the two set off in kayaks from Woods Hole, Massachusetts, to enjoy a day of physical exertion in waters they both have come to love. MacKinnon, an HHMI investigator and Nobel laureate at Rockefeller University, spent his youth in coastal Massachusetts, and over the last three years has accomplished more as a “paddler”—venturing miles out upon the swelling seas, beyond sight of land—than most kayakers ever attempt.

Armstrong, emeritus professor of physiology at the University of Pennsylvania and co-winner (with MacKinnon and Bertil Hille) of a Lasker Award, has summered for years at Woods Hole. In his early 70s, he still

runs marathons and is an accomplished sailor. Nevertheless, that Sunday morning marked only the second time he had ever stepped into a kayak.

They planned to paddle 30 miles over the Vineyard Sound—which separates Martha’s Vineyard from Cape Cod—to Cuttyhunk Island, the westernmost in a chain called the Elizabeth Islands. Much to their delight, says Armstrong, that bright Sunday morning “a 10-knot wind and an ebbing tide were at our backs.” Remarkably, they reached Cuttyhunk

in only three hours. After lunch they would ride the flood tide back to Woods Hole, or so they thought.

“Now we had that good wind against us,” MacKinnon recalls, and passage was getting increasingly difficult as the hours passed.

“We’d been going about five miles, right into the teeth of the wind and waves,” MacKinnon remembers. “Then, suddenly, over Clay went, upside down and under water. I expected he’d pop right up beside the kayak, but he remained upside down, trying to figure out how to do an Eskimo roll—just kind of *deducing* it himself!” (In this classic maneuver of the sport the submerged kayaker, from an inverted position, rolls right-side up, powered by a quick flick of the hip and screw-like sweep of the paddle.)

“I’m just watching this helplessly,” says MacKinnon, “seeing Clay rolling up, maybe 45 degrees . . . and then, boom! Right back under.”



“I’m just watching this helplessly, seeing Clay rolling up, maybe 45 degrees . . . and then, boom! Right back under. ”

ROD MACKINNON

The Sweet Smell of Exhaust

"I've been around sports cars and racing cars for as long as I've been around science," says neurologist Peter St George-Hyslop. "My parents were scientists, and my father had a passion for cars. I distinctly remember the smell of racing fuel and Castrol R from when I was two."

St George-Hyslop is an HHMI international research scholar at the University of Toronto who works on the molecular mechanisms that cause neurons to degenerate in Alzheimer's disease. In his office, the original engine block from a vintage Jaguar he's rebuilding serves as a coffee table.

Sports-car restoration is, refreshingly, "a more constrained problem" than probing the internal machinery of a cell, he says. "When something doesn't work in the car, you can hit it with a spanner, swear at it, and walk away. With science, if it isn't working, you've still got to keep plugging." Still, biology "does give you fewer skinned knuckles."

St George-Hyslop did a bit of racing as a student in the 1970s and never really hung up his driving gloves. "No hairy crashes, but I checked out the grass and weeds in the ditch on several occasions. Severely injurious to one's pride, but not to one's car. Since then, I've owned Jaguars and similar sorts of sporty cars, including a souped-up, Porsche-engined VW and a Triumph TR6.

"What I've always done with my cars has been to take the engine out, tear it apart, and put it together according to [competition] blueprints. The car I'm working on now is a 1974 V12 E-type Jaguar roadster."

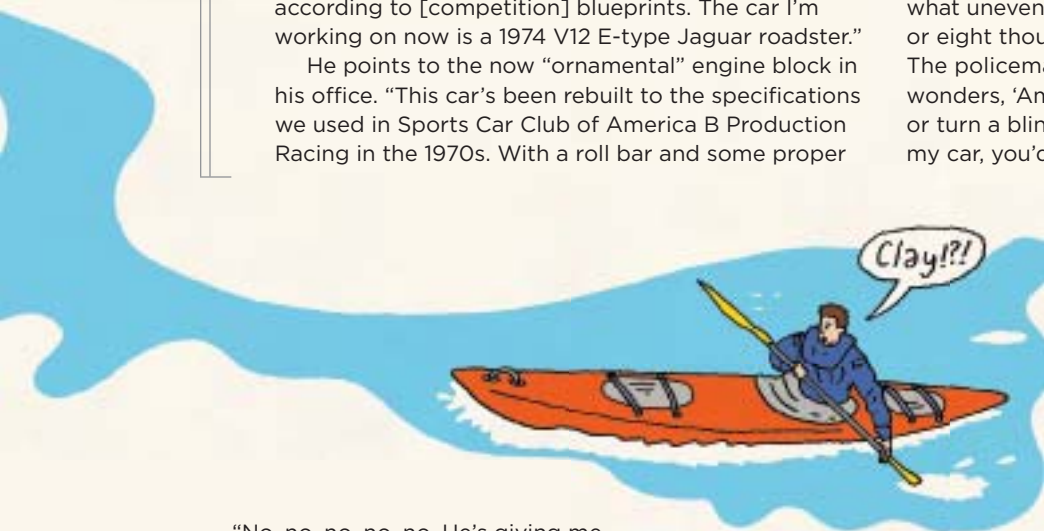
He points to the now "ornamental" engine block in his office. "This car's been rebuilt to the specifications we used in Sports Car Club of America B Production Racing in the 1970s. With a roll bar and some proper



seats, I could qualify it for racing. But I'm not allowed to do that—" a sigh slips out "—due to, ah, family edicts." The family includes a wife and three teenage daughters. He notes with satisfaction, however, that one of the girls "is a car nut."

When he gets time out on the road, the scientist at the wheel is free to revel in other sensations.

"The vibrations, the feeling of acceleration and cornering and wind in your face. The sweet, aromatic smells of hot oil and racing fuel exhaust," he exults, his account picking up speed. "The rumble of the 12-cylinder's exhaust pipe, straight from the headers out. This deep, bass rumble: it starts out low and somewhat uneven and then, as you accelerate up to seven or eight thousand rpm, it becomes a melodious howl. The policeman at the side of the road looks up and wonders, 'Am I going to chase after you and book you, or turn a blind eye because, by the time I could get in my car, you'd be long gone?'" —George Heidekat



"No, no, no, no, no. He's giving me credit that I don't deserve," Armstrong insists. "The fact is, I lost my paddle. That's why I remained upside down. I had nothing to right myself with!" It was a harrowing few minutes, in which Armstrong figured out, at last, "how to get out of the damned kayak," and the two managed to drain his boat and set him in motion again.

But there remained 10 miles to travel, with wind and waves against

them. "Clay kept pushing on but was tiring," remembers MacKinnon, 20 years Armstrong's junior. "I started looking at my watch. I'm constantly calculating things: the time, the current, the wind, hours of daylight left. We paddled up onto a beach. I told Clay we weren't going to make it." He didn't say anything. "What I'm getting at,"

MacKinnon said, "is that I'm going to tow you for a while."

Armstrong glared at MacKinnon: "You're not going to tow me *anywhere*! I'll sleep here on the *beach* if I have to, but you're not going to tow me!"

"His insistence on towing me kind of woke me up," Armstrong admits. He paddled with renewed vigor, and they reached Woods Hole with daylight to spare.

The ordeal "certainly reinforced my recognition of the fact that the sea can kill you," MacKinnon says. "But it also put me in even greater awe of my friend. Clay Armstrong, for 20 years, has been teaching me—and not only about potassium ion channels!"

Armstrong, for his part, reports that he is ready for another go, this summer. —Peter Tarr

A Baroque Biochemist

On Tuesday and Friday evenings, biochemist Alexander Konstantinov walks away from his spectrophotometer and picks up a different fine-tuned instrument. He draws the violin to his chin and slides the bow across its special silk strings, creating the richly decorated sounds of early 17th century Baroque music.

Konstantinov, an HHMI international research scholar at Moscow Lomonosov State University, leads the university's chamber orchestra. They play pieces by Johann Hermann Schein, Johannes Rosenmüller, and William Lawes—lesser-known composers who came before Bach, Beethoven, and Vivaldi.

Baroque compositions encourage a feeling of harmony, Konstantinov says. “This music brings a discipline of one’s ego. You feel yourself happy not just when expressing yourself, like many feel when playing a solo part, but feeling yourself a part of the entire ensemble. Inside the baroque orchestra you do not feel either a romantic hero or a small cog within a large score.”

Konstantinov’s passion for music is a life-long infatuation that predates

even his love of science. He first picked up the violin when he was five, with encouragement from his parents. At age six, he passed up an audition for a spot at the Central Music School in Moscow.

“This would have meant a professional music career,” says Konstantinov. But his father had been expelled from Moscow after World War II, moving the family to Saratov, a day trip away. “My parents did not venture to send me alone to boarding school in Moscow,” says Konstantinov.

Still, in Saratov he attended a seven-year “children’s music school” along with regular classes—where he discovered his knack for science, like his mother, a microbiologist, and his father, an expert on water ecosystems. Soon, the time came for him to choose between science and music. “As both my parents were from the scientific world, it happened that I chose science.”

When he began as a student at Moscow State University in 1967, however, he discovered that studying science didn’t mean giving up his violin. That same year, the university established a chamber orchestra, and Konstantinov signed up. He was placed in the last stand of violins.

Over the next 10 years, he moved up, seat by seat, to the front of the

15-person orchestra—eventually becoming the “konzertmeister” of the first violins. In 1991, he took over as director. Among the university scientists playing in the orchestra, four are from Konstantinov’s research group, which studies how cells harvest energy from oxygen molecules.

While the orchestra occasionally plays for university events—including a ceremony for HHMI President Tom Cech a few years ago—his fondest performances are those in which singers join the orchestra. In 1995, they teamed up with a popular philharmonic ensemble to perform odes and semi-operas by English composer Henry Purcell and oratorios by Italy’s Alessandro Scarlatti. These performances included both singing and speaking parts.

Coordinating the musicians with the singers offered Konstantinov a new challenge. The results—the first authentic performances of many of these masterpieces in Russia—were well worth it, he says.

—Sarah C.P. Williams

WEB EXTRA: To hear clips of performances by the Chamber Orchestra of Moscow State University, visit www.hhmi.org/bulletin/may2008.



“As both my parents were from the scientific world, it happened that I chose science. ”

ALEXANDER KONSTANTINOV

08 Know When to Fold 'Em

When the cell's protein-folding machinery is stressed, timing is everything in the decision to keep going or die.

10 Freeze-Framing a Fidgety Molecule

A sister-brother team conspires to pin down the ultrafast movements of enzymes in action.

12 Baring HIV's Dependencies

New atlas reveals that HIV commandeers almost 300 human proteins to do its dirty work.

They've set their sights on a goal. Formulated a scientific question. Creative juices start to flow. How to reach a solution? Science is becoming more collaborative, and that's certainly true for the researchers spotlighted on these pages. Conversations—with a colleague on campus, a sibling on another continent, maybe even a pet parrot—bring a fuzzy, seemingly unattainable goal into focus. Then there are those who see a challenge and jump in—even if it's outside their field—thinking, “I can contribute something.” They shift direction, apply their know-how in a new way, and decipher an intractable puzzle. Talk about inspired resourcefulness.

Know When to Fold 'Em

When the cell's protein-folding machinery is stressed, timing is everything in the decision to keep going or die.

ON THE MISSION BAY CAMPUS OF THE UNIVERSITY OF CALIFORNIA, SAN Francisco (UCSF), Peter Walter's corner office is distinctive for its tall, elegant cactus-like plants—and its poetry-quoting African grey parrot. After months of effort, Walter has trained the parrot, named Beaker, to badger lab members on what's most important: "We need more data." ¶ That's a common refrain from research leaders, but "in this case, Peter's got a parrot to say it," says postdoctoral researcher Jonathan Lin, laughing.

In keeping with Beaker's command, Walter's team has collected convincing data to explain exactly how the endoplasmic reticulum (ER), a maze-like compartment that fans out around the nucleus inside the cell, can determine whether a cell lives or dies. The ER serves as a cellular factory where newly synthesized proteins are folded into their proper structure. A protein must be folded correctly to do its job.

"Only the perfectly made proteins pass quality control," says Walter, an HHMI investigator. Proteins that fail to attain the right conformation are degraded before they can cause cellular dysfunction and disease.

If the ER machinery is insufficient or defective, however, unfolded proteins pile up. Fifteen years ago, in studies of yeast, Walter and his colleagues discovered that the ER copes with the stress of such overload by triggering a set of biochemical reactions, known as the unfolded protein response, or UPR. Later work by various research groups uncovered a similar mechanism in mammalian cells, including human cells:

three enzymes, molecular sensors called IRE1, ATF6, and PERK, detect the glut of unfolded proteins. They then activate various genes that expand the ER and step up its folding capacity, reduce the synthesis of new proteins, and crank up the protein degradation process.

Those protective measures bring the system back into balance. "It's a feedback loop that adjusts supply to demand," Walter says. Yet, paradoxically, if the ER cannot

regain equilibrium, the UPR prompts the cell to commit suicide. In a study published in *Science* last November, Lin, Walter, HHMI investigator Kevan Shokat, and colleagues explored how the same signaling pathways could cause such diametrically opposite fates.

The team exposed cultured human cells to drugs that prevent proteins from folding and eventually cause cell death. Over 24 to 30 hours, the researchers measured the activity generated by the UPR. Initially, all three pathways rapidly turned on, but results unexpectedly showed IRE1 shutting off after about 8 hours, around the time when cells began to deteriorate. ATF6 activity followed a similar pattern. By contrast, responses triggered by PERK—including production of

A GOOD FIT

IT'S A TALE OF GREAT CHEMISTRY between two HHMI investigators at the University of San Francisco: around 2000, Peter Walter talked to Kevan Shokat about devising a drug that would work in yeast to selectively suppress IRE1, which belongs to a vast family of enzymes called kinases. Shokat already had a designer compound in hand that blocked other kinases after he modified them to respond to it. Taking the same approach, he used genetic tinkering to slightly widen IRE1's active site, the pocket where energy-molecule ATP normally plugs in and activates the enzyme. Those changes permitted Shokat's drug to fit only into that mutated pocket, blocking ATP binding. Shokat expected this chemical-genetics strategy to shut off the enzyme, but the reverse happened. "We've done this with 100 kinases and IRE1 is the only one where the drug actually turned *on* the function of the kinase," he says. "So that was an absolute surprise." A surprise that proved helpful when Walter needed a persistent, rather than a suppressed, IRE1.

Andy Smith



a protein that promotes cell suicide—stayed on the whole time.

The drop-off in IRE1 appeared to be “a switch between the pro-survival versus the pro-death phases of the UPR,” says Lin. To test that hypothesis, he and Walter wanted to see what would happen if IRE1 did not power down. Fortunately, UCSF colleague Shokat gave them a “wonderful trick” to do just that, says Walter. Shokat used genetic methods in yeast to alter IRE1’s structure so that the sensor could be selectively turned on by a designer drug (see sidebar).

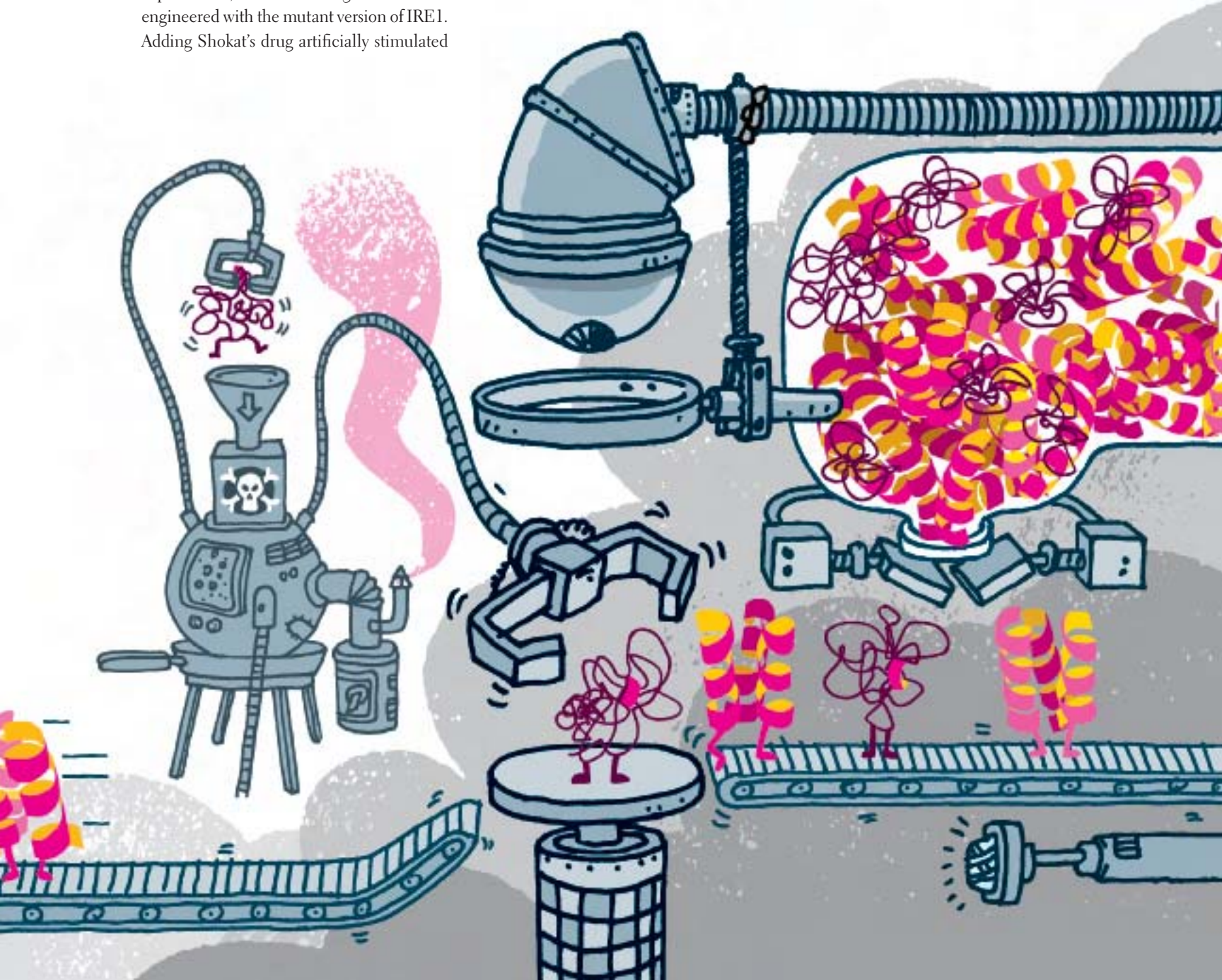
Lin and Walter repeated their cell culture experiments, this time using human cells engineered with the mutant version of IRE1. Adding Shokat’s drug artificially stimulated

and sustained IRE1 levels in the cells—and substantially fewer of them died, confirming the researchers’ theory that the enzyme was pivotal for cell survival.

Going a step further, the researchers examined developing eye cells in rats with retinitis pigmentosa. This inherited form of blindness results from degeneration of retinal cells that make misfolded light-sensing proteins. Those experiments revealed a downturn in IRE1 signaling, typical of cell suicide.

The study raises fresh questions: could future drugs be designed to enhance the UPR’s protective responses or stave off overzealous cell suicide that occurs in this and other diseases—such as diabetes and Alzheimer’s disease—in which cells die from protein-folding glitches? Lin is exploring that possibility in the blind rats.

Walter is investigating the other side of the coin. Could inhibiting the UPR’s protective side within cancer cells, which must crank out many proteins to sustain rapid growth, put an end to a tumor’s growth? Beaker the parrot’s likely response is: “We need more data.” ■ —INGFEI CHEN



Freeze-Framing a Fidgety Molecule

A sister-brother team conspires to pin down the ultrafast movements of enzymes in action.



Dorothee Kern is changing the way scientists think about enzymes—proteins that speed up chemical reactions.

Leah Fasten

DESPITE THEIR HEATED SCIENTIFIC DEBATES AS KIDS IN GERMANY, DOROTHEE

Kern never imagined turning to her little brother to collaborate on her research. But Christian Huebner, three years her junior, is now a physicist and offered just the know-how Kern needed to resolve a puzzle about the behavior of a restless protein. ¶ With his help, Kern, who has previously collaborated with her parents—also scientists in Germany—

made sense of her experimental observations that the protein could adopt three distinct conformations in one crystal. In the process, she turned a long-standing biochemistry assumption on its head.

Kern, an HHMI investigator at Brandeis University, studies the dynamics of enzymes—proteins that speed up chemical reactions by clamping onto one or more substrates and efficiently converting them into products. In this case, she was studying adenylate kinase, an enzyme that processes ATP, ADP, and AMP—molecules that give cells energy and are building blocks of DNA. Adenylate kinase exists in every organism, from bacteria to humans. Kern wanted to know how the enzyme adapted to one of its most extreme environments—inside bacteria that thrive at 220 degrees Fahrenheit in deep ocean vents. Most proteins unravel at such high temperatures.

She already knew the molecular structure of adenylate kinase at more moderate temperatures but not what the heat-loving version looked like. “We really needed a high-resolution structure to see subtle differences,” says Kern.

She and her colleagues turned to x-ray crystallography—they bombarded crystallized protein with x-rays and used the resulting diffraction pattern to determine the protein’s three-dimensional arrangement. “We thought it would be easy,” says Kern.

Not quite. The data gave a jumbled picture of atoms that seemingly existed in three places at once. Kern’s take on this puzzling result: three different structures were present in a single crystal.

“We had one crystal of one unique protein, but three conformations of the protein,” says Kern. “These are just snapshots though—static and frozen in the crystal.”

What Kern needed was a way to measure whether the protein, when it wasn’t stuck in a crystal, actively alternated between these structures. Nuclear magnetic resonance (NMR), which can detect the movement of atoms, provided just such an approach. Using NMR, Kern’s team calculated that the protein switched conformation about every millisecond or so, but they were not able to see exactly which conformations.

To bridge the information obtained by crystallography and NMR, Kern’s team performed a third experiment—a computer simulation that calculated how fast the molecule could move between the structures seen in the crystal. The simulation only added confusion. It indicated that the enzyme could move between the open and partially closed states seen in the crystal in nanoseconds—that is, thousands of times faster than the milliseconds suggested by NMR.

Kern had an idea of what was going on but needed the help of her brother, a physicist at Germany’s Martin Luther University Halle-Wittenberg, to experimentally reveal the inner workings of adenylate kinase. “It was a real collaboration where we were flying back and forth across the ocean,” says Kern, who credits her light-hearted brother for making the partnership lots of fun.

They performed single molecule fluorescence resonance energy transfer (FRET) experiments—tacking fluorescent molecules onto the upper and lower lids of

the clam-shaped enzyme and tracking its opening and closing by measuring changes in fluorescence. Huebner had designed and built a unique ultrasensitive laser that allowed precise measurements and time resolution in microseconds.

The siblings found that every few nanoseconds—as the computer simulation had shown—the enzyme twitches partially shut. But every few milliseconds—as suggested by NMR—it closes all the way.

The traditional view had been that an enzyme snaps shut only when it makes contact with its substrate. But Kern and Huebner showed that an enzyme can constantly fidget and take on a new structure without that contact. And it’s not just in this one instance—Kern has detected twitching in the handful of other enzymes she’s tested so far using NMR. “This is really a paradigm shift,” she says. “We want to encourage scientists to consider that this happens with their own systems. So far, it seems that these short-lived, higher energy states quite often are the biologically active states.”

Returning to her original question on heat-loving adenylate kinase, Kern performed new experiments showing that the hinges between the lids of the enzyme are more rigid in heat-loving bacteria, which slows the enzyme’s movements, presumably keeping it from unraveling at high temperatures. The team’s findings appeared in two papers online in *Nature* on November 18, 2007. Next, Kern wants to find out just how the protein manages to switch states without losing its structure.

“The risk of a protein being flexible is that it can fall apart,” she says. “The fascinating part to me is how nature keeps that from happening. These proteins are really living on the edge.”

As for the sibling team, the collaboration continues. “He’s simply the best,” says Kern of Huebner. Calling the teamwork between the labs “electrifying,” Kern says, “The combination of these different biophysical techniques provided us with a much deeper understanding of the fundamental principles of protein function. We’re already planning new projects together.” ■

—SARAH C.P. WILLIAMS

“The risk of a protein being flexible is that it can fall apart. The fascinating part to me is how nature keeps that from happening. ”

DOROTHEE KERN

Baring HIV's Dependencies

New atlas reveals that HIV commandeers almost 300 human proteins to do its dirty work.

VIRUSES ARE NEEDY. EQUIPPED WITH FEW GENES, THEY LEAN HEAVILY ON their host cells to help them successfully invade. ¶ This is true of the human immunodeficiency virus (HIV), which has just nine genes that make only 15 proteins. With such a sparse molecular tool kit, it is a wonder that HIV can wreak such havoc. But its ability to take over the very immune cells intended to protect us from disease is well known. An atlas of those host cell factors the virus hijacks would provide a deeper understanding of the virus, perhaps providing potential ways to thwart it.

In January, a team led by HHMI investigator Stephen Elledge of Brigham and Women's Hospital in Boston produced just such a roadmap. Writing in the journal *Science*, Elledge's team reported that HIV requires at least 273 human proteins, called HIV-dependency factors (HDFs), to do its molecular dirty work.

"This is a tremendous resource for the entire field of HIV research," says Dan R. Littman, HHMI investigator and an HIV expert at New York University Medical Center. "It's been known for a long time that these host factors were out there, but there had never been a systematic approach to identify them. I don't think

anyone could have imagined how many would turn up."

The study greatly expands the number of known HDFs, painting a newly detailed portrait of the virus and its dependencies. Only 36 of the human proteins commandeered by HIV had been previously identified.

To produce the expanded catalog of HDFs, Elledge's group, which included postdoctoral fellow Abraham Brass and Harvard Medical School's Judy Lieberman, tapped newly available commercial libraries of what are known as small interfering RNAs. These genetic molecules can switch genes off, preventing them from making proteins.

FINDING CANCER TARGETS

IT'S NOT JUST HIV THAT STEVE ELLEDGE is probing for weaknesses. He's taken on cancer too. In a paper published in *Science* on February 1, 2008, Elledge and his collaborator, HHMI investigator Gregory J. Hannon of Cold Spring Harbor Laboratory, revealed a new screening technique to probe tumors for genes that help them thrive. **ELLEDGE AND HIS COLLEAGUES GENERATED** about 8,000 bits of short hairpin RNAs (shRNA)—single strands of RNA that fold back on themselves—that can be inserted into retroviruses. When the altered retroviruses infect either normal or cancerous cells, the shRNA binds to corresponding stretches of RNA in the cells, preventing their translation into proteins. **IF THE shRNA KNOCKS DOWN** production of a protein essential to keeping the cells alive, then the abundance of that particular shRNA quickly diminishes as cells die. If the shRNA corresponds to a gene involved in dampening cell growth, then the cells that carry that shRNA will

multiply and thrive. **BY TRACKING THE ABUNDANCE** of each shRNA from the total pool and comparing breast and colon cancers with normal tissues, Elledge and his colleagues were able to identify genes critical to tumors' growth. **"THE OVERALL IDEA BEHIND** this is that cancer cells reprogram their [molecular] networks," says Elledge. "We're interested in finding what components in these new networks are controlling proliferation." **WHILE HIS STUDIES ON HIV** rely on a slightly different method, in both HIV and cancer Elledge hopes that full-genome scanning will reveal new target proteins for drugs. **"HIV IS A LOT LIKE A CANCER CELL—**cancer cells also mutate, so it's hard to get drugs to them that kill them," he says. Both HIV and cancer are so complicated that selecting genes one at a time to test for importance would likely miss other vital ones, he says. "This is a way to take the guesswork out, because we're testing everything." — Sarah C. P. Williams

By turning off genes in human cells one by one and then observing whether HIV could establish itself and reproduce, Elledge's team plodded through 21,000 disrupted genes to isolate those the virus required.

"We were looking for any genes that HIV needs for its life cycle," Elledge says, pointing out that the proteins those genes make have the potential to be drug targets.

Drugs now in use directly attack HIV, and they must be used in combination since the virus has evolved resistance to individual compounds, explains Elledge. Making drugs that target host proteins could bypass resistance; if a host protein the virus requires is disrupted, the virus would have to do much more to overcome the challenge than simply rearrange a few amino acids of genetic material. The new study reveals that many host proteins play coopted roles throughout the cycle of HIV infection—for example, helping the virus glom onto and enter cells, converting RNA to DNA, and creating new infectious particles.

"Many of the proteins identified in this study would probably be good candidates for screening to find new anti-HIV drugs," notes David Baltimore, a leading HIV authority at the California Institute of Technology, who agrees that the virus will have a harder time developing resis-

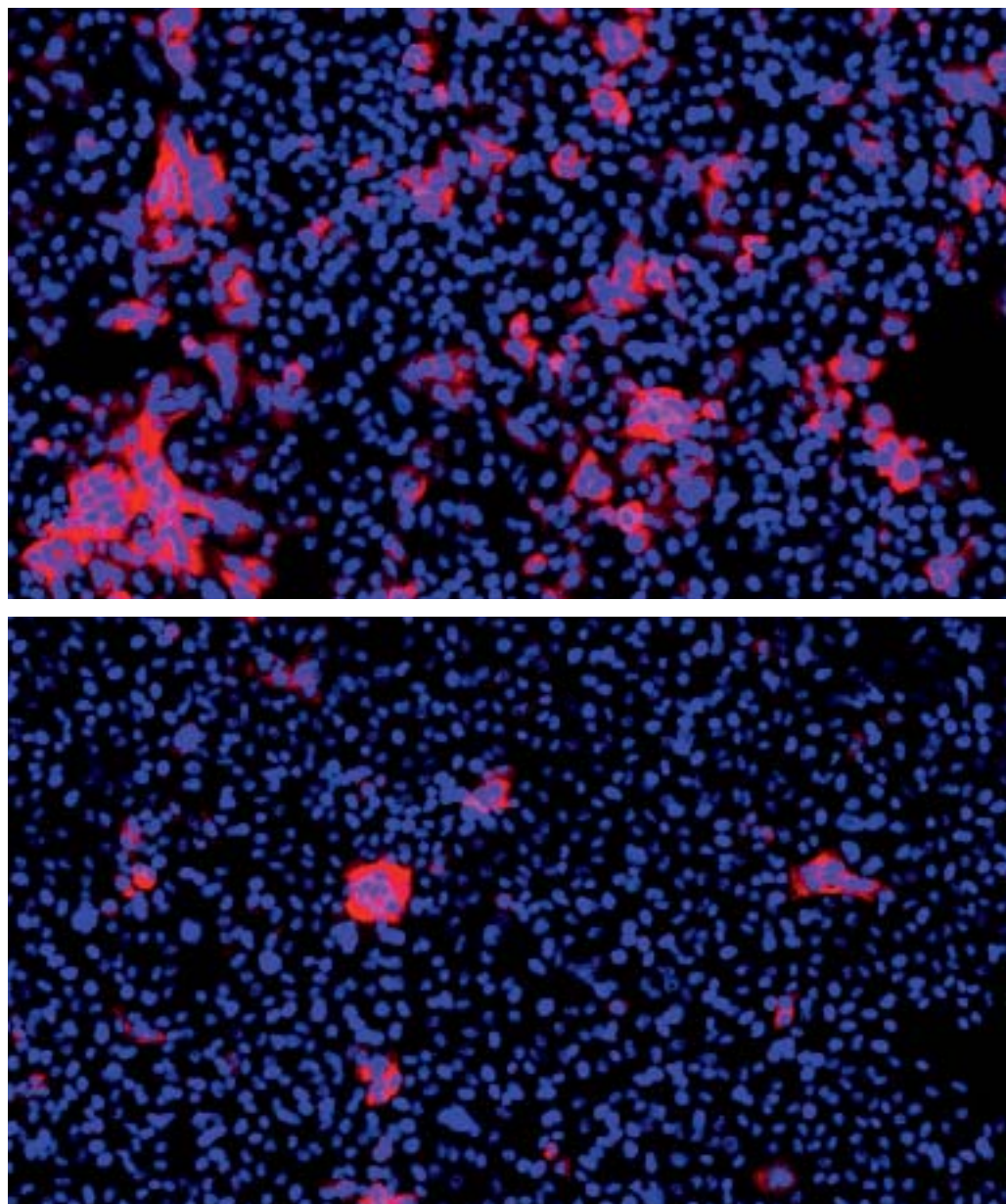
tance to drugs that target cellular proteins. "However," he adds, "for the same reason, the drugs will have to be carefully characterized for toxicity."

Elledge acknowledges that starving viruses of required host proteins could have unintended effects. "The cells do need the proteins," he says. "That needs to be worked out."

For Elledge, who is best known for his DNA cell cycle work, this new study is a

first, if dramatic, foray into HIV biology. His lab also has ongoing gene discovery projects focused on other viral pathogens, as well as cancer, stem cells, and diabetes. He plunged into HIV to spur HIV drug development by industry, which he says is lagging: "I wanted to point out using genetics that there are real targets in cells and get [drug developers] thinking about mining those pathways." ■

—TERRY DEVITT



Among the host cell proteins used by HIV when the virus infects cells is one called human transportin 3. When scientists halted production of that protein in host cells (blue), levels of HIV (red) in the cells were drastically reduced (lower panel) compared to infection under normal conditions (upper panel).

$\sqrt{\text{Teaching} + \text{Young} / \text{Biologists} = \left(\frac{\text{New}}{\text{Tricks}} \right)}$

Long the science where math mattered less, biology increasingly demands powerful quantitative skills. Teaching students the math they'll need, though, is more than just 1+1=2.



by Marc Wortman
illustration by Luke Best



On

the first day of a year-long introductory chemistry course at Atlanta's Emory University, students walked in to find three large round tables surrounded by white boards, no lectern, and a professor who didn't stand still. Their instructor, Tracy Morkin, really baffled them. She projected some charts with numbers on the wall and told the students they would be teaching *one another* chemistry.

"They didn't know what they were getting into when they arrived last fall," Morkin recalls with a smile on an early February morning. She bounds around the room, stopping occasionally to check in with students and their tablemates as they study data sets on the freezing and boiling points of solutions with differing concentrations and under different pressures. They need to figure out, from the data she gave them, which formulas can be used to come up with the numbers. "It's an inversion of the typical way of doing things," says Morkin. The room buzzes as they puzzle through the problem. Finally, she asks a student to step up to a whiteboard. With Morkin's coaching and input from other students, he lays out the equations from which the freezing and boiling points had been derived.

Such nontraditional, active-learning approaches to introductory science and math courses are being tried at other colleges as well, among them North Carolina State University and the Massachusetts Institute of Technology. Morkin's students are delighted to be the first at their school to experience the alternative to a standard lecture-style course. Sitting in a lounge after class, freshman Amol Koldhekar says, "If you talk to people taking the regular chemistry class, they get the right answers, but they don't understand it. They plug 'n chug," putting numbers into memorized equations without knowing where those equations come from.

Fellow freshman Remy Weinberger agrees. "In this class," he says, "you have to understand the theory behind the formulas so you can derive them yourself and know how to use them."

Like most of their classmates, they want to attend medical school or pursue a career in another health care or biomedical science field. Morkin designed her chemistry course to give them a running start in acquiring the quantitative, problem-solving, and interdisciplinary scientific skills they will need.

Recent reports showing an ever-increasing need for biomedical scientists with stronger math skills and a yawning gap between the need and the preparation being offered. So, Emory and a growing number of other academic institutions are experimenting, even at the precollege level, with new ways to integrate quantitative reasoning into the traditional biological sciences curriculum.

Putting more mathematics into biology and related courses, though, is not a simple matter of adding statistics, calculus, and computer science to already challenging subjects. It requires changing minds about the importance of such skills in a field that historically shortchanged them and revamping longstanding attitudes about how to educate future biomedical scientists.

The Rules Have Changed

MANY TEACHERS AND STUDENTS QUESTION THE NEED FOR change. "There's an uphill battle," attests Emory neuroscientist Ronald Calabrese. "I've heard faculty members at department meetings say, 'Why do premed students need differential calculus? They're going to *medical school!*'"

His colleague Dieter Jaeger notes that resistance among students is also a factor. "You have to convince them," he says, "that it's more than just making biology harder."

In fact, even though some students might never need to derive an equation in their biomedical careers, studying math contributes significantly to those careers. A recent study of 8,500 students at 77 U.S. colleges and universities showed that the stronger a student's high school preparation in math, the better he or she is likely to do not only in chemistry and physics but also in biology. Writing in *Science* last July, the study's authors—Philip M. Sadler, director of science education at the Harvard-Smithsonian Center for Astrophysics, and Robert H. Tai, a professor of science education at the University of Virginia—described "more advanced study of mathematics in high school" as one of the "pillars supporting college science."

Perhaps the most important factor driving the increased emphasis on quantitative skills is the changing nature of biology. From discoveries about neural networks, genetics, and cardiac blood flow to understanding disease pathways within cells and throughout entire populations, many of the most important advances in the field now rely on mathematical modeling, quantitative analysis, and bioinformatics (see sidebar).

“I wasn’t good at math in high school,” admits biology professor Karl Joplin, and that influenced his choice of career. “I thought biology was a field with no math. But boy, was I wrong.” Accepting that the rules have changed, he now leads efforts at East Tennessee State University, in Johnson City, and a consortium of other universities to promote more quantitative education in biology.

Fernán Jaramillo, a neuroscientist at Carleton College in Northfield, Minnesota, agrees that “the nature of the problems we study has changed in the past 20 to 25 years. Quantitative issues are much more central, and that is an accelerating trend. Students have to realize they won’t do well without some quantitative competencies.” Jaramillo directs the Interdisciplinary Science and Math Initiative, an HHMI-funded multidepartmental effort to bring more quantitative and interdisciplinary approaches to science courses at his school.

Building Solid Foundations

RECENT SURVEYS HAVE SHOWN THAT AMERICAN COLLEGE students tend to perform poorly in tests of quantitative skills compared with students in other countries. “The rest of the world is catching up, and by some measures has already overtaken us,” according to a 2006 report from a federal Commission on the Future of Higher Education. The problems persist among some with advanced degrees as well. A study published September 5, 2007, in the *Journal of the American Medical Association* found that 75 percent of U.S. physicians-in-training surveyed did not understand the statistics they encountered in medical literature, thereby calling into question their ability to interpret important clinical research data.

Advocates for science education reform at several national organizations, including HHMI, have been urging educational institutions to rethink how they prepare their students in the biomedical sciences. A National Research Council (NRC) committee commissioned by HHMI and the National Institutes of Health to investigate education in the biological sciences issued an influential 2003 report, called *Bio2010: Transforming Undergraduate Education for Future Research Biologists*. It outlines a strategy to improve the quantitative skills and math, chemistry, and physics comprehension of students preparing for biomedical careers. The report encourages faculty to implement teaching strategies that promote the skills required for problem solving in an increasingly interdisciplinary world.

“Biologists of the future are going to need additional skills, more quantitative reasoning being chief among them,” says Adam P. Fagen, a program officer at the NRC’s Board on Life Sciences. “Bioinformatics, for one, didn’t even exist until a few years ago. Now it’s a field in itself and essential to more and more people across the life sciences.”

“All of us are driven by *Bio2010*,” says Joplin. But even before the appearance of the report, HHMI—in conjunction with other supporters of science education reform—invested heavily in helping schools design and implement innovative strategies to bring more math into biology classrooms at all levels.

For example, with HHMI support Joplin helped develop a three-semester introductory biology course at East Tennessee



East Tennessee State University’s Karl Joplin pulled together 30 academic institutions to revamp how biology majors are taught quantitative reasoning skills.



As a science education leader at Emory University, Patricia Marsteller encourages faculty members to cross departmental boundaries to make math come alive for students in the sciences.

State that integrates calculus, statistics, modeling, and other mathematical skills into the traditional curriculum. He also initiated an HHMI-sponsored consortium of 30 academic institutions working to develop strategies and materials for teaching students quantitative methods. (That group will hold its second annual summer workshop at HHMI's headquarters in July.) "We're trying to generate the resources to teach this type of material," says Joplin. "It's so brand-new."

Those working to develop new approaches and teaching materials find themselves facing many other hurdles, including the legacy of mathematical concepts being taught without showing how they apply to biology. "The textbooks haven't changed," Joplin observes. "There's lots of quantitative information, but no connection between the different subjects. It's not conceptual." So he has been developing mathematics-teaching modules based on biological examples. "We want students to look at a data set and not see a blank wall. Instead, they should be able to describe the data and see something interesting in them." However, just coming up with data sets to teach quantitative reasoning skills for biologists requires starting virtually from scratch.

Claudia Neuhauser, an HHMI professor and applied mathematician at the University of Minnesota, is a pioneer in teaching biology undergraduates the calculus and other math they will need (see Perspectives and Opinions, "Making Math Relevant"). "There's a problem with the way we teach," she says. "Teaching is being done in silos"—within traditional departmental boundaries—"but now we're asking faculty and students to do work outside those silos, and it's a challenge."

Building Bridges

PATRICIA MARSTELLER CALLS HERSELF EMORY'S "DIRECTOR of subversive studies" because of her work to build bridges between traditionally distinct departments, courses, and laboratories. That's one of her charges as director of the university's Center for Science Education.

To push faculty members to rethink how and what they teach and get them to reach beyond their traditional disciplinary boundaries takes provocation and rewards, according to Marsteller. "It's difficult," she says, "because it requires collaboration and cooperation between departments that don't work in the same way and don't think in the same way about education. Faculty are torn by their disciplinary loyalties, and of course it's always hard to teach old dogs new tricks."

When *Bio2010* appeared, she saw it as an opportunity to spark interdisciplinary conversations, if not out-and-out insurrection. She sent copies to every faculty member of Emory's biology department and to many in the chemistry, physics, and mathematics and computer science departments. Soon discussions began and an interdepartmental working group on science education started meeting regularly.

Other efforts were already under way to bolster math preparation at the undergraduate level, including a two-semester calculus course, now a requirement for all biology majors. Mathematics professor Dwight Duffus, who created the course a decade ago, covers differential equations, probability and statistics, and modeling by using a range of biological topics—such as predator-prey systems, movement of species across regions, the spread of disease, and the firing of muscle neurons—to make the math immediately relevant.

Duffus is still learning how to teach math for biology students. "The problem that I have, as a mathematician," he says, "is understanding the math and computing skills and knowledge biologists need in their majors. Should they be able to construct a mathematical model on their own or just be familiar with the main concepts? You have to be aware of the diverse math backgrounds and aptitudes of students."

Vaidy Sunderam, who chairs the department of mathematics and computer science at Emory, believes that more interdepartmental dialogue is needed. "There's still this gap," he says. "Mathematicians talk of matrices and equations, and biologists talk about structure and function."

However, Sunderam and chemistry department chair David Lynn come together regularly as codirectors of the university's Computational and Life Sciences Initiative, launched two years

ZEROING IN ON CANCER GENES

What do math and cancer research have in common? A lot, according to Bert Vogelstein. An HHMI investigator at Johns Hopkins University School of Medicine, Vogelstein searches the human genome for genetic mutations that cause cancer. “There are about 20,000 genes, and each gene has on average 2,000 DNA bases,” he says. “That’s about 40 million bases we have to look at” in each tumor cell, and “billions if we’re looking at a bunch of tumors.” With all that data, “mathematical analysis is essential,” he says. ✨ Vogelstein says finding cancer-causing mutations among all these bases is especially tough because a mutation doesn’t necessarily mean cancer. “Genes are mutating all the time in both normal cells and cancer cells,” he says. Furthermore, the mutations that play a causal role in cancer aren’t always the same; most show up in only a small fraction of tumors. The only effective way to identify them, he says, is to use computational tools from the field of bioinformatics. ✨ Vogelstein, who majored in math as an undergraduate at the University of Pennsylvania, calls bioinformatics a way to “distill the wheat from the chaff.” He says the first step is to run computer simulations on DNA sequences from the Human Genome Project to get a sense of how often individual mutations happen due simply to chance. Then, he uses algorithms to compare those mock mutation frequencies with real data from cancer cells. Cancer cells will, on average, have more of some types of mutations than the random baseline created by the simulation, says Vogelstein, and the algorithms flag those mutations. However, not all of them are worthy of further study; some are just “passengers—along for the ride but not actually driving the cancer.” Using researchers’ knowledge of gene function, computers prioritize each mutation based on its precise characteristics and the gene in which it occurs. “At the end you end up with a relatively small list of genes” that are worth examining further in laboratory studies, says Vogelstein. ✨ Those studies, which “knock out” genes of interest from cancer cells or the genomes of mice, require huge effort and time. “Before you invest any time in those detailed studies, you need some sort of overall picture,” he says. “The only way to do that right now is to use bioinformatic tools.” —Benjamin Lester

ago to foster interdisciplinary scholarship. They have begun hiring faculty and bringing in graduate students who pursue interdepartmental research in emerging areas that require powerful bioinformatics and other quantitative capabilities. Lynn, an HHMI professor, has also tapped this workforce as a source of teaching skills geared to the new emphasis in biology. For example, he developed a freshman course in which graduate students from a variety of disciplines teach about their research.

Emory’s Computational and Life Sciences Initiative has quickly “captured the imagination of a broad spectrum of the community,” says Lynn. “We don’t want to weaken the departments, but we do want to catalyze new opportunities between them. That’s where the future discoveries will emerge.”

Active Learning

AT THE BEGINNING OF EMORY’S SCHOOL YEAR, THE STUDENTS taking introductory chemistry had a choice between a regular lecture format and Morkin’s innovative version. Few had ever experienced such an interactive approach to learning, especially in a course considered so fundamental to their future, and “they had to be sold on it,” she recalls.

She showed them studies done at North Carolina State University demonstrating that students who had taken this type of course—so-called “active learning” in a nontraditional classroom setting—earned better grades than their peers in traditional lecture classes. She told them they would need to learn to work together and “figure out the chemistry.” Her enthusiasm—and the data—won them over.

Now, seven months later, the students talk back and forth across the tables. Papers rustle as they share notes and calculations. “If it gets too quiet,” Morkin says, “they know I’m going to bother them. It’s tough for me not to talk too much, but as long as they have each other, they don’t need me.”

Several students confirm what she says. Premed freshman Weinberger says, “It’s nice to have your peers explain concepts to you. Their thinking is more similar to yours than the professor’s is.”

Adds classmate Ryan Makinson, who wants to attend graduate school in neuroscience: “I understand a theory better by explaining it to a classmate. It’s pretty cool.”

According to Lynn, the success of Morkin’s experimental course is spurring the department to “completely change the way chemistry is taught” at Emory. At the department’s urging, the university has begun restructuring its principal lecture hall into an “active-learning environment,” while redesigning courses to fit it.

Marsteller, who helped the chemistry department launch Morkin’s course with HHMI support for its development, thinks active learning is a useful tool for boosting math competence among science students. She hopes other Emory courses will adopt a similar active-learning approach. “We’ve been training a lot of scientists who don’t understand the quantitative methods they are using,” she says. “Students need to struggle with them. If they’re just hearing the solution, all they do is write it down and forget it.”

For students in Morkin’s class and others like it around the country, the numbers have started to add up. They will know how to get the answers they need long after the course grades are in. ■

BARRIERS TO SILENCE

Children with the subtlest form of autism suffer social isolation; those with more severe disease face a much tougher road. New genetic clues put the spotlight on the communication hubs of the brain's neurons—the synapses.

by **Richard Saltus** | photography by Fredrik Brodén



UNLIKE HER TWIN SISTER, CARLY, 12-YEAR-OLD NICOLE BRANCONNIER DOESN'T HAVE CLOSE FRIENDS.

She doesn't share her sister's pre-teen obsession with *High School Musical* or instant messaging. She goes to a special school; she speaks in choppy, incomplete sentences, unable to explain anything complicated; and when she walks past people in her home in Danvers, Massachusetts, she shows little interest and doesn't make eye contact.

Like many children with autism spectrum disorders, Nicole's connection to the world is an extremely narrow one. Her single overwhelming interest is animals—her dog Lulu (she will read out loud only to Lulu), zoo animals, animals in photos, stuffed or plastic ones that she used to arrange in particular, ritualistic ways. “She has her own agenda,” says her mother, Maureen. “She can't take other

people's perspective and doesn't understand why they don't go along with what she wants to do.”

Sixty-five years after child psychiatrist Leo Kanner described the puzzling cluster of cognitive, emotional, and social disturbances of 11 children with what he dubbed “infantile autism,” much about autism remains an enigma. An ever-widening array of disturbed behaviors and developmental obstacles—from mild to devastating—now fits under the umbrella term “autism spectrum disorders” (see sidebar).

Research into this mysterious disease, however, has gotten a recent “kick in the pants,” says Gerald Fischbach, former director of the National Institute of Neurological Diseases and Stroke who has served on the HHMI scientific review board. A convergence of funding, a handful of key discoveries about the autistic brain, and technology advances that enable fine-grained DNA searches have attracted major scientific players who've narrowed

the search for causes, according to Fischbach, now scientific director of the autism-focused Simons Foundation, a private philanthropy launched by billionaire hedge fund manager James H. Simons, whose daughter has mild autism.

Discoveries of gene mutations in some autistic individuals support the growing suspicion that the key problem in autism may lie within the synapse—the tiny, chemical-filled gap between the tip of a transmitting neuron's long, spindly arm and the receiving end of the next.

An estimated 100 billion neurons make up the human brain, connecting at synapses to create powerful information-processing networks that enable humans to think and remember, interpret sensory information from the outside world, and navigate the challenges of social relationships.

Sir Charles Sherrington, the neurologist and Nobel laureate who coined the term “synapse,” famously likened the brain to “an enchanted loom where millions

A SPECTRUM OF BEHAVIORS

In his classic 1943 paper, child psychiatrist Leo Kanner described the peculiar behaviors of 11 children he had observed in the late 1930s and early 1940s. He was most dismayed—and intrigued—by what he called their “profound aloneness.” These self-absorbed children didn't respond to their names or make eye contact, and their isolation was evident from their earliest days. In general, Kanner noted, they showed no more interest in people than in “the desk, the bookshelf, or the filing cabinet.” The disease, it was thought at the time, was rare, the cause unknown.

Today, the label “autism” is applied to a spectrum of what are called “pervasive developmental disorders” (PDD), and it is still based on symptoms and behaviors. Disorders on the spectrum—including autistic disorder, PDD-NOS (not otherwise specified), childhood disintegration disorder, and Asperger's syndrome—range in severity from devastatingly disabling

to so-called “high-functioning autism.” Rett syndrome is sometimes included on the more severe end of the spectrum.

Autistic disorders, once considered rare, are now diagnosed in about 1 in 150 children. The Simons Foundation's Gerald Fischbach believes that the broadening definition of autism accounts for most of the increase. In addition, he says, the expansion of services for children with autism could be promoting more-frequent diagnoses.

Delays in normal development, causing cognitive and social deficit, usually appear in the first two or three years. Autistic children may walk on their toes, flap their hands, or become fascinated with spinning things. They may be absorbed in obsessive rituals, such as lining up toys in peculiar ways, flipping light switches on and off, or unraveling their socks thread by thread. Boys are diagnosed with autism four times more often than girls. —R.S.

of flashing shuttles weave a dissolving pattern, always a meaningful pattern though never an abiding one.” Perhaps the autistic brain is a loom that creates flawed patterns or can’t easily be programmed to weave new designs.

BAD GENES, NOT BAD PARENTS

Though Kanner believed autism was inborn, many psychiatrists blamed it on cold, unloving mothers whose inadequate parenting marred their autistic children’s developing psyches. But studies in the 1970s showed that among twins with autistic disorders, identical twins are very likely to both be affected, whereas fraternal twins—like Nicole and Carly Branconnier—are rarely both affected. These studies provide strong evidence that faulty genes are largely responsible, most likely combined with unknown and unmeasurable inputs from environmental factors.

“Genetic” doesn’t always mean “inherited.” Although the less-disabling forms of autism can often be traced in families, scientists believe severe cases most often arise from spontaneous, or *de novo*, mutations. These are DNA mutations—present in the child but not in the parents—that occurred during the formation of the eggs or sperm before conception.

“This is not surprising, as autistic children rarely marry and have children,” says Christopher Walsh, an HHMI investigator at Children’s Hospital Boston and Beth Israel Deaconess Medical Center, who is searching for relevant genes.

An estimated 5 to 15 percent of cases stem from chromosomal abnormalities causing rare diseases with autistic features, such as Rett syndrome and fragile X syndrome. As for the remainder, no single gene has been linked to a large

portion of cases. A likely scenario is that mutations or slight variants in a handful of genes, or maybe in hundreds of different genes, acting in combination account for most autism.

In the absence of any known biological abnormality or “biomarker” in autism, scientists’ best bet is to hunt for altered genes in patients and families. Just such a discovery, in a rare syndrome caused by a single damaged gene, opened a new window on autism less than a decade ago.

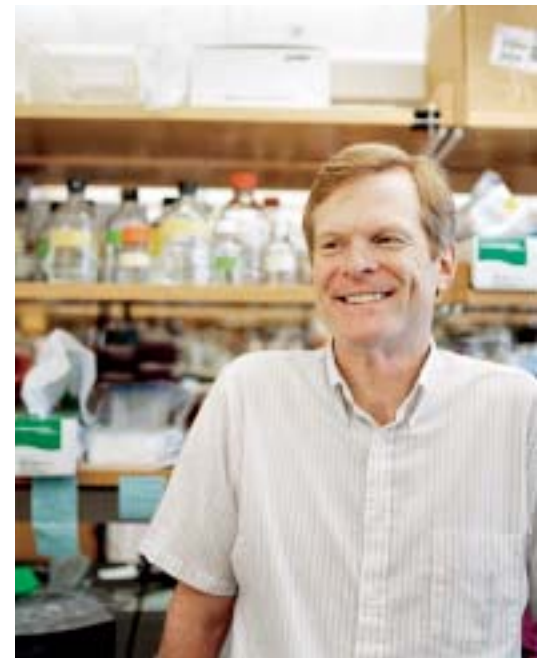
In 1999, HHMI investigator Huda Zoghbi identified gene mutations that cause Rett syndrome—a rare, devastating “autism spectrum” disease that affects girls. After normal development for the first 6 to 18 months, affected girls’ speech, motor control, and social development plateau and then deteriorate, accompanied by the onset of tremors, seizures, and stereotypic hand-wringing movements. Zoghbi, a pediatric neurologist and geneticist at Baylor College of Medicine, found that 95 percent of Rett cases involve mutations in a gene on the X chromosome called *MECP2*. “This was the first identification of a gene [mutation] in any developmental cognitive disorder,” observes Fischbach.

Zoghbi created a mouse model in which she has been studying the effects of the mutant gene. “MeCP2 is present in every mature neuron—thus, it is not surprising that it is important for social behavior and communication,” says Zoghbi. The MeCP2 protein regulates the activity of other genes in its pathway and also controls variable splicing of the genes’ RNA blueprints to make different forms of the protein.

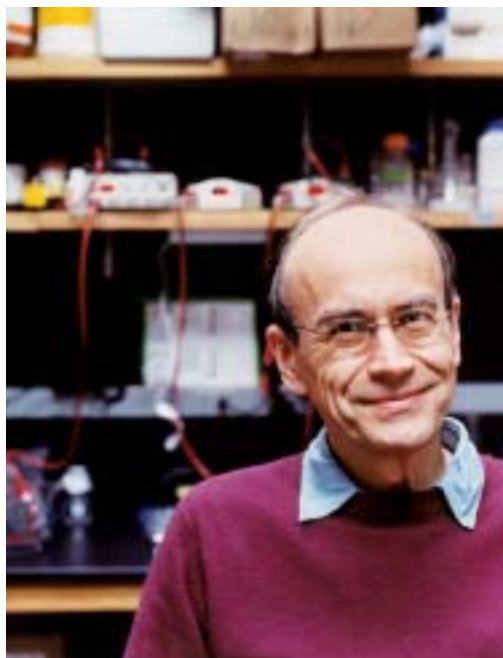
TROUBLE AT THE JUNCTION

Zoghbi’s group later described MECP2 mutations in a handful of girls and boys with autistic features who did not have Rett syndrome. Although *MECP2* mutations don’t appear to be very common in “pure” autism, findings by Zoghbi and others have shown that they can occur. These discoveries point a suspicious finger at synapses.

In 2007, Zoghbi and colleagues reported in *Neuron* that MeCP2 regulates the formation of synapses connecting neurons that secrete glutamate—an “excitatory” chemical messenger that causes neurons to fire. Glutamate is like a green light that encourages nerve signals to jump across the synapse; its opposite “red light” neurotransmitter is GABA, an inhibitor that quickly halts nerve firing when appropriate.



Li-Huei Tsai’s studies of protein interactions and Christopher Walsh’s gene-seeking strategy are beginning to provide clues to autism’s origins.



The relevance of the synapse is becoming clear from Huda Zoghbi's findings in Rett syndrome and Thomas Südhof's mouse model of autism.

A proper balance of excitatory and inhibitory events is key for learning, memory, and other information-processing tasks. If there's a generalized excitatory-inhibitory imbalance, it might well explain why the autistic brain falters in trying to build networks for learning, language, and social awareness. In 2003, Zoghbi proposed that changes in the function of synapses may be a fundamental cause of neurological disorders—including autism.

That same year, another set of mutations in autism came to light, causing excitement because they, too, pointed to components of the synapse-making machinery. In the 1990s, Thomas Südhof, an HHMI investigator at University of Texas Southwestern Medical Center at Dallas, identified genes for two key families of proteins involved in creating the brain's synaptic nerve connections.

The two gene-protein families, called neuroligins and neuroligins, are located on opposite sides of the “cleft” or tiny space where the neurons meet at a synapse. These two protein complexes extend out of the nerve cells and physically bridge the synaptic divide, but they also affect the excitatory-inhibitory balance of nerve signal traffic.

Thomas Bourgeron at the Pasteur Institute in Paris first reported mutations affecting these proteins in autistic patients

in 2003. He found that two pairs of Swedish brothers with autism disorders had mutations in the neuroligin proteins that Südhof had identified seven years earlier.

More recently, a larger international study revealed a gene for one of the neuroligins in a chromosomal region linked to autism. Bourgeron has also found mutations in another gene expressed in synapses, *Shank3*, which interacts with neuroligins. Other scientists have uncovered evidence that links the gene with autism.

“I suspect that *Shank3* is one of at least a dozen genes that have rare variants in them which likely are causative for the disease,” comments Louis Kunkel, an HHMI investigator at Children's Hospital Boston, who is hunting for autism genes. “These will all likely be important in neuronal maturation and learning.”

Another piece of the synaptic puzzle recently emerged from the lab of HHMI investigator Li-Huei Tsai at the Picower Institute for Learning and Memory at MIT. Publishing in *Neuron* in 2007, she reported that a protein, Cdk5, modifies another protein called CASK and promotes the interaction of CASK with neuroligin proteins at newly forming synapses.

“This general process seems to be extremely relevant to autism,” Tsai says, “because a lot of the proteins implicated

in autism spectrum disorders all seem to overlap in this particular area of synapse development.”

A dramatic demonstration of how a single mutation can cause autistic symptoms came when Südhof created lab mice containing the *neuroligin-3* gene mutation previously found in humans. The mutation lowered the amount of neuroligin-3 protein in the animals' forebrains by 90 percent, with a surprising consequence for their behavior.

Compared with control mice, the gene-altered rodents were less social: they spent less time interacting with a new mouse placed in their cage. But they became smarter: they took fewer days to learn the location of a platform submerged in murky water, indicating enhanced spatial memory.

“This is incredibly exciting,” Südhof says. “Usually when you impair mouse cognitive function, they're just stupid. These mice are not stupid—they have a huge positive change in learning along with a modest social deficit. This is the first genetic dissection of circuits that underlie these different effects.”

The events leading from mutation to altered behavior aren't fully understood, Südhof says, but the results “validate the whole idea that autism is related to synapses.”

CASTING A WIDE NET FOR GENES

Further progress in autism research means continuing the gene hunt on a broad front, deploying a variety of strategies. Many different kinds of genetic flaws appear to be involved—mutations, deletions, copy number variations (too many or too few copies of critical genes), large and small chromosome effects. Fortunately, new genomic tools such as single nucleotide polymorphism “chips” can spot increasingly small genetic flaws.

A genome search using these methods led to a January report by the Boston-based

Autism Consortium (a research collaboration involving 14 Boston-area institutions) of an abnormal chunk of chromosome 16 found in several autistic patients. The segment harbors a mixture of gene deletions and extra copies of genes that is estimated to account for 1 percent of unexplained cases of autism spectrum disorder. Several of the genes are known to be expressed in the brain or in early nervous system development. Researchers believe that a large number of rare “copy number variations” such as these may be waiting to be found.

Working with clinical genetics collaborators in several Middle Eastern countries where intermarriage between cousins and large family size are common, HHMI investigator Walsh, a member of the Autism Consortium, has been trying another gene-seeking strategy. He is attempting to find rare recessive gene mutations, which are more likely to be expressed when intermarriage occurs over generations. “My collaborators bring in their tough cases,” says Walsh, who has visited countries including Dubai, Kuwait, Saudi Arabia, and Oman. “Our team of genetic counselors, pediatric neurologists, clinical psychologists, and others provide a second opinion, draw blood for DNA samples, and say that we’ll try to figure out the genetic picture.”

Walsh’s strategy has begun to pay off. He has found several families in which a member with autism is missing both the paternally and maternally inherited copies of genes, making those genes likely suspects.

“We don’t yet know what the deletions do,” he says. But three of the missing genes turned out to be among those being studied by Michael Greenberg, a Children’s Hospital Boston colleague. Greenberg is looking for autism clues by studying nervous system genes that are silent until activated, during learning, to make more synapses and are logical candidates for autism research.

Also collaborating with Greenberg at Children’s Hospital Boston is Kunkel, who previously discovered the gene for Duchenne muscular dystrophy. He and Children’s Hospital colleague Isaac Kohane have devised an unorthodox gene-hunting approach—looking for gene “signatures” of autism in blood cells, rather than in the brain.

“We’re using microarrays to see if there is a signature of gene expression in whole blood that distinguishes autism, and we are starting to see such signatures,” says Kunkel. If gene expression in the blood cells is similar enough to that in brain cells to be a useful surrogate measure, researchers could avoid the need to obtain and test brain tissue, which is impossible in living humans. Ultimately, a “proxy” signature for autism could be used diagnostically and in testing candidate drug therapies.

With a variety of intensive behavioral therapies, the capabilities and lives of many autistic children, like Nicole Branconnier, have improved. There is no definitive treatment for the multifaceted, complex disorders of autism, and a cure seems a distant prospect.

However, the biological underpinnings of autism are becoming clearer. And among the recent findings, one in particular may bode well for reversing symptoms with future therapies.

In 2007, Adrian Bird, a geneticist at the University of Edinburgh, showed that Rett syndrome mice with a silenced *MECP2* gene could recover many of their lost functions when the gene was reactivated in adulthood. This couldn’t be attempted in humans with Bird’s experimental methods, but the implications are cause for optimism: apparently the *MECP2* mutations don’t irreparably harm the neurons themselves, which develop very early; rather, the mutations cause malfunctions in the synapses that form later on.

“SHE CAN’T
TAKE OTHER
PEOPLE’S
PERSPECTIVE
AND DOESN’T
UNDERSTAND
WHY THEY
DON’T GO
ALONG WITH
WHAT SHE
WANTS
TO DO.”

—Maureen Branconnier

Bird’s finding offers another reason why the synapse makes sense as the culprit in autism, says Walsh. “It may explain the later age of diagnosis of autism, and its preferential effects on later-appearing skills—like language and social behavior—and perhaps its greater likelihood of improvement in some fortunate children,” speculates Walsh. “If we can develop medications to modulate these synaptic changes, we may be able to provide better therapies for this devastating disorder.”

Fischbach agrees: “The possibilities of reversal are very real.” That’s encouraging, and so is the quickening pace of gene discovery, which ultimately should shed further light on causative mechanisms. How long this will take, though, is anyone’s guess.

“The hunt is on,” says Fischbach, “but it’s going to be a while.” ■

FOR MORE INFORMATION: To learn more about the latest approaches to autism, visit the Websites for the Simons Foundation (<http://www.simonsfoundation.org/>) and the Autism Consortium (<http://www.autismconsortium.org/>).

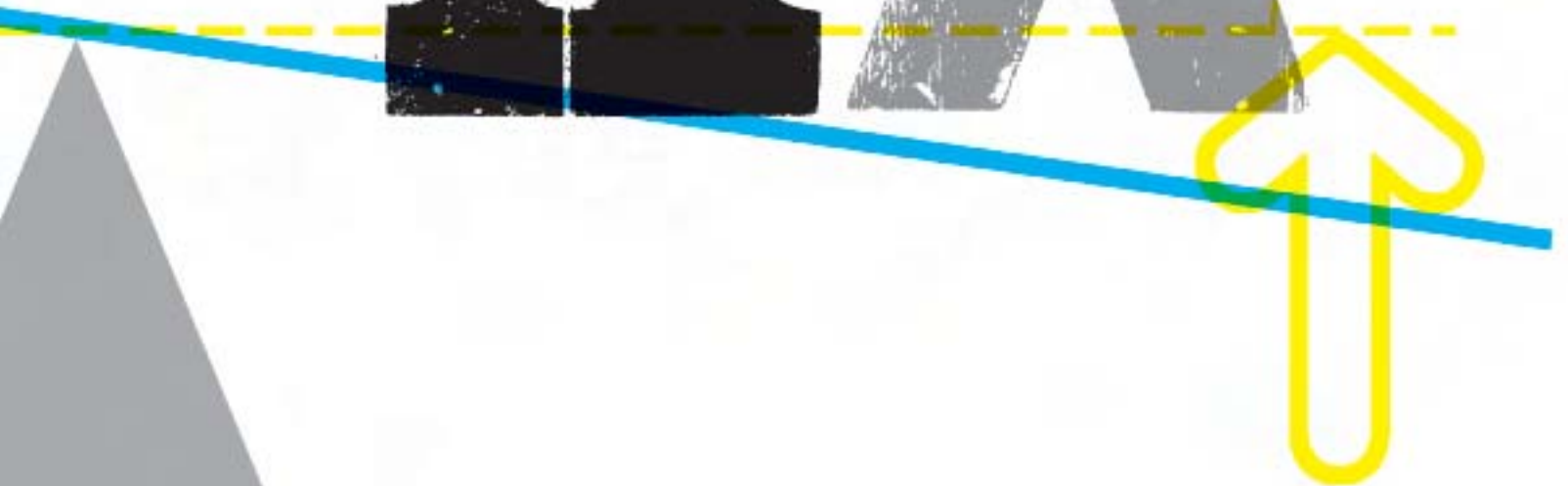


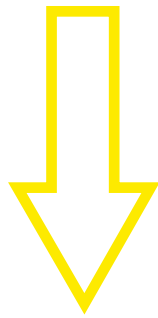
GENETIC
BALANCING
ACT

**BY
MITCH
LESLIE**

ILLUSTRATION
BY
PATRICK
THOMAS

ANIMALS
HAVE
EVOLVED
INTRICATE
WAYS TO
ENSURE
THAT GENE
ACTIVITY IS
THE SAME IN
MALES AND
FEMALES
DESPITE THE
INHERENT
IMBALANCE
OF X CHRO-
SOMES.





When it comes to chromosomes, men get short-changed. Among their 23 pairs of chromosomes, women have two strapping X chromosomes, while men sport an X chromosome and a Y. It might be the ultimate source of maleness, but the Y chromosome is downright diminutive, with only about one-third as many DNA bases as the X chromosome and fewer than one-tenth as many genes.

This disparity leaves our cells in a jam. A woman's cells carry twice as many copies of most X chromosome genes as a man's cells and—if the genes work at full throttle—can crank out correspondingly more of their protein products. Although genes on the X chromosome are essential for everything from filtering blood to repairing DNA (most of them have nothing to do with sex differences), a double dose of their proteins is disastrous for cells.

And that's not only true in humans. Animals as different as roundworms, fruit flies, and opossums face the same problem. "It's essential to have gene balance" between the sexes, says HHMI investigator Barbara J. Meyer of the University of California, Berkeley. If the numbers are out of whack, "the animals are dead, it's that simple," she says.

Necessity, however, is the mother of adaptation. Animals have evolved myriad mechanisms to keep gene activity the same in the two sexes. Known as dosage compensation, this type of gender equality can work in several ways. For example, female mammals shut down one X chromosome, preventing most of its genes from yielding any proteins—what's termed X chromosome inactivation. This process is complete by the early stages of embryonic development, and the inactivated chromosome remains off for life. Fruit flies use the opposite approach: responsibility for dosage compensation falls on the male, where the output of the genes on its single X chromosome doubles, thereby ensuring the same gene expression as in XX female flies.

Two HHMI investigators, Meyer and Jeannie T. Lee of Harvard Medical School and Massachusetts General Hospital, are at the forefront of research on the intricacies of dosage compensation. In more than 20 years of experiments on nematodes, or roundworms, Meyer has shown how cells count the number of X chromosomes they contain and revealed the workings of a protein cluster that adjusts gene activity on the X chromosome. Studying mice, Lee has deciphered details of the molecules that regulate X inactivation. Both scientists say their results have surprised them at every turn.

Now their work is having an impact on biologists in other fields. Researchers are looking to dosage compensation for clues about how cells orchestrate changes to large tracts of DNA or entire chromosomes. And Lee's recent findings of peculiarities in X inactivation among embryonic stem cell lines have heightened concerns about the safety of these stem cells when used to develop replacement tissues and organs.

CELLULAR ABACUS

To pull off dosage compensation, cells need basic math skills. They have to be able to count the number of X chromosomes and non-sex chromosomes (called autosomes) they harbor. Meyer uncovered the basis for this arithmetic ability in roundworms.

The discovery began with an Alexander Fleming moment. Fleming, a Scottish bacteriologist, stumbled on penicillin in 1928 when he took a close look at some moldy bacterial cultures, rather than tossing them out as he usually did. The Meyer lab version of this event occurred 60 years later and involved a flask of misbehaving worms.

In the late 1980s, the lab was studying animals with a genetic mutation called *dumpy* that disables dosage compensation

and sickens or kills the worms. But the nematodes in one container were hale, and their population was booming. Thinking that the culture had been contaminated, Meyer's lab technician started to throw the animals away. "He was literally pouring the culture down the sink," recalls Meyer, "when I said, 'Stop!'" Under the microscope, any nematodes that survived the mutation should have appeared short and squat, but she noticed that they were long and sleek. Investigating the animals' mysterious survival led Meyer and colleagues to a gene called *xol-1*. "That broke it open for us," she says.

As she and her co-workers have revealed over the last 20 years, *xol-1* serves as a cellular abacus, with its tallies dictating not only whether dosage compensation kicks in, but also the animal's sex. Worm sexuality differs from our own. Male worms carry one X chromosome, but no Y. The other sex, with two Xs, is a hermaphrodite (an organism with male and female reproductive systems).

By hunting down mutated genes that turn *xol-1* on or off at the wrong time, Meyer's team identified the "counters" that tell worm cells how many sex chromosomes they have. Several genes on the X chromosome, the researchers found in a series of studies, code for proteins called X signal elements that can shut down *xol-1*. The researchers also found genes on the autosomes whose proteins, known as autosomal signal elements, can activate *xol-1*. In other words, worm cells simultaneously fashion proteins that can turn *xol-1* off and proteins that can turn it on.

The fate of an X chromosome depends on a molecular scuffle between the X signal elements and the autosomal signal elements. The battle occurs at *xol-1*'s promoter, its on-off switch. "They're all duking it out for *xol-1*'s promoter," says Meyer. In the free-for-all, numbers prevail. Male worms have one X chromosome but two copies of each autosome. So their "on" signals from the autosomes overwhelm their "off" signals from the solitary X. Thus, *xol-1* is active and thwarts dosage

compensation. This allows cells to turn on another gene that triggers the animal to develop male characteristics.

With a pair of X chromosomes, a hermaphrodite produces twice as many X signal elements as does a male. In this case, the "off" proteins from the X chromosomes win out over the "on" proteins from the autosomes, quieting *xol-1* and permitting dosage compensation.

Meyer and colleagues continue to refine this story. In a November 2007 paper, for instance, they reported identifying another X signal element, the fifth found so far.

They have also tracked down the molecular "dimmer switch" responsible for dosage compensation in roundworms.

Meyer's lab first learned that the process operates differently in worms than in mice and humans. Instead of turning off one of its two X chromosomes, a hermaphroditic worm dials down the activity of all genes on both Xs by half, on average. Meyer's group went on to discover that worms rely on a cluster of proteins called the dosage compensation complex (DCC) to achieve this feat. The complex attaches to the X chromosome and turns gene activity down. But the DCC's proteins don't unite and start working until the cell inactivates *xol-1*.

Some of the DCC's proteins are similar to proteins that help chromosomes compact and then separate during cell division. That finding suggests that the DCC is derived, evolutionarily, from a protein combination that performs a completely different task. In other words, when cells in some ancestral worm needed



THROUGH HER WORK IN ROUNDWORMS, BARBARA MEYER HAS REVEALED HOW A PROTEIN CLUSTER ADJUSTS GENE ACTIVITY ON THE X CHROMOSOME, A PROCESS CALLED DOSAGE COMPENSATION.



JEANNIE LEE HAS IDENTIFIED KEY GENES INVOLVED IN X CHROMOSOME INACTIVATION AND HAS UNCOVERED DETAILS OF HOW MOLECULES REGULATE THAT INACTIVATION IN FRUIT FLIES.

a dosage compensation system, “they stole it” from another cellular mechanism, Meyer says, thus avoiding the need to evolve entirely new molecular machinery to do the job.

Her lab has answered the key question of how the DCC distinguishes X chromosomes from autosomes. To find out, the researchers attached different chunks of an X chromosome to an autosome and determined which ones attract the DCC. In 2004, the team first revealed that the DCC homes in on several DNA stretches along the X chromosome. One stretch was about 800 nucleotide bases long. In a

2006 follow-up study, Meyer and colleagues whittled that segment down and dissected others, demonstrating that the DCC recognizes two specific DNA sequences, or motifs, each about 8 nucleotide bases long.

The surprising fact, Meyer says, is that these motifs appear on autosomes as well as on the X chromosome. The arrangement of motifs, not just their presence, might dictate whether the DCC latches on, a possibility Meyer’s lab is now exploring.

TWISTS, TURNS, AND MORE TWISTS

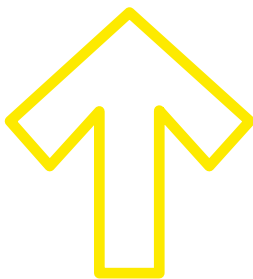
Once a cell has finished counting chromosomes, it’s ready to take action. In female mammals, that means muffling one of the X chromosomes. How cells turn on dosage compensation when it’s needed—and keep it off when it’s not—has occupied Jeannie Lee for 13 years. When she began her experiments as a post-doc in Rudolph Jaenisch’s lab at the Massachusetts Institute of Technology, she didn’t envision that the work would take

this long. “I thought it would be solved by now,” she says. Yet scientists are far from having all the answers, in part because their studies keep throwing them curve balls, including odd molecular pathways, backward DNA silencing, and unexpected chromosomal liaisons.

British geneticist Mary Lyon galvanized research into dosage compensation in 1961, when she determined that the Barr body, a shadowy structure lurking at the edge of the nucleus in mammalian cells, was an inert X chromosome. On the basis of that finding, researchers posited that cells must have an “X inactivation center” that closed down the chromosome. But the responsible genes were elusive. It wasn’t until 1991 that Huntington Willard, now an HHMI professor at Duke University in Durham, North Carolina, and colleagues identified a gene, named *Xist*, that instigates dosage compensation (see sidebar). Lee cites that finding as one of her motivations for switching to X inactivation research in 1995.

Her first big discovery came four years later, when she and her colleagues pinpointed the gene *Tsix*, which blocks *Xist*. *Tsix*’s existence makes sense—cells need to keep *Xist* under control to prevent it from shutting down both X chromosomes in females or the only X in males. *Xist* and *Tsix* aren’t just opposites in function. Their nucleotide sequences are mirror images, what researchers call complementary. The name Lee’s team chose for their new gene reflects this inverse relationship: *Tsix* is *Xist* backward.

How *Tsix* and *Xist* work caught researchers off guard. Unlike the genes that control the color of our eyes or allow our cells to break down sugars, *Tsix* and *Xist* don’t encode proteins. When a cell needs to perform a task, it typically makes an RNA copy of a gene, which in turn codes for a protein that takes care of business. But for *Xist* and *Tsix*, the RNA molecule itself is all that’s needed to carry out functions in



the cells. Willard's lab demonstrated this unusual behavior for *Xist*, whereas Lee's group showed it for *Tsix*.

That wasn't the last surprise that awaited Lee and colleagues as they delved into *Tsix*'s actions. Because strands of *Tsix* and *Xist* RNA are complementary, if they meet they'll stick together. A reasonable hypothesis is that *Tsix* RNA prevents X chromosome inactivation by grabbing and disabling the *Xist* RNA.

Reasonable but wrong, Lee and colleagues concluded in a 2006 *Molecular Cell* paper. Inside the cell, a chromosome's DNA wraps around spool-like proteins called histones. To shut down a specific gene, cells typically coil the DNA strands tighter, denying access to essential DNA-reading proteins. But to silence the *Xist* gene in fruit flies, Lee and colleagues found, *Tsix* appears to do the opposite; it loosens the DNA. Cinching up the DNA, by contrast, turns on *Xist*. Only a few fruit fly genes are known to operate in this way, she says.

In retrospect, the seemingly backward mechanism makes sense, she adds. To close down an entire X chromosome, a cell might compress all the chromosome's DNA. But if *Xist* were a conventional gene, that tightening would also shut it down—and once *Xist* stopped working, other X chromosome genes might start up again. So the unusual method for switching off *Xist* might permit the gene to remain active even when the rest of the chromosome is silenced, Lee says.

Thanks to her team's report two years ago in *Science*, researchers have another oddity to ponder that involves *Tsix*. Lee's group discovered that before one X is inactivated, the two X chromosomes in female mouse cells line up and briefly touch at the X-inactivation center. Such

pairing doesn't normally occur once X inactivation is complete, and it requires at least three genes, including *Tsix*. Without the contact, the cell can't figure out how many X chromosomes it contains or which of them to inactivate, so it might shut down both or neither. During their brief dalliance, the two chromosomes appear to be communicating. What information passes between them "is something we're vigorously pursuing," Lee says.

A FLAG FOR STEM CELLS

Findings by Lee and others also raise concerns about the safety of embryonic stem cells. Researchers have high hopes that these flexible cells, which can specialize into heart cells, liver cells, or any of the body's other cell types, can be

directed to repair or replace damaged tissue and organs.

But only if X inactivation proceeds normally. Lee's group assessed the amount of *Xist* RNA, viewed as an indicator of chromosome shutdown, in 11 stem cell lines being maintained in the lab. The samples included "approved" lines that scientists can study with federal government money and other lines provided by HHMI investigator Douglas A. Melton, co-director of Harvard's Stem Cell Institute, that had been developed without federal funds.

"When we looked at their X inactivation state, they were all over the map," Lee says.

(continued on page 56)

A MYSTERY THAT GRABS HOLD


"This is a subject that people fall in love with," says Huntington Willard, an HHMI professor at Duke University in Durham, North Carolina. → Willard, who describes himself as "the old guy" in the field, says that his attraction to dosage compensation and X chromosome inactivation began in the early 1970s, when he was a Harvard undergraduate working on a lab project with the late HHMI investigator Sam Latt. During mitosis, cells copy their chromosomes and then divide, and Latt and Willard wanted to determine whether silenced and active X chromosomes behaved differently during this process. They did—the inactive X was slower to duplicate and copied its sections in a different order than did its active partner. → When he moved to Yale University for grad school, Willard kept up his work on X inactivation—but only by moonlighting. By day, he worked on his official dissertation project, studying the genetics of an inherited metabolic disorder called methylmalonic acidemia. Willard has divided his effort between X inactivation and other genetic projects ever since. He was part of the team that nailed down the DNA sequence of the X chromosome in 2005, for instance, and in 1997 his group designed the first artificial human chromosome. In 1991, he and his co-workers jump-started molecular investigation into X inactivation by identifying the first mammalian gene that controlled the process. They called it *Xist*.

→ Three years ago, Willard and his colleague Laura Carrel of Pennsylvania State University gave the field something else to explore. They measured the activity of 95 percent of the X chromosome genes—the most comprehensive survey so far. Ten to 15 percent of the genes escaped inactivation entirely, and another 10 percent sometimes shut down. → How cells manage to inactivate most X chromosome genes but leave some working is a mystery. Scientists had thought that *Xist* RNA spurs shutdown by enveloping the X chromosome. In fact, if researchers tag the RNA with a fluorescent marker, these *Xist* molecules show up as a luminous cloud swaddling the chromosome. The question is how this cloud clears over the genes that remain active. One of the many seductive mysteries, Willard says, that captivate and keep researchers coming back for more. —M.L.





Sources of Renewal



As scientists learn more about how to produce and manipulate stem cells—amid high expectations and close scrutiny—no one is ready to choose any one approach over another.

by **Robin Mejia**
illustration by Shout



Above the desk of HHMI investigator
George Q. Daley hangs a striking
impressionistic painting of a person
lost in thought, called
“On Learning My Diagnosis.”

It's by an attorney Daley began treating in 1993 for a chronic and sometimes fatal blood disorder. With that diagnosis, the man put down his law books. He has been doing so well for so long, however, that he's returned to his legal practice. This is the kind of outcome that a doctor hopes for.

Not every patient is that lucky. “I just spent the last week in the hospital taking care of kids with all kinds of blood diseases,” says Daley, a physician-researcher at Children's Hospital Boston. “Sickle cell anemia, for example, is exquisitely painful, and we don't have a treatment for it. We give them narcotics and we hydrate them, but what we'd ideally like to do is repair their cells.”

He envisions a day when he'll be able to take cells from his patients, repair the damaged genes, and grow new blood cells to treat them. Daley, a leader in studying stem cells—embryonic stem (ES) cells, adult stem cells, and the tantalizing but still very new induced pluripotent stem (iPS) cells—has already managed to do that kind of cellular repair in mice.

For Daley and others who study stem cells, recent discoveries make this an exciting and challenging time. They are learning how to manipulate these cells to produce the building blocks of various organs. And the development of iPS cells opens the possibility of producing patient-specific stem cells without using human embryos, an achievement that could defuse many of the ethical and political tensions that surround this area of biology. But there is much work to do before researchers know how well these stem cells will deliver on their promise.

Working within Limits

To achieve cellular repair in mice, a team led by senior colleague Rudolf Jaenisch and Daley, when he was at the Whitehead Institute, took the nucleus from the cell of an adult mouse with a genetic defect and inserted it into a mouse egg. (An egg cell can

reprogram its nucleus to create a cell with the potential to produce any part of the body.) If the egg is grown to a blastocyst—an early stage embryo—researchers can harvest stem cells from it. Daley's team did that and more. They repaired the defective gene in the cultured stem cells, coaxed the repaired cells to become blood stem cells in a Petri dish, transplanted the healthy blood cells into diseased mice, and partially restored immune function in the animals; they published the results in 2002 in *Cell*.

This is the ultimate promise of stem cell research—fixing illnesses at the genetic level and then using the modified cells to treat patients. No one has yet succeeded in creating human stem cell lines with the technique Daley's team used in mice, called somatic cell nuclear transfer or therapeutic cloning. Researchers have created human stem cell lines from embryos donated by in vitro fertilization patients, but these cell lines are not patient specific.

There are groups with ethical or religious concerns that consider the use of embryos destruction of human life or a step toward reproductive cloning. President George W. Bush agrees, and announced in 2001 that the U.S. government would fund research only with human embryonic stem cell lines created before August 9, 2001. Researchers like Daley cannot apply for National Institutes of Health (NIH) grants, or any other federal funds, to support development of new human embryonic stem cells, nor can they use equipment funded by federal grants to work with newer stem cell lines.

Voters in some states as well as private donors have provided a few alternatives. In 2004, Californians passed Proposition 71, authorizing \$3 billion in state bonds to fund stem cell research through a granting agency called the California Institute for Regenerative Medicine. A handful of other states followed, with much smaller amounts. For its part, Harvard relied on private philanthropic donations to create the Harvard Stem Cell Institute, which now encompasses labs at the medical school, other parts of the university, and 11 teaching hospitals. Daley, a member of the Stem Cell Institute along with several HHMI colleagues, lined up private funding to support his embryonic stem cell work, supplemented in February 2008 when he became an HHMI investigator.

However, the administration's position on human embryonic stem cells is seen as a barrier, as many researchers limit themselves to projects that are eligible for funding from the NIH.

“I have a junior investigator in my lab who's a driving force behind our human ES research, which uses all private funding,”

says Daley. “As he’s trying to get an independent faculty position, other mentors are saying, you need an NIH grant, you better focus on a mouse program.”

Excitement Tempered with Caution

*At the end of 2007, a new door swung open. Scientists in Japan and Wisconsin, and Daley at Harvard, reported that they had successfully turned human adult skin cells into stem cells. In November, researchers in the lab of Shinya Yamanaka at Kyoto University documented in *Cell*, and, separately, a team led by James Thomson at the University of Wisconsin reported in *Science*, that they had created stem cells by inserting four genes into human adult skin cells. The genes appeared to perform the same function that insertion into an egg does in other animals: resetting the cell’s genetic state back to day one.*

Daley’s paper, published online in *Nature* in December 2007, described his ability to reprogram human adult skin cells using even fewer genes. Scientists refer to the new cells as induced pluripotent stem (iPS) cells, meaning they have been coaxed to regress to a state in which they could become any of the various cell types that make up the body. In human cell lines, such pluripotency is shared only by embryonic stem cells, although the iPS technique bypasses one of the steps that has hampered the development of patient-specific human lines; the new process created stem cells without the costly and difficult step of harvesting eggs. And no embryos are required.

Researchers around the world are elated by this apparent breakthrough. “Clearly, this work is a very big step,” says Alan Trounson, president of the California Institute for Regenerative Medicine, which was founded to support embryonic stem cell research but now expects to fund efforts using the new technique as well.

Some researchers are even transcending their usual penchant for understatement. In *The New England Journal of Medicine*, Douglas R. Higgs, of Oxford’s Weatherall Institute of Molecular Medicine, pointed out iPS cells’ clinical implications, particularly their potential for overcoming the immune system incompatibility issues of existing transplant technology. Reprogramming a patient’s own somatic cells, he wrote, is “the biologic equivalent of an alchemist’s dream of turning lead into gold.”

But iPS cells are a new discovery with plenty of questions that need exploring. Daley notes that while iPS work holds promise as

TWO STEPS CLOSER

In type 1 diabetes, the body attacks and kills its own insulin-producing pancreatic beta cells, leaving patients dependent on insulin injections for life. Unlike the liver and skin, the pancreas does not have a well of adult stem cells at the ready to repair damage. When the supply of beta cells is exhausted, as happens in type 1 diabetes, there’s no place to turn for more.

HHMI investigator Douglas A. Melton’s sights are set on curing type 1 diabetes by regrowing beta cells, and he is trying to do so the only way he can, from embryonic stem cells. Guiding the ES cells through several developmental steps is a long, slow process.

“Figuring out how to tell these cells, which we know can do anything, what to do is a challenge,” he says. Researchers can tease stem cells into some types of neurons and blood cells, but Melton estimates that five to seven steps are required to create beta cells—and it has taken three years to figure out how to guide ES cells through just two of those steps.

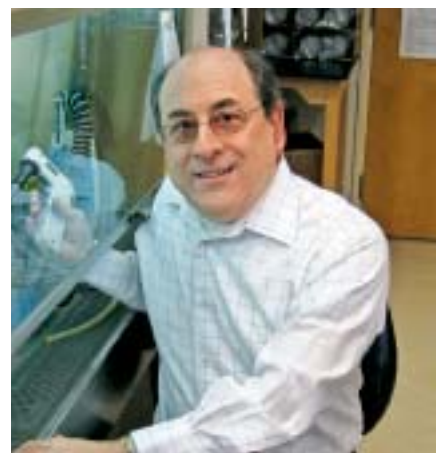
This is stem cell research as developmental biology. Melton explains that the most immediate benefits of most stem cell research will likely come from what these cells can teach scientists about how cells differentiate, how tissues develop, and how disease occurs.

For example, once Melton discovers how to grow a beta cell, he could create stem cells from someone with type 1 diabetes and from someone else with healthy beta cells. Then he could grow both stem cells into beta cells and watch to see where the diabetic’s cells go wrong.

By producing the disease in a Petri dish, scientists can run experiments—and even test drugs—in ways they never could in people. This is why the Harvard Stem Cell Institute has spent more than \$6.5 million creating a lab to facilitate precisely that kind of work.

Thus, in the basement of the building where Melton conducts his research, a collection of boxy robots is at work. They hold plates barely larger than drink coasters, with each containing up to 384 cultures in separate tiny wells. Mechanical limbs move the plates around and drop different chemicals into each—creating, in effect, 384 distinct experiments. Next door, an automated microscope reader scans the results of these experiments; it takes up to two hours to scan all 384 wells on a plate.

That’s still a lot faster than a postdoc. “In fact, a person couldn’t really do that assay,” says Lee Rubin, director of translational medicine at the Harvard Stem Cell Institute. This is why most academic labs have traditionally studied only a few compounds at a time. Harvard researchers are using Rubin’s robotic set-up—a stripped-down version of a drug-company screening lab—to ask a broad array of scientific questions. And it is how Melton figured out how to coax his embryonic stem cells two steps closer to a pancreas. —R.M.



From left:

George Q. Daley, HHMI investigator

Douglas A. Melton, HHMI investigator

Sean J. Morrison, HHMI investigator

Stuart H. Orkin, HHMI investigator

HHMI scientists and others are exploring the possibilities and limitations of stem cells to understand tissue development with hopes of curing disease.

PRINCIPAL PLAYERS

Embryonic stem (ES) cells are derived from embryos that develop from eggs fertilized in vitro. Each embryo, typically four to five days old, is a hollow microscopic ball of cells called a blastocyst. To generate cultures of specific types of differentiated cells—heart muscle cells, blood cells, or nerve cells, for example—researchers change the chemical composition of the culture medium, alter the surface of the culture dish, or modify the cells by inserting specific genes. Through years of experimentation, scientists have established some basic protocols, or “recipes,” for the directed differentiation of embryonic stem cells into specific cell types.

Adult stem cells are undifferentiated cells found among the differentiated cells that constitute a tissue or organ. The primary roles of adult stem cells in a living organism are to maintain and repair the tissue where they are found. The adult tissues reported to contain stem cells include brain, bone marrow, skeletal muscle, skin, and liver.

Somatic cell nuclear transfer reprograms adult cells, a feat that has been accomplished only with animal cells. An adult cell’s nucleus is inserted into an egg cell, which creates an embryo. Stem cells are then derived through a process similar to that used to create embryonic stem cells.

Induced pluripotent stem (iPS) cells are derived from adult somatic cells such as skin cells. These cells are reprogrammed, with the insertion of a handful of genes, to act as stem cells. In fact, they then display embryonic stem cell-like abilities.

an easier route to his goal of making patient-specific stem cells, none of his physician colleagues would consider using iPS cells as a treatment, at least for now. To insert new genes into cells to form iPS cells, researchers attached the genes to fragments of a virus that can cause cancer.

Scientists are working on methods to revert cells to a pluripotent state without using such viruses—by employing drugs, for example, or by injecting proteins directly into the cell.

“I think we’ll see this happen soon,” says Konrad Hochedlinger, a colleague of Daley’s at the Harvard Stem Cell Institute who also works on iPS cells. “Then the big question will be how similar these induced cells are to embryonic stem cells.”

“People take it for granted that they are identical,” he says, but iPS cells are not yet as well understood as their ES counterparts. Thus, Hochedlinger wants to grow human iPS cells alongside human ES cells and then direct both to become adult tissues such as muscle or nerve cells. He points out that with mouse cells, molecular analysis of the two cell types found no major differences, but when he attempted to grow adult heart cells from the mouse iPS cells using a protocol developed with ES cells, the iPS cells didn’t seem to form tissue as easily.

“Superficially, things look okay, but as you look more closely, the iPS cells don’t develop quite normally,” says HHMI investigator Stuart H. Orkin, at Children’s Hospital Boston. “They’re pretty close but they ain’t perfect.”

Orkin studies ES cells, trying to understand exactly what processes keep them from differentiating into adult tissue. The initial iPS experiments reported last fall created something of a “black box,” he explains. Scientists know that four specific genes cause the cells to regress to a pluripotent state, but they don’t

“We’re gathering a complete parts list of the things that are involved and required.”

Stuart Orkin

know how they do it, or why the process takes weeks longer than transferring a nucleus from an adult cell into an egg.

In a series of experiments described in the March 21, 2008, issue of *Cell*, Orkin examined nine genes that are known to help ES cells maintain themselves, including the four used in the recent iPS experiments and five others. Each of those nine genes produces a transcription factor, a protein that causes other genes to turn on or off. Orkin identified hundreds of genes that are targeted by one or several of the transcription factors, work that he hopes will help scientists tease out the cellular-level processes that help ES cells maintain and reproduce themselves. Similar work on iPS cells could help explain the differences between the two types of stem cells.

“We’re gathering a complete parts list of the things that are involved and required,” Orkin says. “Maybe adding something that hasn’t been considered yet might make it better.”

Science Informing Policy

With each new discovery, stem cell researchers have learned to provide perspective and context to help a hopeful public and those eager to find alternatives to using human embryos to understand the implications of the findings and the questions that remain.

HHMI investigator Sean J. Morrison, director of the University of Michigan Center for Stem Cell Biology, testifies to state and national officials about stem cell research, writes op-eds, and frequently talks with reporters.

Douglas A. Melton, a co-founder of the Harvard Stem Cell Institute, has discussed stem cell policy with President Bush. The HHMI investigator doesn’t particularly enjoy policy work, but thinks it is important.

The iPS findings added to the ongoing debates. In his State of the Union Address on January 28, 2008, President Bush noted, “In November, we witnessed a landmark achievement when scientists discovered a way to reprogram adult skin cells to act like embryonic stem cells. This breakthrough has the potential to move us beyond the divisive debates of the past by extending the frontiers of medicine without the destruction of human life.” He urged Congress to pass a ban on cloning, which would preclude

the development of stem cell lines created by somatic cell nuclear transfer, the technique Daley used in his mouse work and continues to explore with human cells.

This ban is a long-standing goal of groups opposed to human embryonic stem cell research. They praised the iPS work and used it as a reason to call (again) for a ban on therapeutic cloning.

“These are the same political lobbyists that have been looking for reasons to end this research all along,” says Morrison, who studies the mechanisms involved in adult stem cell renewal and aging. “The positions they’ve taken in the past were not credible, and the position they’re taking on iPS cells is not credible.” He points to earlier claims by several groups that 65 diseases had been treated with adult stem cells. That claim, “has been roundly dismissed.”

“We haven’t had a logical debate,” adds Melton, who argues that if stem cell research were truly unethical, “you would never say it’s okay as long as you don’t use federal funds.”

Both Melton and Morrison are hopeful that scientists may eventually be able to focus only on iPS cells, but they argue that it’s too early to close the door on embryonic stem cell research. “Certainly from a patient perspective that would be wrongheaded,” says Melton, whose position is echoed throughout the field.

This caution is based on history. In the early 1990s, researchers thought they were closing in on treatments for genetic diseases when they figured out how to insert working copies of genes directly into patients’ cells. “We could get genes to express in bone marrow,” says Daley, but those genes were inserted into cells by using viral vectors much like those involved in creating iPS cells. Because that treatment led to an unanticipated side effect—leukemia—iPS cells are not considered safe for human transplants.

“Some people believe that these are just technical obstacles we will overcome,” says Morrison. “Others believe that the Food and Drug Administration will never approve these lines, even if we improve the technology and eliminate the viruses.” He pauses

(continued on page 56)

PERSPECTIVES & OPINIONS

Stuart L. Schreiber

SMALL
MOLECULES
FOR BIG
MEDICINES

Leah Fasten

Drug discovery is an expensive process that can take dozens of years and result in only a few compounds making it to market. Stuart L. Schreiber, an HHMI investigator at the Broad Institute of Harvard and MIT, hopes to improve those odds using small molecule “bioprobes” to study the causes of diseases and to find better therapeutic targets for drug companies to explore.

The search for new drugs took an exciting turn in the past year when scientists linked variations in more than 50 genes in humans to their predisposition to develop more than a dozen diseases. While the ability to identify such links opened the door for potentially discovering the role of thousands of genes in diseases, it also posed new challenges to pharmaceutical companies. That’s because insights from research in human genetics aren’t made in a way that is compatible with how pharmaceutical companies traditionally practice drug discovery.

The pharmaceutical industry typically looks for treatments by first forming a hypothesis that a certain “target” molecule might be responsible for a disease, and then launching drug discovery efforts based on that assumption. Human genetics research, however, tends not to point to therapeutic targets, but rather to processes in the body that may have gone awry or imbalances that need to be corrected, such as insufficient insulin secretion.

Our lab has developed a set of tools and techniques we believe can help bridge this gap using small-molecule bioprobes. Small molecules have critical roles in all levels of biology—including cell growth, proliferation, sensing, and signaling—so researchers in academia and at pharmaceutical companies alike have a great interest in them. But instead of using small molecules as therapeutics, we are using them as bioprobes to examine the underlying causes of diseases. Our approach is discovery-based rather than hypothesis-driven. We want to define the properties of a cell in a particular state so we can study how they change as the cell becomes diseased. To understand a bodily process, it helps to perturb it with the bioprobes and determine the consequences. We believe this approach could lead us to choose more effective compounds as candidate probes, and as a result uncover more relevant therapeutic targets for drug discovery.

There already is evidence to indicate our method is working. Using small-molecule bioprobes, we identified two proteins that pharmaceutical companies subsequently developed as therapeutic targets with new mechanisms of action. Both won FDA approval in 2007. One drug, Torisel (temsirolimus) from Wyeth, treats renal cell carcinoma by inhibiting

mammalian target of rapamycin (mTOR) proteins. The other drug, Zolinza (vorinostat) from Merck, treats cutaneous T-cell lymphoma by modulating histone deacetylase (HDAC) proteins. We discovered that the mTOR and HDAC proteins might be good targets by using the small-molecule probes rapamycin and trapoxin, respectively, which cause behavioral changes in cells.

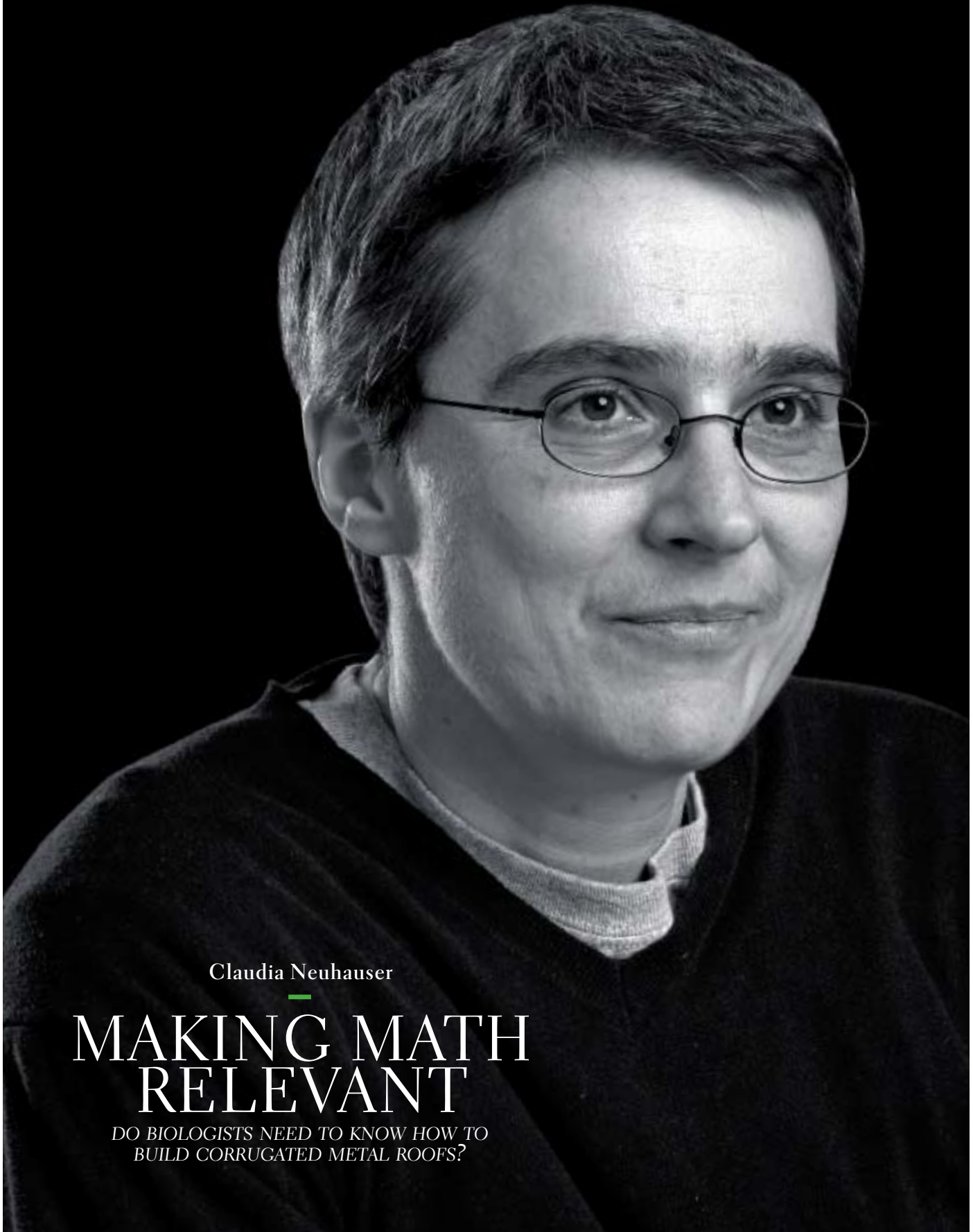
The field of biological research that focuses on the science of small molecules, called chemical biology, is relatively new. Its coemergence with modern human genetics is leading to enormous opportunities for synergies between these fields. In addition to information on genotype, or the inherited instructions carried by a human or other organism, modern human genetics is shining a bright light on the phenotype of diseases—that is, the change in morphology, development, or behavior of an organism as a result of disease. With chemical biology, we can provide powerful methods for studying phenotypes of disease and making sense of what we find. That’s where we can provide valuable information on likely targets to pharmaceutical companies.

Under a new chemical biology program called the Novel Therapeutics Initiative, the Broad Institute is further refining our thinking on effective drug discovery processes. Rather than trying to convince the pharmaceutical industry that an experimental lab technique is useful, we prefer to demonstrate the capabilities of our overall process in the context of especially challenging diseases, such as schizophrenia, diabetes, and cancers. If we can find highly unusual, highly effective therapeutic agents for these diseases that could not have been discovered otherwise, the pharmaceutical industry likely will pay attention. We’re encouraged by the outcomes with mTOR and HDAC inhibitors.

We don’t expect the pharmaceutical industry, which has spent millions of dollars and decades on its current drug discovery approaches, to slow down their process and switch to a new one. My hope is that applying chemical biology research and methods to drug discovery may serve as a way for the academic community to broaden its ties with the pharmaceutical industry, and demonstrate a path forward for developing better and safer drugs.

INTERVIEW BY LORI VALIGRA. *The head of the chemical biology program at the Broad Institute, Stuart Schreiber received his B.A. in chemistry from the University of Virginia and his Ph.D. in organic chemistry from Harvard University.*

PERSPECTIVES & OPINIONS



Claudia Neuhauser

MAKING MATH RELEVANT

DO BIOLOGISTS NEED TO KNOW HOW TO
BUILD CORRUGATED METAL ROOFS?

John Christenson

Math education needs to adapt to the needs of future biologists, says Claudia Neuhauser, an HHMI professor at the University of Minnesota, Twin Cities. According to Neuhauser, biology students are often fed math designed for engineers—with little relevance to their field of study. Now, she's one of a number of faculty members around the country teaching her students quantitative skills within the context of biology.

What's driving the movement to revamp math education for undergraduates?

The math we teach biology students is not the math they need. First, the quantitative education we give them is often restricted to calculus, but the calculus we teach them is usually geared more toward engineering than biology. Second, biologists need to be able to sift through large data sets, but right now, we don't teach undergraduates data analysis. Today, we can sequence entire genomes, and we're developing networks of sensors that can continuously record things like temperature and moisture from the environment. These systems produce huge amounts of data, and to work with them students need a good grounding in statistical analysis and computer science tools like data mining.

However, I don't think we can just send them out to take a semester of computer science or a semester of statistics. We need to integrate these tools into the biology curriculum, because when you learn something in context, you can see its relevance immediately, and it sticks a lot better.

When did it hit you that students weren't getting what they needed?

When I first came to Twin Cities, I taught the first-year calculus course. The students just hated it; there was this sense of hostility in the classroom. So I asked, "Who am I teaching here?" It turned out the class was full of biology majors. We used examples like calculating the amount of material you need to build a corrugated metal roof, and the students had no idea why we were teaching them this stuff. So I designed a calculus course based on biological scenarios they may encounter later in school or once they graduate. The students got much more interested. After I did that, I started working with other faculty members to make math—especially data analysis—part of their own courses.

Can you give another example of making math relevant?

Fishing is big in Minnesota, and there is a differential equation that describes fish growth, used by some states' departments of natural resources to determine minimum catchable sizes. I used this sort of real-world system to teach differential equations in the calculus course.

Are other schools making similar changes in their courses?

There's been a shift at many schools toward focused calculus courses for biology students. Lou Gross at the University of Tennessee integrates data analysis and statistics into the first-year calculus course. He's been one of the driving forces behind the push for more statistics. In general, though, there has been much less done with data analysis than with calculus. I think part of the reason is that calculus is the traditional bedrock in the curriculum, and it's very hard to make the dramatic changes necessary to shift the focus to data analysis. Especially at large universities, courses are taught in different departments, and the faculty may or may not know each other. It's sort of a silo structure. You have to have a biology person and a math person or a computer science person sit down and decide they want to break out of the silos. It's a slow process.

Is there a quicker way to make it happen?

The best way is to create an integrated curriculum from the ground up. The big project I'm working on now is the health sciences major at our new campus in Rochester. Many of these students will go on to become doctors, dentists, nurses, and veterinarians. We're going to build a module-based curriculum, where the quantitative, life, and physical sciences and the humanities are taught in modules, and then students will have to combine what they learn in integrative lab courses. Biological information is increasingly stored in big, minable databases. It's going to be important for these students to be able to analyze those databases and do experiments based on the data.

Our goal is to give students the quantitative tools they'll need in 10 to 15 years, when they finish their schooling and take jobs. We know it's going to be a data-intensive world, and we know that the standard tools we currently teach them are not good enough.

INTERVIEW BY BENJAMIN LESTER. *Claudia Neuhauser, author of the textbook, Calculus for Biology and Medicine (Second edition; Prentice Hall), studies theoretical population biology.*

Q&A

How does chemistry affect the average person?

Four HHMI scientists with chemistry backgrounds tell the Bulletin why modern life owes much to chemistry. — EDITED BY SARAH C.P. WILLIAMS



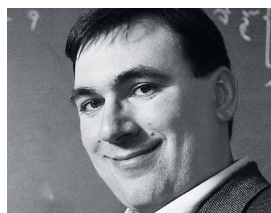
Linda C. Hsieh-Wilson
HHMI INVESTIGATOR
CALIFORNIA INSTITUTE OF
TECHNOLOGY

“Chemistry is all around us—it’s the reason we exist. From the enzymes that break down our food to blood clotting and skin healing after we scrape our elbow, everything is chemical and biochemical reactions—molecules dancing with one another that give rise to everything we are and do.”



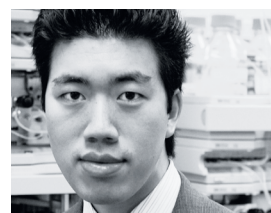
Jennifer A. Doudna
HHMI INVESTIGATOR
UNIVERSITY OF CALIFORNIA,
BERKELEY

“Two specific ways that chemistry affects (improves!) the average person’s life come to mind: one is the Haber-Bosch method of ammonia production, developed in the early 20th century. It enables the abundant nitrogen gas in the atmosphere to be converted to ammonia, which can then be turned into nitrates and nitrites for fertilizer. The process has revolutionized modern agriculture. The second is the production of antibiotics, which came about initially through careful isolation and analysis of natural compounds. The challenge to scientists and to society is to use such advances wisely.”



Milan Mrksich
HHMI INVESTIGATOR
THE UNIVERSITY OF CHICAGO

“Chemistry touches on every aspect of our lives. We live in a material world, and molecules are the stuff of materials. Over the next 20 years, the need to develop new sources of energy and to manage the impact of energy use on the environment will drive profound developments in catalysis—the process of making reactions more efficient. Chemists will pave the way toward lowering the energy it takes to produce materials and enabling recycling of unwanted by-products.”



David R. Liu
HHMI INVESTIGATOR
HARVARD UNIVERSITY

“Chemistry is a body of knowledge, a set of tools, and a philosophy. The knowledge and the tools have enabled life-saving drugs, computers, higher crop yields, ways to harvest and store energy, and all the non-natural substances used by society including steel, paint, suntan lotion, and modern anesthesia. The philosophy embraces manipulating the structure of molecules to change their properties. It encourages us to improve upon nature’s substances and dares us to design and make our own molecules that might fight a disease, make a better battery, or shed light on life’s origins.”

44 Science Education

Cue the Crickets / Strategizing to Diversify Science

47 Institute News

In Memoriam: Richard Gordon Darman /
HHMI Offers Boost to Early Career Scientists

48 Lab Book

Of Fish and Men / Fixing Fragile X / A New Clarity

51 Ask a Scientist

Do only the “long bones” of the arms and legs
contain growth plates? And if so, how do all of the
body’s bones stop growing at maturity?

52 Up Close

Hearing Through the Din

54 Nota Bene

Lifton Wins Wiley Prize /
Hynes Awarded Wilson Medal

These mitochondria of a monkey kidney cell were visualized using 3D-STORM microscopy, which allows scientists to pinpoint fluorescently-tagged molecules in three-dimensional space (see page 50). Mitochondria colored red and yellow are in the foreground, while those shaded purple and blue are deeper within the cell.

Cue the Crickets

AN INTERNATIONAL COLLABORATION AMONG TEEN RESEARCHERS GOES CHIRPINGLY.

THE BELL SIGNALING THE PERIOD'S END HAD RUNG FIVE MINUTES earlier, but students in this morning biology class were still tightly huddled around lab tables. What could possibly keep teenagers so riveted that teachers need to shoo them out?

A dozen students at the Academy of Science (AOS)—an HHMI-supported effort at Dominion High School in Loudoun County, Virginia—were in deep consultation with 12 colleagues from the prestigious Hwa Chong Institution (HCI) in Singapore. These students, all 11th graders, had been collaborating for weeks via e-mail. For 10 days in early November, they finally had the chance to interact face to face.

This program is the brainchild of AOS director George Wolfe, a former Peace Corps volunteer who includes among his professed goals to produce “globally aware citizens.” Three years ago, Wolfe was invited to Singapore to train teachers. While there on a later visit, he suggested to Har Hui Peng, HCI’s principal research consultant, that “our students collaborate like real scientists.” AOS requires that students undertake a two-year research project after their sophomore year. With the exchange program in place, those interested could apply to work with their counterparts at HCI.

Two students from each school teamed up to tackle one of six scientific ventures, which ranged from comparing the antibacterial properties of Western and Asian herbs to looking at a possible link between fish feminization and estrogen concentrations in waste water.

Devin Bowers, an AOS junior and avid guitarist, wanted to study something that involved acoustics. After consultation with Wolfe, he and classmate Aliya Jamil decided to investigate the evolutionary divergence of cricket song, on scales both local and—thanks to the involvement of Singapore students Cedrych Beh and Tse Yean Teo—global. The cricket song project also pulled in Gus Lott, a scientist at HHMI’s nearby Janelia Farm Research Campus, who is interested in educational applications of software he developed that can be used to analyze insect sounds (see sidebar).

Both groups faced some real-world obstacles. For one thing, urban Singapore does not have big cricket populations, so Beh and Teo regularly traveled a mile-long causeway to cross the border to Malaysia and its cricket-rich fields. Because they could not carry live insects across the border, the boys, crouched side by side in the tall grass that crickets favor, made their recordings on site.

Back in Virginia, Bowers and Jamil had their own problems. One batch of crickets died in an unsanitary aquarium. As winter approached and temperatures dropped, so did the

young researchers’ hopes when crickets outside started dying off. Then, Jamil’s mother took time during a family gathering in Pennsylvania to collect crickets from a field near her relatives’ home. And Bowers reports that “my mom and I went to the National Arboretum, which was like the Garden of Eden: crickets everywhere!” The aquarium was then thoroughly cleaned, and “most of them lived long enough for us to record them,” says Bowers, adding that, “One escaped during recording. I still haven’t found him....”

Next, the vast differences in scientific approach between the young scientists’ countries had to be reconciled when Beh and Teo visited AOS, where the style tends to be “hands-on,” fostering an

READY FOR PRIME TIME

Janelia Farm’s Gus Lott received a rock star’s welcome when he showed up at the Academy of Science (AOS)—on a Harley Davidson 2005 Sportster, no less—during the Singapore students’ visit. At first the group appeared mesmerized, listening reverently and nodding their heads as the tall, confident, and undeniably hip scientist—cowboy boots peeking from the hem of faded blue jeans—demonstrated his “g-PRIME” software.

When AOS student Devin Bowers admitted to finding the program “intimidating,” Lott, an engineer in Janelia’s instrument design and fabrication shop, gathered AOS’s Bowers and Aliya Jamil and Singapore’s Cedrych Beh and Tse Yean Teo at the whiteboard to show in layman’s terms just how g-PRIME, which turns a computer’s sound card into a tool that can acquire and analyze signals, does its thing. For someone who believes, as Lott does, that “the engineering is easy, while the relating of it to scientists—both established and budding—is difficult,” he made that look easy too. **“In the case of cricket chirps,** the software measures parameters such as chirp frequency, duration, amplitude, and time between chirps,” he said, jumping at once into colleague-to-colleague discourse with the students. In mere minutes, neither Lott nor his complex computer program seemed quite so intimidating. Bowers later described g-PRIME as a “godsend” in their analysis of cricket mating songs. By the time Lott headed back to Janelia Farm, the students were well-versed in g-PRIME, and their awestruck stares had turned into smiles of comprehension and gratitude.



Clockwise from lower right: Students Cedrych Beh, Devin Bowers, Aliya Jamil, and Tse Yean Teo learn about each others' cultures while analyzing cricket song; Teo and Beh are students at Singapore's Hwa Chong Institution; Gus Lott (right) created software the students are using to analyze the insect sounds; George Wolfe, director of Loudoun County's Academy of Science, set the collaborative project in motion.

appreciation of scientific inquiry for the sake of discovery. Tradition (and financial realities) in Singapore means that all science projects there, even in high school, must aim for practical applications; embracing a less rigid approach was an adjustment for the visitors.

"They both have pros and cons," Teo says of the schools' differing methods. "I'd prefer a combination of their relaxed environment and our task-oriented approach."

Between experimenting and drafting reports, the hosting and visiting students ate, toured, and lived together. Each boy from HCI, an all-male institution, stayed with an American family.

What did the Singaporean students find most surprising about America? For one thing, they got to sleep in. At HCI, students

often are expected to be at school before sunrise. With time to kill every morning, Beh and Teo usually could be seen in the mist-covered fields alongside Dominion High School collecting the season's last crickets.

Back in Singapore, the HCI students now no longer have free time in the morning, but they stay in touch with their Virginia collaborators—via e-mails that contain, in equal measure, details of their projects and typical teenage banter. In August, using funds from an annual HHMI grant to Loudoun County Public Schools, the American students are planning a trip to Singapore, so the six teams can present their final results together. ■

—LINDSAY MORAN

Strategizing to Diversify Science

EDUCATIONAL INSTITUTIONS SHARE EXPERIENCES, AND AGREE TO CONTINUE THE COLLABORATION, IN ATTRACTING UNDERREPRESENTED MINORITIES TO SCIENCE AND RETAINING THEM.

THE FUTURE LOOKS BRIGHT FOR BRIAN LEÓN. “I AM IN A POSITION where I cannot be disappointed about anything,” says the University of California, Irvine (UCI), senior. He has already been accepted into five graduate programs and is waiting to hear from three more.

After transferring from a local community college, León joined UCI’s Minority Science Programs (MSP), which provide a combination of study-skill courses, research experiences, and career advice to selected students from minority groups that are underrepresented in the biomedical sciences. “I never go two days without some form of contact [with program staff],” says León, whose family emigrated from Peru. Such steady interaction seems to pay off: in 2007, 15 seniors completed the MSP and 11 entered a Ph.D. program.

UCI’s programs were among the initiatives spotlighted in “Diversifying Science: From Concept to Practice”—a workshop held at HHMI headquarters January 27–29, 2008, and the last in a series. These four diversity symposia, sponsored by HHMI and the National Institutes of Health, brought together institutions committed to increasing the number of underrepresented minority students in the sciences.

All the symposia showcased model programs at colleges and universities with impressive records of graduating minority students in science fields, but the fourth symposium went further by discussing challenges that participants have faced in implementing their programs and the strategies they’ve used to overcome them.

For example, one accomplishment of the earlier symposia was that participating institutions began measuring minority-student attrition from science majors—in large part by tracking the number of minorities in each biology and chemistry class and their grades over a three-year period.

A preliminary analysis of these data indicated that, at least for university biology, how students do in their first year determines whether they will graduate with a science major. “If students have bad experiences in the first science courses, they typically leave science,” says Mount Holyoke College’s Craig Woodard, one of the organizers of the final symposium.

This result helped convince Pam Baker and other administrators at Bates College to start a bridge program that brings incoming freshmen to the Lewiston, Maine, campus for six weeks during the summer to acclimate them to campus life and to accelerate their academic experience by taking chemistry and math courses.

In her presentation at the last symposium, Baker explained that, of the 11 students who participated in the new program, 9 have continued in science courses in the second semester—a good indication that they will pick a science as their major. Because of this initial success, the college has committed to fund the program when the HHMI grant currently supporting it runs out.

Given the fact that the data identified a significant problem and inspired a seemingly effective solution, the symposium participants were enthusiastic about continuing to collect data. And if institutions use commonly agreed-upon measures, it will provide a way to compare them with their peers. “The data were difficult to collect because we had not done it before,” says Woodard, “but everyone agreed it is helpful and important.”

The symposium organizers—others include Robert Lue of Harvard University, Barbara Wakimoto of the University of Washington, and John Matsui of the University of California, Berkeley—are now gathering feedback from participants to determine how best to



Speakers at the fourth diversity symposium, sponsored by HHMI and the NIH, included John Matsui, University of California, Berkeley, and Pamela Baker, Bates College.

continue this multi-institutional collaboration. “One possibility is to hold smaller regional meetings and invite institutions that did not take part in the diversity symposia,” says Woodard. “Also, participants could in effect become consultants, making site visits and giving presentations about their programs.” The long-term goal would be to enlist many more colleges and universities in similar efforts, thereby triggering change at the national level.

For now, success lies in individual stories. “I will be the first to earn a doctorate degree in the family,” says León. “When I think of that I have a real sense of accomplishment, and I hope to inspire my brother to do the same.” ■ —LAURA BONETTA

In Memoriam

Richard Gordon Darman

1943–2008

Richard Gordon Darman, a financial executive with a distinguished career in public service and a Trustee of the Howard Hughes Medical Institute, died January 25, 2008, in Washington, D.C. He was 64.

Darman was a partner of The Carlyle Group, a global private equity firm, and chairman of the board of AES Corp., an international power company. He became an HHMI Trustee in 2005, served as chairman of the Institute's Audit and Compensation Committee, and was also a member of its Finance Committee.

Throughout his tenure in the federal government, Darman played key roles in the development of tax, spending, and economic policies. He served four presidents, holding positions in the White House, the Office of Management and Budget (OMB), and six cabinet departments.

As deputy treasury secretary during the Reagan Administration, Darman was recognized with the Treasury's highest

award, the Alexander Hamilton medal, for his contributions to the 1986 Tax Reform Act and two international monetary policy accords. Darman subsequently served as director of OMB in the administration of President George H.W. Bush from 1989 to 1993 and was the principal executive branch negotiator for the 1990 budget agreement.

Darman joined The Carlyle Group in 1993. He became a member of the AES board in 2002 and was elected chairman in 2003. Darman was also a trustee of several publicly traded mutual fund groups and was the current chairman of the board of the Smithsonian National Museum of American History.

Darman graduated with honors from Harvard College in 1964 and from the Harvard Business School in 1967. A former fellow of the Woodrow Wilson International Center for Scholars, he wrote widely about public policy and politics. He is survived by his wife, Kathleen Emmet, and three sons, William T.E. Darman, Jonathan W.E. Darman, and C.T. Emmet Darman.

HHMI Offers Boost to Early Career Scientists

MANY EARLY CAREER SCIENTISTS LAUNCH their own labs with start-up funds from their host institution. That support is provided with the expectation that the scientist will establish his or her own research program with independent funding. In the current funding climate, that transition has become a daunting hurdle. Now, a \$300-million HHMI initiative aims to eliminate that stumbling block for as many as 70 of the nation's best early career faculty, chosen through a national competition.

The new program is directed at researchers who have run their own labs for two to six years and who may be ready to move their research in creative, new directions. The scientists will come from any of approximately 200 eligible U.S. medical schools, universities, and research institutions.

The six-year, nonrenewable appointments to HHMI will allow the scientists,

most of whom will be assistant professors at the time of the award, to receive full salary and research support from HHMI. In selecting the early career scientists, HHMI will be guided by the principle of people, not projects—providing the early career scientists with the freedom to pursue their scientific interests wherever they lead.

This initiative comes at a critical time. Funding for the National Institutes of Health (NIH), the nation's largest supporter of basic biomedical research, has remained essentially flat during the last five years, unable to keep up with inflation. Nowhere has the impact of this constrained funding been felt more intensely than by early career scientists who are competing with their peers and more experienced researchers to win research project (R01) grants from NIH.

HHMI President Thomas R. Cech and his advisors saw this as a clear opportunity.

“Many of these scientists who have led their own laboratories for a few years are at a high point of their creativity just as they see their start-up funds and other early career awards ending,” says Cech. “Some of them may still be in line for their first NIH R01 grant, while others may have their first grant but are facing the very challenging first renewal of that grant. It is this period of career vulnerability that the HHMI Early Career Scientist Program aims to bridge.”

HHMI is seeking scientists from all areas of basic biology and biomedicine as well as areas of chemistry, physics, computer science, and engineering that are directly related to biology or medicine. Candidates can apply directly to HHMI, a new approach the Institute used successfully in 2006 and 2007 competitions, broadening its pool of applicants by moving away from accepting nominations only from applicants' host institutions.

Detailed information about the competition, including the list of eligible institutions, may be found on the HHMI Website (www.hhmi.org/earlycareer2009/). ■

Of Fish and Men

THE SAME GENE DRIVES SKIN COLOR EVOLUTION IN STICKLEBACK FISH AND HUMANS.

At the end of the Ice Age, 10,000 years ago, marine stickleback fish colonized the newly formed freshwater lakes and streams that dotted North America, Europe, and Asia. In each new, isolated habitat, the fish evolved traits that would help them thrive.

Among these changes were darkening and lightening of skin color, helping fish blend in or stand out in different water colors. By comparing modern-day stickleback from around the globe, HHMI investigator David Kingsley of Stanford University has revealed a gene responsible for those color changes. What's more, he discovered that the same gene has likely played a role in changing skin color during human evolution.

Taking advantage of genetic crosses and the recently sequenced stickleback genome, Kingsley and colleagues first identified a region of a chromosome, encompassing 12 genes, that seemed to differ distinctly in fish of varying shades. From there, they narrowed the color control down to one gene, *Kitlg*, which is involved in a number of developmental processes—including the development of pigment cells.

The researchers found that lighter-colored fish had a mutation in the regulatory part of the gene, which decreased the gene's expression in gills and skin cells. Since skin color can be slightly affected by many genes, it was surprising that one single gene could have such a large effect.

"If we look at multiple [stickleback populations] along the West Coast where light skin color had evolved, the same mechanism was used over and over," says Kingsley. Such a striking pattern suggested to him that perhaps the gene was involved in

skin color evolution of other species, including humans.

Indeed, when the scientists compared the *Kitlg* gene sequence of Africans and Europeans, they found regulatory differences in *Kitlg* that contribute to skin color variety. They reported the work in the December 14, 2007, issue of *Cell*.

"It may be that the general mechanisms producing major changes during adaptation to a new environment are pretty constrained," says Kingsley. "Mechanisms you find when studying how one organism has evolved may help predict mechanisms used in very different animals." ■ —SARAH C.P. WILLIAMS



A stickleback from the ocean (upper) and one from a freshwater creek (lower) show different skin colors and body types.

IN BRIEF

MALARIA PARASITES PUNCH THROUGH SKIN CELLS

Detailed observations of malaria parasites moving through mice have revealed that the parasites power straight through cells on their journey from the skin to the liver. While scientists had previously observed the parasites jabbing through liver cells, the new work led by HHMI international research scholar Robert Ménard is the first to show that this so-called cell traversal begins at the skin.

Ménard, of the Pasteur Institute in Paris, and his colleagues took advantage of a mutant form of *Plasmodium berghei*—the malaria parasite that infects mice—which cannot make holes in cells. They engineered these mutated parasites to carry a fluorescent molecule they could follow in the mice.

The parasites, which were expected to travel through the body, got immediately stuck in skin cells, the researchers reported February 14, 2008, in *Cell Host & Microbe*. When they were injected straight into the liver, the parasites had no problem growing, dividing, and making the mice sick—obliterating the theory that the ability to dive through liver cells is vital to the parasites' life cycles, and instead suggesting that the long-overlooked skin phase of the malaria parasite deserves closer scrutiny.

Ducking in and out of cells likely helps the parasite avoid immune cells, says Ménard, who next hopes to deduce exactly how the parasites are able to punch through the cells. "It's a pretty aggressive behavior," he says, "and we have no idea yet how they do it."

TRACING THE PATH TO A MELANOMA

Scientists trying to determine what makes a benign mole different from a deadly skin cancer have found a protein that halts the growth of tumors.

Moles and cancerous melanomas both start out as melanocytes—pigment-producing skin cells. When a mutation in melanocytes causes an increase in expression of a protein called BRAF, the cells proliferate faster. This abnormal growth sends cells down one of two routes—it either shuts the cells down and forms a mole, or triggers an unstoppable cancer.

Researchers led by HHMI investigator Michael Green, of the University of Massachusetts Medical School, searched the genome for proteins needed to send cells down the more tame pathway. Their search led them to insulin-like growth factor binding protein 7 (IGFBP7), a protein that melanocytes make when BRAF expression increases. The IGFBP7, it turns out, leaves

the cell where it's made and signals neighboring cells to enter hibernation, preventing tumor formation.

When the researchers injected IGFBP7 into mice that already had tumors, the protein stopped tumor growth. The tumor cells, Green found, had turned off production of IGFBP7. Moles, on the other hand, actively expressed the protein, keeping surrounding cells from becoming cancerous. "It's an extremely powerful anti-cancer mechanism," says Green, who hopes the findings, published in *Cell* on February 8, 2008, will lead to a new melanoma treatment.

SYNDROME CAUSED BY MISSING CHROMOSOME SEGMENT

A chunk of missing DNA explains some cases of mental retardation that have never before been diagnosable, a team of scientists, led by HHMI investigator Evan E. Eichler of the University of Washington School of Medicine, has discovered.

While screening 700 people with mental retardation and seizure disorders, the researchers noticed two unrelated patients with identical large missing areas on chromosome 15. When the researchers looked at a larger group of patients—more than 2,000—nine people turned up with the same missing genetic material. Tests of

Fixing Fragile X

KNOCKING OUT A SINGLE GENE IN NEURONS ELIMINATES SYMPTOMS OF FRAGILE X SYNDROME IN MICE.

By probing the network of genes and proteins active at the junctions between neurons in the brain, HHMI researchers have unearthed a new strategy for treating fragile X syndrome—the most common inherited form of mental retardation.

Mark Bear, an HHMI investigator at the Massachusetts Institute of Technology, studies how the connections between neurons are strengthened or weakened as new pieces of information are retained



The connections between neurons in the brain hold a surprising clue to fragile X syndrome.

in the brain, and other memories fade. He previously found that a neurotransmitter receptor called mGluR5 plays a role in weakening neural connections, by increasing the amount of proteins made at the synapses between neurons.

Most recently, Bear discovered that fragile X mental retardation protein (FMRP), which is mutated in fragile X syndrome, counterbalances mGluR5. “The fragile X protein is normally putting a brake on the protein synthesis stimulated by mGluR5,”

explains Bear. This means that in fragile X syndrome, FMRP is not around so the protein synthesis goes unchecked.

Bear and his colleagues, in a study published in the December 20, 2007, issue of *Neuron*, showed that getting rid of one copy of mGluR5 in fragile X-affected mice eliminates the seizures and memory impairments that such mice typically exhibit.

The researchers bred mice with fragile X syndrome—characterized by developmental delay, structural changes in the brain, and epilepsy—with mice engineered to produce half the normal level of mGluR5. Their offspring showed few symptoms of fragile X, despite the mutation in FMRP they had inherited.

Since mGluR5 and FMRP do not directly interact, but influence neural protein synthesis in opposite directions, the results suggest that it is the increased protein synthesis in fragile X patients that leads to the syndrome. The brains of fragile X-affected mice typically have an excess of particularly weak neural connections. Bear hypothesizes that the excess protein synthesis could be leading to this high density of connections.

“Now we have a lot of work ahead of us to figure out which proteins are producing the pathology,” says Bear, who is also now studying whether blocking mGluR5 receptors in humans can counter fragile X syndrome. ■ —SARAH C.P. WILLIAMS

IN BRIEF

people without mental retardation found no instances of the deletion. The results appeared online in *Nature Genetics* on February 17, 2008.

In most of the affected cases, the large segments of DNA were deleted spontaneously, not inherited. “These kinds of events are happening all the time when sperm and egg are being generated,” says Eichler. “We think everyone has some areas of the genome that are duplicated or deleted but it doesn’t always cause disease.”

The missing chromosome segment spans six genes, and the scientists hope to pinpoint which of those deleted genes is linked to the epilepsy commonly seen in the patients.

While this missing segment explains only 3 in 1,000 cases of mental retardation, Eichler says other spontaneous deletions and duplications of large chunks of chromosomes could explain more.

“Collectively all of these rare sites probably account for 10 percent of mental retardations,” he says.

NUCLEAR NEIGHBORHOODS CONTROL GENE EXPRESSION

By forcing bits of genetic material to the edge of the nucleus, scientists have shown that placement of genes within the nucleus

can affect whether the gene is expressed as a protein.

HHMI investigator Harinder Singh, University of Chicago, was studying how B and T cells of the immune system assemble their receptor genes using the same machinery but never mix up their parts—the proteins that stud the surface of B cells are never expressed in T cells, for example. He and his colleagues noticed that in T cells, the genes encoding B cell receptor proteins were positioned at the edge of the nucleus.

To study the significance of this placement, the researchers designed a molecular tool to tether genes at the nuclear edge. They found that genes bound there were not expressed. Moreover, in research that appeared February 13, 2008, online in *Nature*, they identified membrane proteins that could connect unneeded genes to the nuclear envelope.

Singh says their results suggest that location in the nucleus could be as important as location in the rest of the cell—where compartments process and sort proteins in a specific order.

“We should be thinking of genes in the nucleus as having to be moved and sorted too,” he says. “There’s a degree of organization in the nucleus that we haven’t paid much attention to.”

STRUCTURE OF SPASTIN ENZYME REVEALS HOW IT SLURPS UP MICROTUBULES

HHMI researchers have pieced together the structure of spastin, a protein that remodels networks of microtubules, which transport molecules inside cells. When spastin malfunctions, as it does in hereditary spastic paraplegias, people experience progressive weakness and stiffness. Understanding spastin’s control of microtubules could lead to treatments for these disorders.

Ronald D. Vale of the University of California, San Francisco, has now confirmed the suspicion that spastin is a ring composed of six identical subunits. The details of spastin’s structure appeared in *Nature* on January 17, 2008. By complementing the x-ray techniques used to determine this structure with further tests, Vale and his colleagues shed light on how the protein works.

“Spastin appears to grab a loose tail region of the microtubule and mechanically ratchet it through the pore,” says Vale, who described it as “a kind of noodle-slurping mechanism.”

This breaking apart of microtubules is probably necessary to the constant remodeling inside cells, says Vale. When this

A New Clarity

APPLYING A NEW MICROSCOPY TECHNIQUE TO DETECT INDIVIDUAL MOLECULES IN THREE DIMENSIONS.

The intricate molecular insides of cells are coming into focus, thanks to HHMI investigator Xiaowei Zhuang at Harvard University. Zhuang has developed a three-dimensional version of her high-resolution stochastic optical reconstruction microscopy (STORM) technique, allowing scientists to find the location of cellular molecules with better resolution than conventional light microscopy.

To get a glimpse of cells' inner workings, scientists typically tag molecules with proteins or dyes that give off fluorescence. But images of this fluorescence have a resolution that's limited to a few hundred nanometers by the diffraction of the light in all directions.

With this conventional method, "if you have a very interesting structure but it's smaller than the resolution, it just looks like a

featureless dot," says Zhuang. Multiple fluorescent molecules blur together. So she and her colleagues came up with a trick.

Zhuang's STORM technique, first described in 2006, had been used only for two-dimensional imaging until now. STORM involves tagging molecules with a fluorescent label, or fluorophore, that can be switched on and off.

Her team avoids the problem of overlapping fluorescence by using low amounts of light to switch on only a small percentage of fluorophores at once. Through the microscope, researchers can pinpoint a molecule's location by calculating where the center of each dot is.

Now, Zhuang has also developed a way to determine where the molecule is in the third dimension—by analyzing the size and shape, or blurriness, of each dot. Repeating the process many times, randomly turning on fluorophores during each iteration, can reveal the precise location of all the tagged molecules in a cell.

"These cellular images are now 10 times sharper in all three dimensions," says Zhuang, who described the technique in the February 8, 2008, issue of *Science* and has used it to look at the proteins that help viruses enter cells—a process involving miniscule complexes of molecules that had never before been resolved by light microscopy.

"We can solve many problems that were previously beyond our reach, but there are still things that we can't reveal," she says. "And the closer the resolution gets to true molecular scale, the more questions arise." ■ —SARAH C.P. WILLIAMS



Microtubules visualized with a new microscopy technique (right) appear sharper than when viewed using conventional microscopy (left).

IN BRIEF

process is hindered by mutations in spastin, the microtubules in neurons—where spastin may be especially active—can't rearrange themselves.

DECODING THE MOLECULAR SIGNATURE OF PROSTATE CANCER

Prostate cancers display a molecular signpost that alerts the body to their presence, HHMI researchers have discovered. Understanding how this flag signals the immune system to respond to tumor cells may help researchers develop new ways to fight cancer.

Scientists led by HHMI investigator James P. Allison of Memorial Sloan-Kettering Cancer Center chopped up tumors and exposed the tissue to a mixture of immune T cells, all carrying different random receptors. T cells carrying one receptor in particular replicated in response to the tissue, indicating that this receptor recognized a marker on the tumor cell surface.

To the researchers' surprise, however, the T cells carrying the tumor receptor were also activated by other types of chopped-up tissue, suggesting that the

marker was not specific to cancer cells. Yet in living mice, T cells carrying the receptor of interest attacked just prostate tumors.

To clear up this discrepancy, the scientists searched for the molecule that the T cells recognize. They pinpointed histone H4, a protein found ubiquitously inside the nuclei of cells—explaining why all ground-up tissues activate the T cells. In prostate tumor cells, they determined, histone H4 leaves the nucleus and is displayed on the surface of the cells.

The team next hopes to learn exactly how histone H4 goes from being inside cell nuclei to being a flag for the immune system, as well as whether the protein has value in predicting prostate cancer.

DNA EXPANSION LEADS TO NEUROLOGICAL DISORDER

The inherited neurodegenerative disease spinocerebellar ataxia type 1 (SCA1) can be explained by a molecular tug-of-war, researchers have discovered. It was known that an expansion of a repetitive region of DNA in the gene ataxin 1 (ATXN1) led to SCA1, characterized by neural degeneration

that begins at around age 30. Now, HHMI investigator Huda Zoghbi of Baylor College of Medicine has shown why this expansion is so toxic to cells.

Zoghbi and her colleagues wanted to explore a recent observation that a separate, single mutation in ATXN1 can cancel out the disease-causing effects of the expansion. So they screened proteins to find ones that interacted preferentially with one form of ATXN1 or the other.

The researchers found two proteins—RBM17 and CIC. The disease-causing version of ATXN1 prefers to bind to RBM17, leaving CIC with less ATXN1 to bind to. RBM17 and CIC are constantly struggling to spend time with ATXN1, says Zoghbi. The inherited expansion shifts this balance in RBM17's favor, and that is what causes disease, the researchers conclude in a report published online in *Nature* on March 12, 2008.

Next, Zoghbi and her collaborators hope to deduce the functions of both RBM17 and CIC, to determine why ATXN1's increased interactions with RBM17 cause neural degeneration.

Q

**Do only the
“long bones”
of the arms and
legs contain
growth plates?
And if so, how
do all of the
body’s bones
stop growing at
maturity?**

Bret, a curious adult from Illinois

A

Most of the bones with growth plates are indeed the long bones—those that make up your arms, legs, fingers, and toes. If you think about it, this makes sense: most of an individual’s growth, especially during the spurt that occurs in adolescence, involves the lengthening of his or her limbs.

Growth plates, found at both ends of the long bones, consist of cartilage, which is made by special cells called chondrocytes that inhabit the growth plate zone. During adolescence, chondrocytes make cartilage at a very fast pace in growth plate zones at both ends of the long bones, adding length and creating a scaffold upon which specialized cells called osteoblasts can form bone. The process, which results in a very rapid lengthwise growth of the bones in your limbs, is jump-started during adolescence by a growth factor in the body called insulin-like growth factor (IGF).

However, this process doesn’t last forever. The osteoblasts replace cartilage with bone faster than new cartilage is created at the far end of the growth plate. As this happens, the growth plates gradually thin, until eventually they disappear. At the same time, IGF concentrations in the body

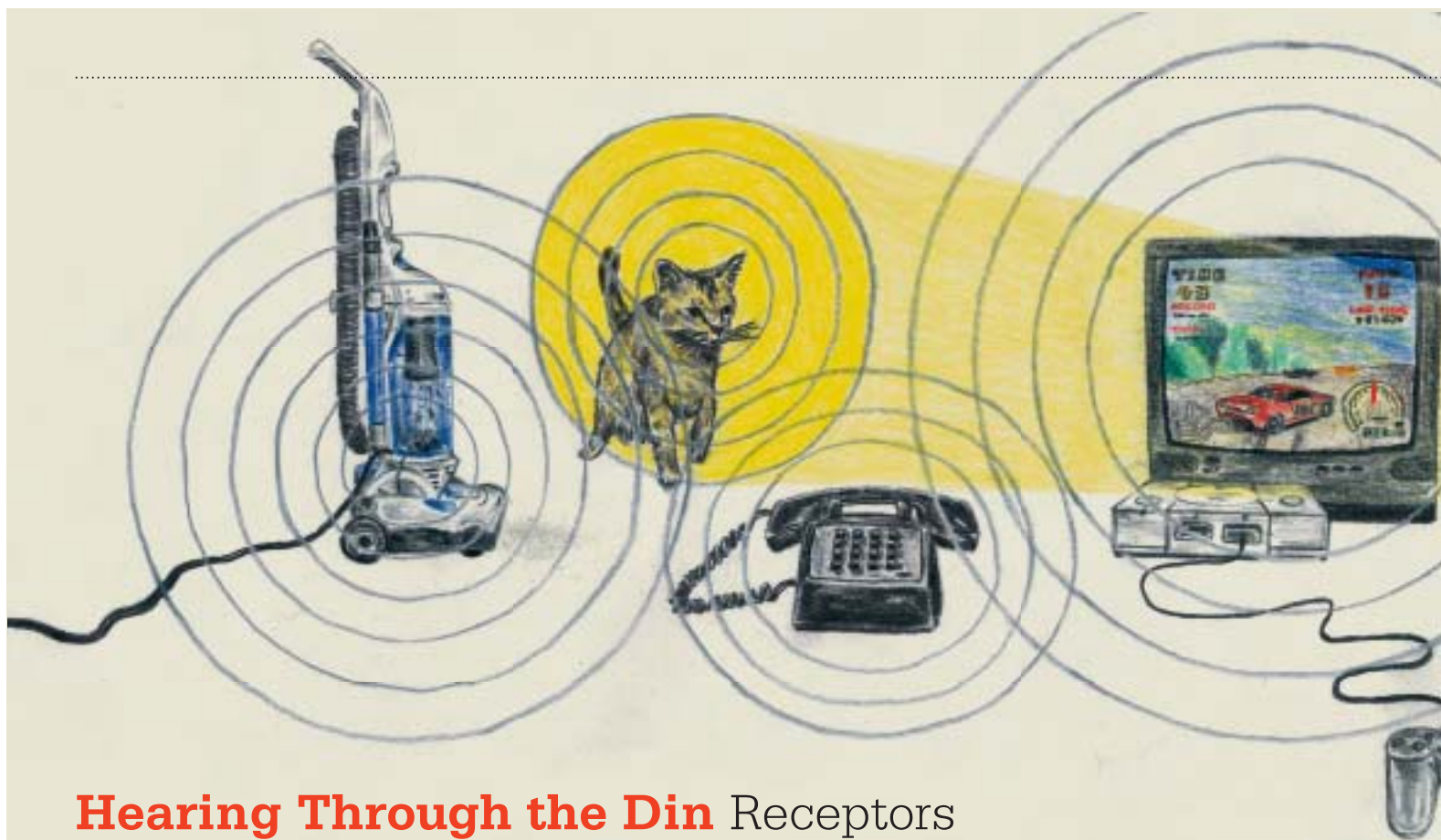
also decrease (but not to zero). As that happens, this type of lengthwise growth in the long bones stops and you are as tall as you will ever be.

Does this mean the long bones are done growing or that other bones never grow? Absolutely not. All bones grow constantly, even *after* maturity, but they are constantly being reabsorbed as well. As cells called osteoclasts destroy the older bone tissue, the osteoblasts make new bone to replace it. During childhood and adolescence, signals are being released throughout your body that tip this balance toward the positive, so that all your bones generally do grow. In healthy adults, who have all the bone they need, the rates of destruction and of creation of bone net out to zero. The bones grow only as fast as they are destroyed and so stay the same length and thickness. Amazingly, 30 percent of bone in any human is generally destroyed and re-created every year.

ANSWER RESEARCHED BY STEPHEN PAN, M.D., former HHMI-NIH research scholar, now a resident in internal medicine at Stanford University Hospital & Clinics, Stanford, California.

FOR MORE INFORMATION on bone growth, visit <http://www.endotext.org/parathyroid/parathyroid1/parathyroidframe1.htm> (Baron, R. “Anatomy and Ultrastructure of Bone – Histogenesis, Growth, and Remodeling,” 2006).

Science is all about asking questions, exploring the problems that confound or intrigue us. But answers can’t always be found in a classroom or textbook. At HHMI’s *Ask a Scientist* Website, working scientists tackle your tough questions about human biology, diseases, evolution, animals, and genetics. Visit www.hhmi.org/askascientist to browse an archive of questions and answers, find helpful Web links, or toss your question into the mix. What’s been puzzling you lately?



Hearing Through the Din Receptors embedded in the ear's hair cells might explain mammals' selective hearing.

A CHICKEN WAITING TO CROSS A NOISY ROAD CAN'T BE blamed if it doesn't hear the fox sneaking up behind it. Chickens—and a host of other vertebrates—don't have as sophisticated hearing systems as mammals.

One protein makes the difference, says HHMI international research scholar A. Belén Elgoyhen. It's a component of a receptor in a part of the auditory system of mammals, called the efferent pathway, that modulates whether sounds reach the brain or are filtered out.

Basic sound detection is a one-way process that begins when sound waves strike the eardrum and jiggle hair cells in the cochlea of the inner ear. That jiggling generates neural signals that speed to the brain's auditory center, where they register as sound.

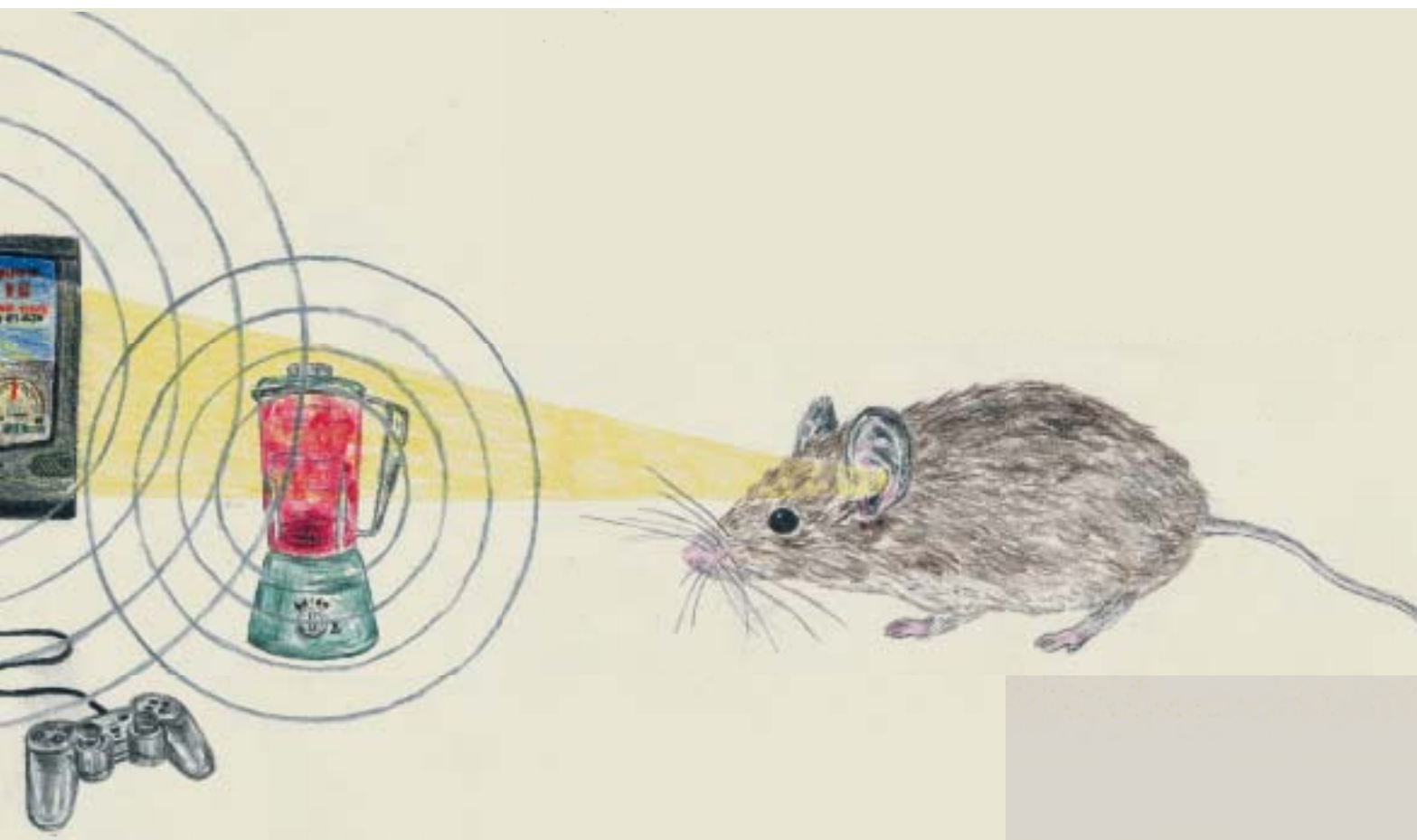
But really sophisticated sound processing in mammals also depends on efferent signals that travel in reverse, from the brain back to the cochlea. These signals control specialized, adjustable outer hair cells that can modulate sound signals traveling to the brain. The exact function of this feedback system largely remains a mystery. But researchers believe it enables mammals, including humans, to filter background noise so they can

concentrate on relevant sounds such as hearing a knock at the door over the sound of music. The efferent system also may dampen loud sounds to protect ears from injury.

The efferent system's neurons control outer hair cells by launching bursts of the neurotransmitter acetylcholine at receptors embedded in the surface of these hair cells. These receptors latch onto the acetylcholine, which triggers a gush of calcium into the outer hair cell, inhibiting the cells' ability to modulate auditory output and dampen sound signals. So by adjusting the amount of acetylcholine it pumps out, the efferent system can change how the outer hair cells function, and how much they dampen sound.

In earlier work, Elgoyhen discovered that these "nicotinic acetylcholine receptors" are built of two basic protein components, the alpha-9 subunit and the alpha-10 subunit, which differ subtly in structure.

"We know these receptor subunits exist in all vertebrates; however, only the mammalian cochlea has this fine-tuning of outer hair cells," says Elgoyhen, who is at the Institute for Research on Genetic Engineering and Molecular Biology, CONICET, in Buenos Aires, Argentina. "We believe that



mammals and nonmammals may have slightly different alpha-10 subunits that enables this tuning in mammals.”

In their latest study, Elgoyhen and her colleagues tackled the key question of whether the alpha-10 subunit is really critical to the receptor’s function. To their puzzlement, earlier test-tube studies had revealed the opposite—that even without the alpha-10 subunit, the receptor appeared to function normally.

So the researchers explored what would happen when they created “knockout” mice that had no alpha-10 subunit. They reported their findings in the December 18, 2007, *Proceedings of the National Academy of Sciences*.

Their first measurements of the physiological functioning of the basic machinery of the animals’ auditory system indicated that it functioned perfectly well. Then they conducted more subtle physiological tests that specifically measured whether the animals’ efferent auditory systems were working properly. To determine how the ears of the mice responded to different sound frequencies, the researchers inserted electrodes into the auditory nerves of the mice and placed delicate microphones next to the outer hair cells. Those tests revealed that, although the animals’ basic hearing was intact, the efferent systems in the

mice lacking the alpha-10 subunit were not fully functional at the electrophysiological level. The outer hair cells in those mice did not respond normally to bursts of acetylcholine from efferent neurons.

Establishing the physiological role of the alpha-10 subunit represents only the beginning of their explorations into its function, says Elgoyhen. Her lab has already started cloning the chicken versions of the alpha-10 and alpha-9 subunits.

“We are comparing the properties of the chicken receptor to the mammalian receptor,” she says, “to see if there is some functional difference between them that can tell us why this alpha-10 subunit uniquely evolved a special role in mammals compared with nonmammalian vertebrates.” What’s more, Elgoyhen and her colleagues plan to explore the more subtle hearing consequences of loss of the alpha-10 subunit and thus a fully functional efferent system.

“So far, we only know that without the subunit, the efferent system does not work,” she says. “Now, we are investigating the consequences at the level of behavior—whether the knockout mice show a difference in protection from sound injury or in attentional behavior.” ■ —DENNIS MEREDITH

SPOTLIGHT

Lifton Wins Wiley Prize



RICHARD LIFTON

HHMI investigator **Richard P. Lifton** of Yale University School of Medicine received the 2008 Wiley Prize in Biomedical Sciences. Awarded by the Wiley Foundation, the annual prize recognizes a scientist whose contributions have opened new fields of research or advanced new concepts within biomedicine. Lifton was chosen for his discovery of the genes that cause many forms of high and low blood pressure in humans. The genes, involved in regulation of salt balance by the kidney, highlight the link between dietary salt and hypertension.

HHMI investigator **ANGELIKA AMON**, Massachusetts Institute of Technology, received the 2008 National Academy of Sciences Award in Molecular Biology, awarded annually for a recent notable discovery in molecular biology by a young scientist. Amon was recognized for her groundbreaking studies on the mechanism of chromosome segregation.

KELLI CARROLL, a 2006 HHMI summer research fellow at Davidson College, was awarded a Barry M. Goldwater Scholarship. Carroll, now a junior at Davidson, will work as a 2008 summer research intern in Rex Kerr's lab at Janelia Farm Research Campus. Kerr is developing new techniques for monitoring the neural activity of the nematode worm *Caenorhabditis elegans*.

SEAN B. CARROLL, an HHMI investigator at the University of Wisconsin-Madison,

received the Distinguished Service Award from the National Association of Biology Teachers. Presented annually, the award honors a nationally recognized scientist who has made major contributions to biology education through research, writing, and teaching.

HHMI investigator **ARUL M. CHINNAIYAN**, University of Michigan Medical School, received the 2008 Award for Outstanding Achievement in Cancer Research from the American Association for Cancer Research. Chinnaiyan's research aims to pinpoint chromosomal abnormalities responsible for tumors.

HHMI international research scholar **PASCALE F. COSSART**, Pasteur Institute of Paris, received the 2008 Louis-Jeantet Prize for Medicine from the Louis-Jeantet Foundation. The prize, awarded annually to a European researcher, recognizes Cossart's

groundbreaking work on the bacterium that causes listeriosis and her coordination of a European consortium that decrypted the bacterium's genome.

DIEGO DE MENDOZA, an HHMI international research scholar at the Institute of Molecular and Cellular Biology of Rosario, received a Bernardo Houssay prize from Argentinean President Nestor Kirchner. The Argentine Secretariat awards the prize for Science, Technology, and Innovation annually for outstanding work in one of six scientific fields.

HHMI investigator **DAVID EISENBERG**, University of California, Los Angeles, received the 2008 Emily Gray Award from the Biophysical Society for significant contributions to education in biophysics.

ELLEN H. FANNING, an HHMI professor at Vanderbilt University, was elected to the German National Academy of Science (Deutsche Akademie der Naturforscher Leopoldina). Fanning studies how DNA tumor viruses take advantage of cells to replicate.

HHMI investigator **RICHARD A. FLAVELL**, Yale University School of Medicine, received the 2008 Invitrogen Meritorious Career Award from the American Association of Immunologists. The award recognizes his numerous contributions to the field of immunology. Flavell also received the Hebrew University-Hadassah Medical School's 2008 Rabbi Shai Shacknai Memorial Prize in Immunology and Cancer Research for his research on autoimmune diseases.

TODD R. GOLUB, an HHMI investigator at the Dana-Farber Cancer Institute, won the 2008 E. Mead Johnson Award. Given by the Society for Pediatric Research, the award recognizes clinical and laboratory research achievements in pediatrics. Golub studies how to use genomic methods to improve the understanding, diagnosis, and treatment of diseases, including childhood cancers.

HHMI investigator **JUDITH KIMBLE**, University of Wisconsin–Madison, was elected to serve a three-year term on the Council of the National Academy of Sciences.

HHMI investigator **SUSAN L. LINDQUIST**, Massachusetts Institute of Technology, received the 2008 Otto Warburg Prize from the German Society for Biochemistry and Molecular Biology. She also received the Protein Society's 2008 Stein and Moore Award, which recognizes her groundbreaking discoveries in understanding the wide array of biological processes governed by protein folding, and the 2008 Genetics Society of America Medal.

HHMI investigator **SEAN J. MORRISON** of the University of Michigan Medical School received the 2008 Harland Winfield Mossman Award from the American Association of Anatomists. The award, presented annually to a young investigator who has made important contributions in the field of developmental biology, recognizes Morrison's research on stem cell biology.

HHMI international research scholar **LÁSZLÓ NAGY**, University of Debrecen, Hungary, received the 2008 European Society for Clinical Investigation Award for Excellence in Biomedical Investigation. Nagy studies nuclear hormone receptors and their roles in infectious and chronic inflammatory diseases.

HHMI professors **REBECCA RICHARDS-KORTUM**, Rice University, and **DAVID WALT**, Tufts University, were elected to the National Academy of Engineering.

RENEE RIVAS and **ALEXANDER HERTEL-FERNANDEZ**, former HHMI research interns at the University of Alabama, and **HENRY SWOFFORD**, an HHMI-supported undergraduate biotechnology scholar at Georgia State University, were named to the 2008 USA Today All-USA College Academic Team.

SPOTLIGHT

Hynes Awarded Wilson Medal



RICHARD HYNES

The 2007 E.B. Wilson Medal went to HHMI investigator **Richard O. Hynes** of the Massachusetts Institute of Technology. The medal, the highest science honor of the American Society for Cell Biology, is awarded for lifetime contributions to cell biology. Hynes studies the molecules that govern how cells adhere to one another and how they reach their proper locations in the body.

Hynes, a native of Great Britain, was also recently appointed as one of ten governors of the Wellcome Trust, the United Kingdom's largest non-governmental source of funds for biomedical research.

HHMI investigator **JOHN D. SCOTT**, Oregon Health & Science University, was elected a fellow of the Royal Society Edinburgh, the national science academy of Scotland.

HHMI professor **DAVID R. WALT**, a chemist at Tufts University, was elected a fellow of the American Institute for Medical and Biological Engineering for his development of optical sensors and arrays used in biochemistry.

ISIAH M. WARNER, an HHMI professor at Louisiana State University, received the 2008 American Chemical Society Division of Analytical Chemistry Award in Spectrochemical Analysis.

WILLIAM N. ZAGOTTA, an HHMI investigator at the University of Washington School of Medicine, received the 2008 K.S. Cole

Award from the Biophysical Society. Zagotta studies the molecular workings of ion channels—pore-forming proteins that control voltage gradients across cell membranes.

HHMI professor **RICHARD N. ZARE**, Stanford University, was elected a fellow of the Association for Women in Science, making him one of only a few men ever honored by the association for achievement in promoting women in science, through mentoring, leadership, and advocacy.

HHMI investigator **XIAOWEI ZHUANG**, Harvard University, received the 2008 Coblentz award from the Coblentz Society. The award recognizes contributions that have had an impact on the field of vibrational spectroscopy.

CONTINUED FROM PAGE 31
(GENETIC BALANCING ACT)

As her team revealed this March in the *Proceedings of the National Academy of Sciences*, two of the stem cell lines carried out X inactivation just fine. But in six lines, after one X was inactivated the cells stopped producing *Xist* RNA. Although the team found no evidence that an entire X chromosome reawakened in these cell lines, it's possible that some—perhaps many—genes on the X could fire up again. The scientists have already found evidence that this happens in mouse stem cells.

Reactivation might just kill the cells, but it could spell trouble for another reason. Some tumor cells carry an extra X chromosome, so it's not unreasonable to wonder whether a partially reactivated X might prompt similar abnormal growth. "It's extremely disconcerting," says Lee. "There's nothing we can do to restore X inactivation once reactivation occurs." The findings, she says, indicate that researchers need to do more experiments to determine

whether stem cells induce tumors if they are transplanted into patients.

Other stem cell experts praise this work. Although researchers have previously pinpointed X inactivation mishaps in stem cells, "this is the most thorough study" to date, says Renee Reijo Pera, director of the Center for Human Embryonic Stem Cell Research at Stanford University. "It definitely raises a red flag," though we need more information about X inactivation in the early embryo to judge how serious the problem is, she says.

Expect the Unexpected

What intrigues Meyer these days is the connection between dosage compensation and other cellular events that involve large-scale alterations to chromosomes. One example is crossing over, which occurs during meiosis, the type of cell division that leads to sperm and eggs. During crossing over, chromosomes pair up and swap DNA. The exchange is important from an evolutionary standpoint because it boosts the genetic diver-

sity of offspring. But it's also important to get the chromosomes in position for meiosis.

Meyer and colleagues revealed this January that a protein that's part of the all-important dosage compensation complex has another job—helping govern the number of times crossing over happens. According to Meyer, this link is "completely unexpected" and suggests that crossing over and dosage compensation in worms use a similar molecular mechanism to make big changes to the chromosomes.

As they've investigated the details of dosage compensation, Lee, Meyer, and other researchers have wandered into strange territory. They've come across molecular battles, take-charge RNA molecules, and furtive liaisons between chromosomes. And that's just the beginning. Plenty of unknowns remain. Mammal cells, for example, count their X chromosomes and randomly pick one for inactivation. Nobody knows how they manage either task. Whatever the answers turn out to be, Lee and Meyer say they're expecting more surprises. ■

CONTINUED FROM PAGE 37
(SOURCES OF RENEWAL)

before making a broader point. "It's worthwhile to bear in mind," he says, "that we would not have iPS cells except for the ability to study embryonic stem cells. The same people who are now crowing that we don't need embryonic stem cell research tend to forget that we would never have gotten to this point without it."

Advances are coming so quickly that it's difficult to get top scientists to speculate about where the field will be a year from now. Orkin expects that Hochedlinger's

work comparing both types of cells will raise a "cautionary note" for researchers. And Orkin hopes his research will provide the tools needed to create iPS cells that more closely mimic ES cell lines.

Daley is making more iPS cells, creating lines of cells with various blood diseases. In the near term, he hopes that, by transferring diseases from patients into Petri dishes, he'll be able to learn more about disease progression and possibly identify therapies, as he can conduct experiments in cell cultures that he wouldn't do with patients. Looking further

ahead, he remains committed to the possibility of doing for people what he's already done for mice.

"We think that these disease-specific lines will ... help lay the foundation for using genetically repaired cells to replace disease tissues," he says.

Of course, before he can do that, scientists will have to learn to reprogram cells without using viral vectors, a challenge that everyone seems to be pursuing but that no one wants to discuss in any detail. Daley will only say, "That's the hottest area of research in the lab right now." ■



Mixed Sources
Cert no. SW-COC-1855
© 1996 FSC



This paper is certified by SmartWood for FSC standards, which promote environmentally appropriate, socially beneficial, and economically viable management of the world's forests.