FOCUS

News from Harvard Medical, Dental and Public Health Schools

July/August 2011 ▶ focushms.com

ADVOCACY HONORED: Neir Eshel and Michael Honigburg, page 4

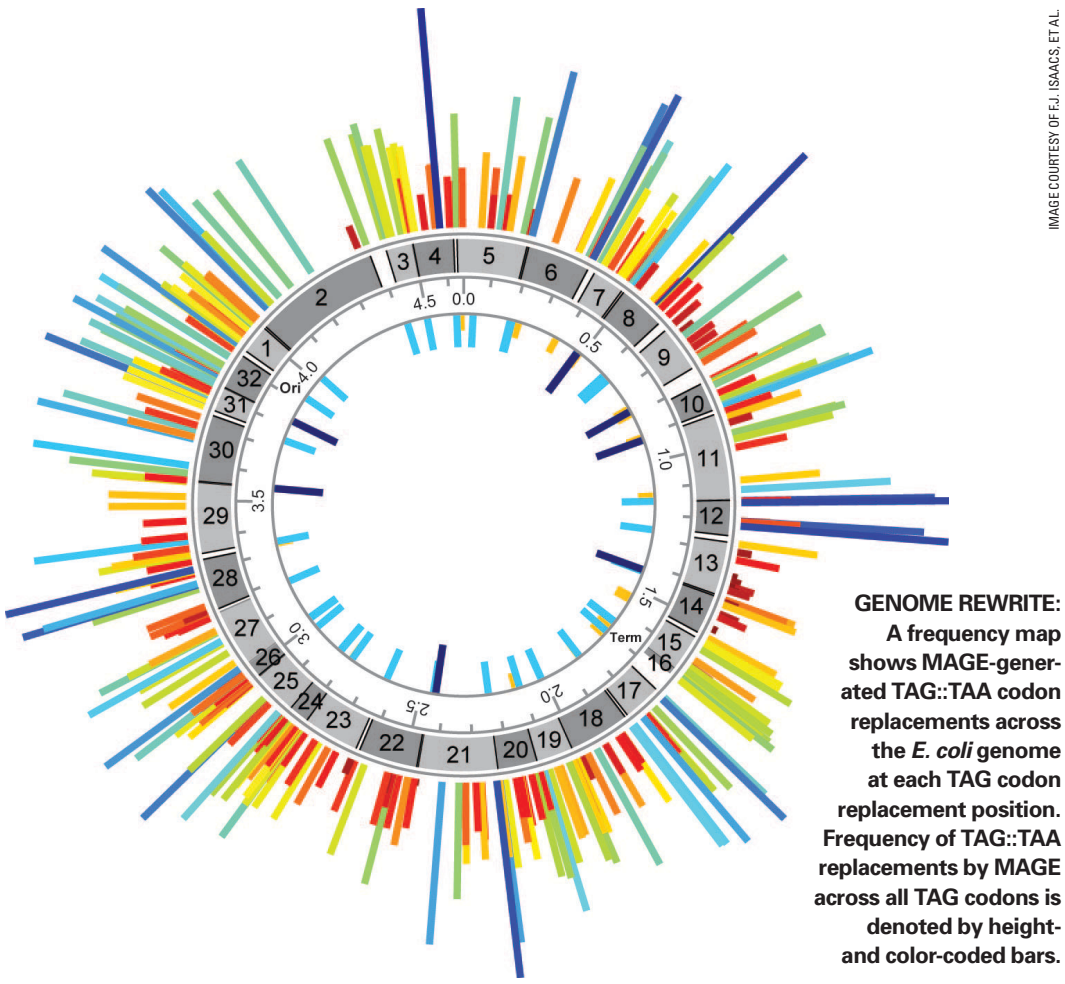


IMAGE COURTESY OF F.J. ISAACS, ET AL.

GENOME REWRITE: A frequency map shows MAGE-generated TAG::TAA codon replacements across the *E. coli* genome at each TAG codon replacement position. Frequency of TAG::TAA replacements by MAGE across all TAG codons is denoted by height- and color-coded bars.

A Genome Editor Gets Its Rewrite

Synthetic biologists re-engineer genomes from nucleotide to megabase scale

The power to edit genes is as revolutionary, immediately useful and unlimited in its potential as was Johannes Gutenberg's printing press. And like Gutenberg's invention, most DNA editing tools are slow, expensive, and hard to use—a brilliant technology in its infancy.

Now, Harvard researchers developing genome-scale editing tools as fast and easy as word processing have rewritten the genome of living cells using the genetic equivalent of search and replace—and combined those rewrites in novel cell strains strikingly different from their forebears.

"The payoff doesn't really come from making a copy of something that already exists," said Professor of Genetics George Church, who led the research in collaboration with Joe Jacobson, an associate professor at the Media

See "Genome Editor," page 6

The World's Most Advanced Genetic Map

New biological atlas focuses on African-American genomics

STEPHANIE MITCHELL

David Reich

A consortium led by scientists at the University of Oxford and Harvard Medical School has constructed the world's most detailed genetic map to date.

A genetic map specifies precise areas in the genetic material of a sperm or an egg wherein DNA from a mother and a father has been reshuffled through a process of recombination to produce a single reproductive cell. While almost every genetic map built so far has been developed from people of European ancestry, this new map is the first constructed from African-American recombination genomic data.

"This is the world's most accurate genetic map," said David Reich, a professor of genetics at HMS who co-led the study with Simon Myers, a lecturer in the Department of Statistics at the University of Oxford. The researchers were surprised to find that positions where recombination occurs in African-Americans are significantly different from those of non-African populations.

See "Genetic Map," page 6

Under 6 and Overweight

How do we fight early childhood obesity?

Focus sat down with Elsie Taveras, an assistant professor of population medicine and of pediatrics at HMS, to discuss a new Institute of Medicine report on early childhood obesity prevention. Slightly more than 20 percent of U.S. children between the ages of 2 and 5 are already overweight or obese, and nearly 10 percent of infants and toddlers carry excess weight for their length. Following is an excerpt from the conversation with Taveras, who

See "Overweight," page 8

COURTESY OF MICHAEL OVERHOLTZER

Michael Overholtzer sheds light on entosis.

When Cell-In-Cell Invaders Spell Aggressive Cancer

Discoverers of entosis propose a sinister new role for cells within cells

Pop! A mammary epithelial cell detaches from the extracellular matrix, the collagenous material that binds the cells of tissues together. Such outcasts usually die quickly, but not this one. Instead, the vagabond burrows into a healthy cell, a phenomenon called entosis. Most entosed cells soon die—but again, not this one. Instead, this cell squats in a vacuole until the time comes

See "Cell-in-Cell," page 8

INSIDE

GREGORY OLSEN/ ISTOCK

Massachusetts Health Care Law Boosts Access to Care

One state's success may steer national debate

Recent research conducted at Harvard Medical School and the Harvard School of Public Health may have strong implications for informing the controversial debate currently surrounding national health care reform.

In a study published in the July edition of the *American Journal of Preventive Medicine,* the Harvard research team, led by first author Aakanksha Pande, a doctoral student in the Department of Population Medicine at HMS and at the Harvard Pilgrim Health Care Institute, found that Massachusetts health reform has effectively increased access to health care and reduced disparities. Massachusetts health reform is structurally similar to the 2010 Patient Protection and Affordable Care Act (PPACA), the federal statute signed into law by President Obama last year.

"As the political rhetoric heats up in advance of another presidential election cycle," said senior author Joshua Salomon, associate professor of international health at HSPH, "it's important to understand what the experience in Massachusetts tells us about the effects of health reform on access and affordability of care."

The researchers found that, three years after being enacted in 2006, Massachusetts health reform was associated with a 7.6 percent increase in health insurance among residents, a 4.8 percent decrease in those forgoing health care due to cost, and a 6.6 percent increase in residents having a primary care physician. These improvements were most evident among socioeconomically disadvantaged groups.

LESSONS FOR THE NATION?

Does Massachusetts health reform provide a good proxy for national reform? "Yes and no," Pande said. The terms of each act are similar, including the provision of a health mandate that requires all residents to obtain health insurance. However, Massachusetts health reform was passed with very little opposition in the state legislature, whereas the PPACA has been met with contention. For this reason, implementing health reform at the national level might prove more difficult.

According to Salomon, Massachusetts health reform's success cannot be ignored. "Our study confirms a dramatic rise in health care coverage in Massachusetts since health reform was passed," he said.

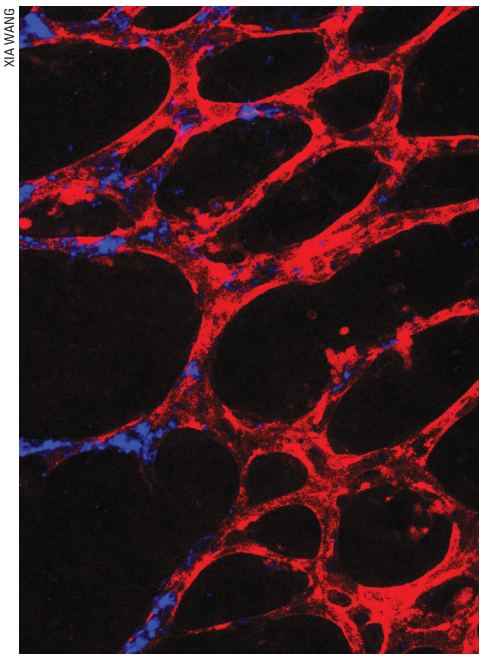
"There had been lots of discussion in the media about the political and ethical aspects of requiring health insurance," Pande said. "But evidence of whether or not a health mandate works had not been established in a rigorous manner. We approached the issue from a neutral perspective and determined that, in Massachusetts, it does."

—Joanna Logue

Building Blood Vessels

HMS Assistant Professor of Neurobiology Chenghua Gu and colleagues have discovered how two proteins—plexin-D1 (blue in the image below) and semaphorin3E—help modulate the formation of new blood vessel networks, a process called angiogenesis. The findings could make it possible to improve upon existing cancer therapies that starve tumors by blocking angiogenesis. The study also has implications for treating macular degeneration and diabetic retinopathy, both of which involve uncontrolled proliferation of blood vessels. Gu explains her lab's discovery in a video at **focushms.com.**

XIA WANG



VIDEO EXTRA

See "Building Blood Vessels" at focushms.com

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HARVARD MEDICAL SCHOOL

A Publication of Harvard Medical School Communications and External Relations

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Conflict disclosures and funding sources appear online.

Recent books written or edited by members of the HMS, HSPH and HSDM faculty or staff may be submitted to *Focus* at the address above. Books received by Aug. 23, 2011, will be featured in the next book section.

We invite letters from our readers, which should be brief and include a signature, address and daytime phone number.

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The Smell of Danger

Rodent olfaction and the chemistry of instinct

The mechanics of instinctive behavior are mysterious. Even something as simple as the question of how a mouse can use its powerful sense of smell to detect and evade predators, including species it has never met before, has been almost totally unknown at the molecular level until now.

David Ferrero and Stephen Liberles, neuroscientists at HMS, have discovered a single compound found in high concentrations in the urine of carnivores that triggers an instinctual avoidance response in mice and rats. This is the first time scientists have identified a chemical tag that lets rodents sense carnivores in general from a safe distance. The authors write that understanding the molecular basis of predator odor recognition by rodents will provide crucial tools to study the neural circuitry associated with innate behavior.

Their findings were published online June 20 in the *Proceedings of the National Academy of Science.*

The search began in 2006, when Stephen Liberles, now Assistant Professor of Cell Biology at HMS, was working as a post-doc in the lab of Linda Buck. Buck was part of the team that won the Nobel Prize for identifying the receptors that allow olfactory neurons to detect odors. While in her lab, Liberles identified a new type of olfactory receptor, the trace amine-associated receptors (TAARs).

Mice have about 1200 kinds of odor receptors, and 14 kinds of TAARs. In comparison, humans —who rely more on vision than smell— have about 350 odor receptors and five TAARs.

Liberles's initial findings indicated that several of the TAARs detect chemicals found in mouse urine, including one with enriched production by males. He wondered, could TAARs (which appear to have evolved from neurotransmitter receptors that mediate behavior and emotion) play a role in the social behavior of rodents? What other naturally occurring odors might they be able to detect?

In Liberles's lab at HMS, graduate student David Ferrero began a search for other natural compounds that were detected by the TAARs. Working with commercially available predator and prey urine (used by gardeners to keep pests out of crops and by hunters to mask their scent or as bait), Ferrero discovered that one of the 14 TAARs, TAAR4, detected the odor of several carnivores.

LIKE A SUPER-PHEROMONE

It seemed they had found a kairomone, a chemical that works like a pheromone, except that it communicates between members of different species instead of members of the same species. Prior to this discovery, the only known rodent-carnivore kairomones were a volatile compound produced by foxes, but not other predators, and two non-volatile compounds produced by cats and rats (which prey on mice).

Volatile compounds aerosolize and can be smelled at great distances; non-volatile compounds need to be sniffed more directly, something that would help in avoiding not a predator directly but rather its terrain.

"One of the things that's really new here is that this is a generalized predator kairomone that's volatile," said Ferrero.

For rodents, it's the smell of danger.

Ferrero identified the compound that activates TAAR4 as 2-phenylethylamine, a product of protein metabolism. He then obtained specimens from 38 species of mammals and found elevated levels of 2-phenylethylamineby 18 of 19 species of carnivores, but not by non-carnivores (including rabbits, deer, primates, and a giraffe).

"It's been known so long that predator odors are great rodent deterrents, but we've discovered one molecule that's a key part of this ecological relationship," Ferrero said.

In a series of behavior tests, rats and mice showed a clear, innate avoidance to the smell of 2-phenylethylamine. The behavioral studies were repeated using a carnivore samples that had been depleted of 2-phenylethylamine. Rats failed to show full avoidance of the depleted carnivore urine, indicating that 2-phenylethylamine is a key trigger for predator avoidance.

MILD OR REPUGNANT?

Lacking the gene for TAAR4, humans can't experience anything like what rodents do when they smell 2-phenylethylamine. To us, it has a mildly inoffensive odor. But trimethylamine, a related organic compound that activates TAAR5, a receptor found in humans, is deeply repugnant to people.

What happens between the receptors and the parts of the brain that trigger that avoidance behavior remains a mystery, one with direct medical relevance.

"In humans, the parts of the brain that deal with likes and dislikes go awry in many diseases, like drug addiction, and predator odor responses have been used to model stress and anxiety disorders," Liberles said. "Going from chemicals to receptors to neural circuits to behaviors is a Holy Grail of neuroscience."

"The neural circuits are like a black box, but here we have identified a chemical stimulant and a candidate receptor that trigger one behavior," Ferrero said. "We feel this is an important first step to understanding the neural circuitry of innate behavior."

This research was funded by the National Institute on Deafness and Other Communication Disorders.

—Jake Miller

To learn more, students may contact Stephen Liberles at stephen_liberles@hms.harvard.

Paper Chase

RECENT PUBLICATIONS FROM HMS

The index below is a selection of new studies and review articles by researchers from across the HMS community. It represents a small sample of research at **focushms.com.**

GENETIC BASIS FOR DAPTOMYCIN RESISTANCE IN ENTEROCOCCI

Palmer KL, Daniel A, Hardy C, Silverman J, Gilmore MS. Harvard Medical School (HMS).

The emergence of multidrug-resistant enterococci is a public health concern. The authors evolved daptomycin-resistant strains of the multidrug-resistant *E. faecalis* strain V583. Based on the availability of a fully closed genome sequence for V583, the authors used whole-genome resequencing to identify mutations that became fixed over short time scales (about two weeks) upon serial passage in the presence of daptomycin. Seven candidate daptomycin resistance genes and three different mutational paths to daptomycin resistance in *E. faecalis* were identified. Results demonstrate a mechanism of enterococcal daptomycin resistance genetically distinct from that occurring in staphylococci. *Antimicrobial Agents Chemotherapy.* 2011 Jul; 55(7):3345-56.

GENOME-WIDE REGULATION OF 5HMC, 5MC, AND GENE EXPRESSION BY TET1 HYDROXYLASE IN MOUSE EMBRYONIC STEM CELLS

Xu Y, Wu F, Tan L, Kong L, Xiong L, Deng J, Barbera AJ, Zheng L, Zhang H, Huang S, Min J, Nicholson T, Chen T, Xu G, Shi Y, Zhang K, Shi YG. Brigham and Women's Hospital, HMS.

DNA methylation at the 5 position of cytosine (5mC) in the mammalian genome is a key epigenetic event. The ten-eleven translocation (Tet) family of 5mC-hydroxylases, which convert 5mC to 5-hydroxymethylcytosine (5hmC), offers a way for dynamic regulation of DNA methylation. The authors report that Tet1 binds to unmodified C or 5mC- or 5hmC-modified CpG-rich DNA through its CXXC domain. Genome-wide mapping of Tet1 and 5hmC reveals mechanisms by which Tet1 controls 5hmC and 5mC levels in mouse embryonic stem cells (mESCs). The authors also uncover a comprehensive gene network influenced by Tet1. *Molecular Cell.* 2011 May 20; 42(4):451-64.

HIV-1 ADAPTATION TO NK-CELL-MEDIATED IMMUNE PRESSURE

Alter G, Heckerman D, Schneidewind A, Fadda L, Kadie CM, Carlson JM, Oniangue-Ndza C, Martin M, Li B, Khakoo SI, Carrington M, Allen TM. Ragon Institute at MGH, MIT and Harvard; Massachusetts General Hospital; HMS.

Recent studies suggest that natural killer (NK) cells can contribute to the control of HIV-1 infection through recognition of virally infected cells by killer immunoglobulin-like receptors (KIRs). The authors describe KIR-associated amino-acid polymorphisms in the HIV-1 sequence on a population level. They show that these polymorphisms can enhance the binding of inhibitory KIRs to HIV-1-infected CD4(+) T cells and reduce the antiviral activity of KIR-positive NK cells. These data demonstrate that HIV-1 can evade NK-cell-mediated immune pressure by selecting for sequence polymorphisms. NK cells might therefore have an underappreciated role in viral evolution. *Nature.* 2011 Aug 3; 476(7358):96-100.

Clockwise from top left: Jonathan Crocker, Patrick Aquino, Anita Vanka, Kathleen Wittels, Shaida Sharifi, Eileen Dillon, Bhargavi Yalamarti, Mary Buss and Susan Abookire

Fellowships in Medical Education

Faculty fellowships improve skills as educators

Seventeen HMS faculty members and clinical educators have been awarded fellowships in medical education in 2011-2012. Their training is made possible by three fellowship programs: the Rabkin Fellowship at Beth Israel Deaconess Medical Center, the Mount Auburn Fellowship and the HMS Academy Fellowship.

"These fellowships provide dedicated time to further develop the expertise and skills needed to launch or advance academic careers in medical education," said Lori Newman, HMS teaching associate in medicine, who co-directs the Rabkin fellowship with Christopher Smith, HMS associate professor of medicine.

The fellowships aim to help junior and mid-career physicians and educators to develop as teachers, as well as to provide an opportunity to conduct mentored research or a project in an important area in medical education. The Mount Auburn fellowship is open to all clinical educators, while the Rabkin and Academy fellowships require an academic appointment at HMS. Qualified applicants from any HMS affiliate may apply for any of the three fellowships.

"Our evaluation study of the fellowships showed that they foster graduates' sense of identity as medical educators as well as the skills necessary to enhance their professional development," said Beth Lown, associate professor of medicine and director of faculty development and the Mount Auburn medical education fellowship.

"Historically, we have assumed that because people are good clinicians or good researchers, they are good teachers—and some are," said Charles Hatem, the Harold Amos Academy Professor of Medicine and director of medical education at Mount Auburn Hospital, who began spearheading the fellowships in 1998. "But teaching is a skill that can be studied and learned."

Fellows meet two to four times a month to discuss principles of adult learning, curriculum development and assessment as well as to master concepts in medical education research and study design. Fellows present their findings each fall at HMS Medical Education Day.

Graduates have led significant curricular and programmatic changes and served as education leaders in the HMS community and around the world. "Each of the fellows' projects has the potential to make a real impact on the continuous improvement of teaching and learning at HMS and our affiliated hospitals," said Edward Hundert, the new director of the HMS Academy Center for Teaching and Learning and a senior lecturer in medical ethics in the Department of Global Health and Social Medicine.

—Angela Alberti

Academy Fellows in Medical Education

Cynthia Cooper

Curtis Prout Fellow
Instructor in Medicine, Department of Medicine, Massachusetts General Hospital
Student-Initiated Feedback

Amin Sabet

Morgan-Zinsser Fellow
Instructor in Medicine, Department of Medicine, Beth Israel Deaconess Medical Center
Impact of Progress and Cumulative Achievement Testing on Medical Student Stress and Readiness for the USMLE Step 1 Examination

Kathleen Wittels

Morgan-Zinsser Fellow
Instructor in Medicine, Department of Emergency Medicine
Brigham and Women's Hospital
A Simulation-Based Assessment Tool to Measure Emergency Medicine Resident Competency

Karen Wood

Curtis Prout Fellow
Assistant Clinical Professor of Population Medicine, Department of Medicine, Harvard Vanguard Medical Associates
Designing a Curriculum for Patient Safety in the Ambulatory Setting

Mount Auburn Hospital Fellowship in Medical Education

Recipients are at Mt. Auburn Hospital unless noted.

Susan Abookire

Assistant Professor of Medicine and Chair, Department of Quality and Patient Safety
Developing Mount Auburn as a Primary Site for the Newly Created HMS Fellowship in Quality and Patient Safety

Patrick Aquino

Instructor in Psychiatry and Director of Consultation and Emergency Services, Department of Psychiatry
Psychiatry Curriculum for Primary Care Residents in a Patient-Centered Medical Home

Eileen Dillon

Executive Director, Department of Quality and Patient Safety
Introduction to Principles and Concepts of Quality and Patient Safety for Nurses

Laila Khalid

Instructor in Medicine, Chief Resident, Deptartment of Medicine
Enhancing the Teaching Skills of Medical Residents

Pooja Rutberg

Instructor in Pediatrics, Department of Pediatrics, Cambridge Health Alliance
Preparing Residents with Practical Tools for Establishing a Career in Pediatrics

Shaida Sharifi

Medical Director of Cytology and Microbiology, Department of Pathology
The Utility of a Secured Web-based Application as an Educational Tool in Gynecologic Cytology

Bhargavi Yalamarti

Clinical Fellow in Medicine and Hospitalist, Department of Medicine
Enhancing Residents' Communication Skills in Family Meetings

Rabkin Fellows in Medical Education

Recipients are at Beth Israel Deaconess Medical Center unless noted.

Mary Buss

Assistant Professor of Medicine and Medical Oncologist and Palliative Care Consultant, Department of Medicine
Making the Most of the MOLST (Medical Orders for Life-Sustaining Treatment) — Improving Communication Around Goals of Care

Jonathan Crocker

Instructor in Medicine and Attending Physician, Department of Medicine
Filling the Global Health Training Gap: A Resident Curriculum Introducing a Social, Economic, Political and Ethical Framework for Global Health Practice

Alok Gupta

Instructor in Surgery and Attending Trauma Surgeon, Surgical Intensivist, General Surgeon, Department of Surgery
Prospective Randomized Study of High-Fidelity Simulation in Resident Trauma Training

Carolyn Kloek

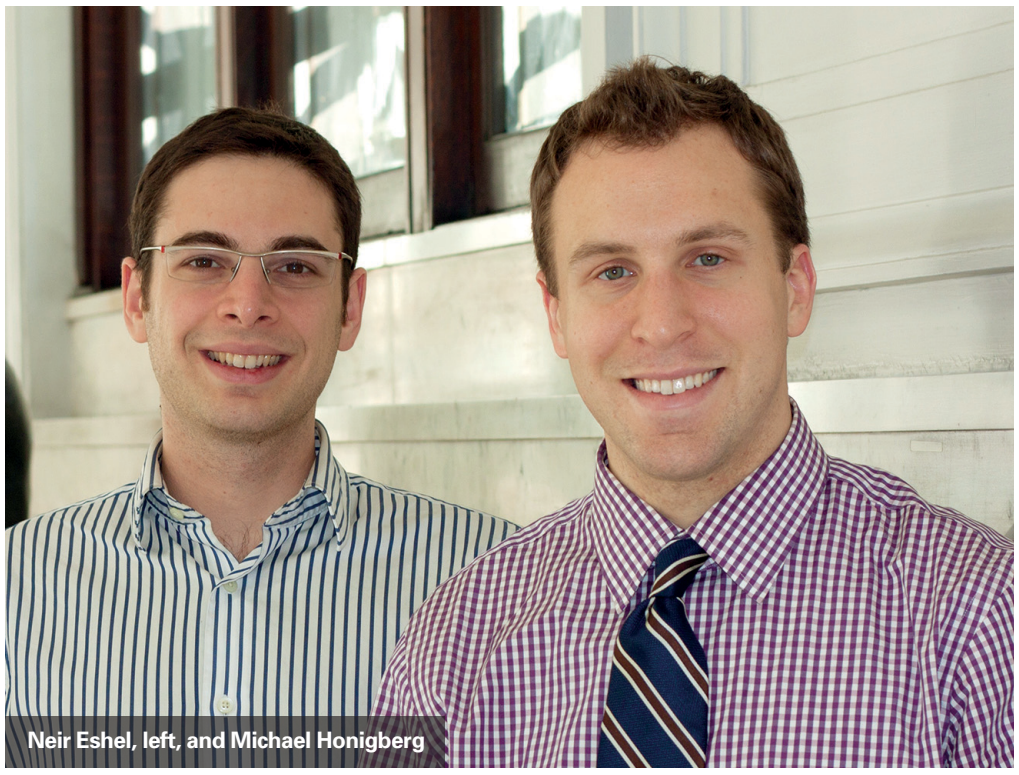
Instructor in Opthalmology and Assistant in Surgery, Department of Ophthalmology and Clinical Associate, Massachusetts Eye and Ear Infirmary
Creation of a Standardized, Case-Based Ophthalmology Curriculum for Medical Students

Jeremy Richards

Instructor in Medicine and Attending Physician, Department of Medicine
Developing a Curriculum to Promote Curiosity in Third-Year Medical Students

Anita Vanka

Instructor in Medicine and Attending Physician, Department of Medicine
Transitions in Care Curriculum



MELIS ANAHTAR

Neir Eshel, left, and Michael Honigberg

LGBT Advocates Honored

HMS students Neir Eshel and Michael Honigberg have been recognized nationally for their achievements in advocating for lesbian, gay, bisexual and transgender issues at the School.

Eshel and Honigberg received the 2011 LGBT Health Achievement Award from the American Medical Student Association. The award recognizes achievements of a medical school, student group or individual in advocating for the inclusion in their institution of LGBT health issues or concerns.

"Neir and Michael deserve to be recognized for their dedication and success in enhancing the visibility of LGBT concerns at Harvard," said LGBT faculty adviser Graham McMahon, HMS associate professor of medicine. "Few could have imagined that the work of a few volunteers could so quickly make such a tangible impact."

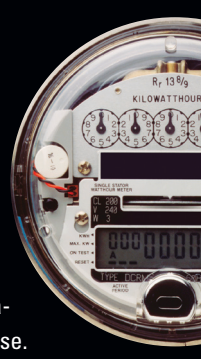
Eshel and Honigberg, co-presidents of the LGBT student group at HMS, expanded support systems for LGBT students. Their outreach to student society leaders and senior faculty contributed to the appointment of two LGBT advisers. And they are working to develop faculty groups at HMS affiliates to advise students during clinical rotations.

"We're grateful that we've had the chance to make a small difference at HMS," Eshel said, "and we look forward to continuing our efforts."

To read more about the new LGBT advisers, visit hms.harvard.edu/org.asp?orma.

—Angela Alberti

Green Tip



ISTOCK PHOTO

The hottest days of the year often require using the most energy to cool and dehumidify buildings at Harvard. You can help save energy at HMS by turning off electronics and laboratory equipment when not in use. For more Green Tips visit http://green.harvard.edu.

Three HMS Affiliates Earn Perfect Scores on LGBT Survey

Participants working toward health care equality

A June report released by the Human Rights Campaign (HRC) Foundation rated three Harvard affiliates as "Leaders in LGBT Healthcare Equality." Beth Israel Deaconess Medical Center, Brigham and Women's Hospital and Dana-Farber Cancer Institute were among only 27 U.S. institutions to receive perfect scores on an annual survey administered by the HRC Foundation.

These facilities were recognized for providing LGBT cultural competency training for all staff, for explicit policies protecting LGBT patients and employees from discrimination, and for ensuring equal visitation access for same-sex couples and parents.

In Boston, seven facilities, including five HMS affiliates, voluntarily participated in the Healthcare Equality Index 2011, including Harvard Vanguard Medical Associates and Massachusetts General Hospital.

"Any facility that participates in this study is showing a level of concern for healthcare equality," said Paul Guequierre, an HRC spokesman. "The fact that even facilities that do not have perfect scores are participating voluntarily shows that they care."

Nationally, the HRC survey found that although nearly 90 percent of participants include sexual orientation in their Patient's Bill of Rights and/or non-discrimination policy, only 60 percent include gender identity in these policies. Additionally, 49 percent grant equal visitation access for same-sex couples through an explicitly inclusive policy; 52 percent have such a policy inclusive of same-sex parents.

"We hope all health care facilities will achieve a perfect score, and we feel that the participants in this survey are working toward that," Guequierre said.

—Angela Alberti

Notable

Excellence in Hispanic Mental Health Research, Advocacy and Leadership Award to Margarita Alegría ▼

Doris Duke Charitable Foundation Clinical Scientist Development Award to David Friedman

▲ American Society of Magazine Editors' National Magazine Award to Atul Gawande • Cambridge Health Alliance Art of Healing Awards to David Osler, Hilary Worthen and Erika Fellinger • Schweitzer Fellows Program Awards to Abhiram Bhashyam, Alister Martin, Raymond Deng, Ted Henson, Jonathan Lee, ▼ Patricia McClory and Mariah Rich

• Helmsley Pilot Grants Program in Crohn's disease to David Breault, Jeffrey Karp, Laurie Glimcher, Dimitrious Iliopoulos, Jonathan Kagan, Bruce Paster, Shiv Pillai, Vijay Yajnik and Matthew Meyerson • The President's Council on Fitness, Sports & Nutrition Community Leadership Award to the Institute of Lifestyle Medicine based at the Spaulding Rehabilitation Network • Program of Excellence Award from the National Heart, Lung, and Blood Institute of the National Institutes of Health to the laboratory of Robert Sackstein ▼

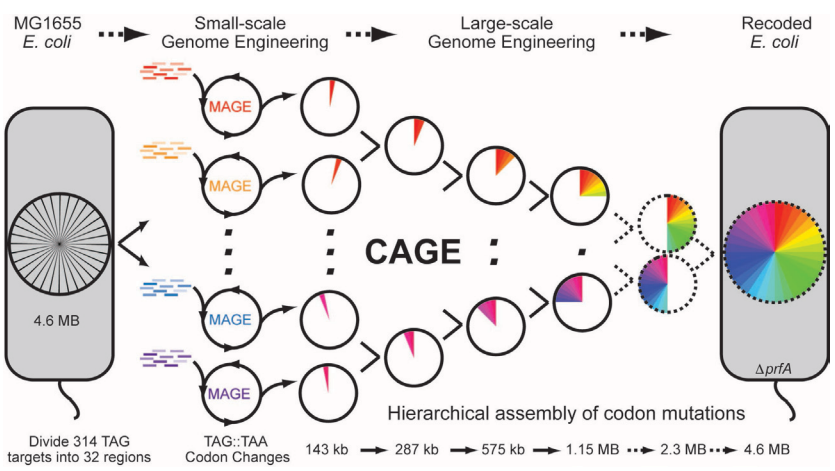


IMAGE COURTESY OF F.J. ISAACS, ET AL.

Researchers in the lab of HMS Professor of Genetics George Church describe a strategy for reassigning all 314 TAG codons to TAA in *E. coli.* Left to right: Multiplex automated genome engineering (MAGE) was used to convert all TAG codons to TAA codons. Hierarchical conjugative assembly genome engineering (CAGE) was used to assemble codon changes into higher ordered strains.

Genome Editor

Continued from page 1

Lab at the Massachusetts Institute of Technology. "You have to change it—functionally and radically."

Such change, Church said, serves three goals. The first is to add functionality to a cell by encoding for useful new amino acids. The second is to introduce safeguards that prevent cross-contamination between modified organisms and the wild. A third, related aim is to establish multi-viral resistance by rewriting code hijacked by viruses.

In industries that cultivate bacteria, including pharmaceuticals and energy, such viruses affect up to 20 percent of cultures. At the biotech company Genzyme, estimates of losses due to viral contamination range from a few hundred million dollars to more than $1 billion.

'EVOLUTION MACHINE'

In the July 15 issue of *Science*, the researchers describe how they replaced instances of a codon—a DNA "word" of three nucleotide letters—in 32 strains of *E. coli*, and then coaxed those partially edited strains along an evolutionary path toward a single cell line in which all 314 instances of the codon had been replaced.

That many edits surpasses current methods by two orders of magnitude, said Harris Wang, a research fellow in Church's lab at the Wyss Institute for Biologically Inspired Engineering who shares lead-author credit on the paper with Farren Isaacs, an assistant professor of molecular, cellular and developmental biology at Yale University and former Harvard research fellow, and Peter Carr, a research scientist at the MIT Media Lab.

In the genetic code, most codons specify an amino acid, a protein building block. But a few codons tell the cell when to stop adding amino acids to a protein chain, and it was one of these "stop" codons that the Harvard researchers targeted. With just 314 occurrences, the TAG stop codon is the rarest word in the *E. coli* genome, making it a prime target for replacement.

Using a platform called multiplex automated genome engineering, or MAGE, the team replaced instances of the TAG codon with another stop codon, TAA, in living *E. coli* cells. Unveiled by the team in 2009, the MAGE process has been called an "evolution machine" for its ability to accelerate targeted genetic change in living cells.

While MAGE, a small-scale engineering process, yielded cells in which TAA codons replaced some but not all TAG codons, the team constructed 32 strains that, taken together, included every possible TAA replacement. Then, using bacteria's innate ability to trade genes through a process called conjugation, the researchers induced the cells to transfer genes containing TAA codons at increasingly larger scales.

The new method, called conjugative assembly genome engineering, or CAGE, resembles a playoff bracket—a hierarchy that winnows 16 pairs to eight to four to two to one—with each round's winner possessing more TAA codons and fewer TAG, explains Isaacs, who invokes "March Madness."

"We're testing decades-old theories on the conservation of the genetic code," Isaacs said. "And we're showing on a genome-wide scale that we're able to make these changes."

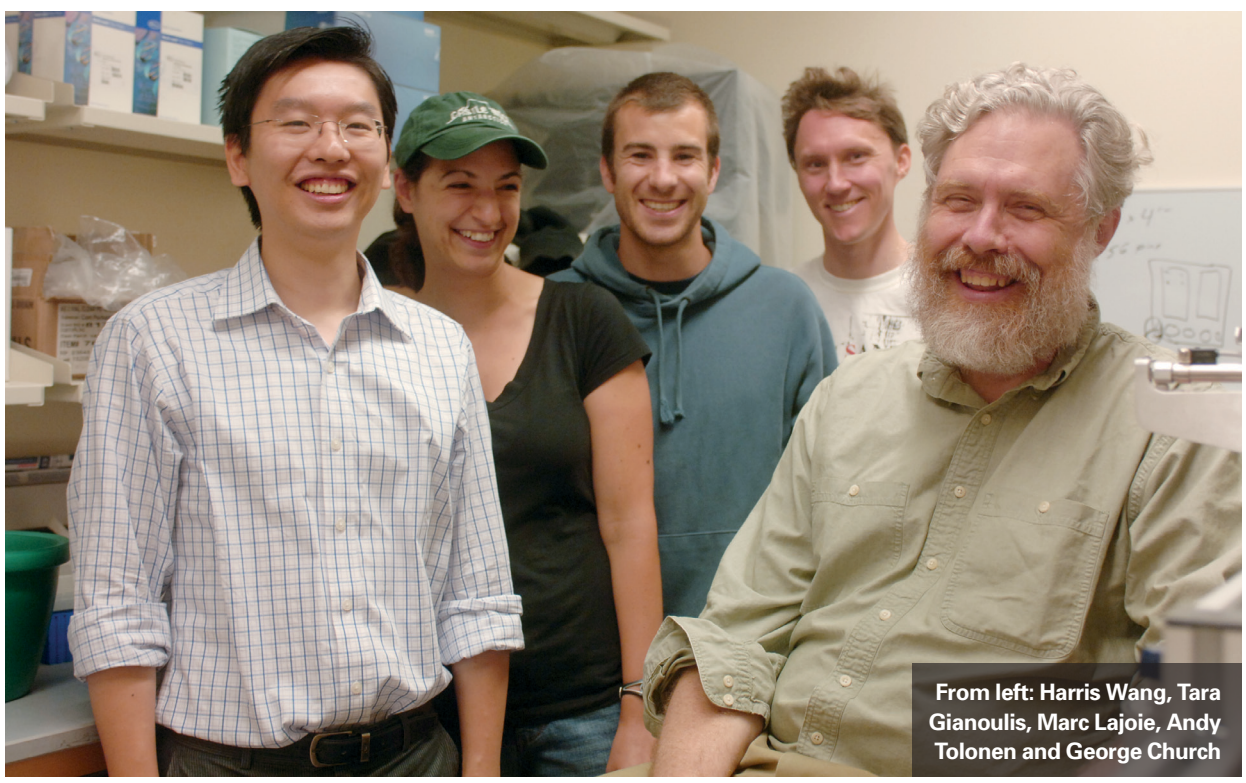
Eager to share their technology, the team published their results as CAGE reached the semifinal round: the final four strains were healthy, even as the team assembled four groups of 80 engineered alterations into stretches of the chromosome surpassing 1 million DNA base pairs.

"We encountered a great deal of skepticism early on that we could make so many changes and preserve the health of these cells," Carr said. "But that's what we've seen."

The researchers are confident that they will create a single strain in which TAG codons are completely eliminated. The next step, they say, is to delete the cell's machinery that reads the TAG gene—freeing up the codon for a completely new purpose, such as encoding a novel amino acid.

"We're trying to challenge people," Wang said, "to think about the genome as something that's highly malleable, highly editable." —*R. Alan Leo*

To learn more, students may contact George Church at gmc@harvard.edu or Farren Isaacs at farren.isaacs@yale.edu.



ANGELA ALBERTI

From left: Harris Wang, Tara Gianoulis, Marc Lajoie, Andy Tolonen and George Church

Genetic Map

Continued from page 1

"The landscape of recombination has shifted in African-Americans compared with Europeans," said Anjali Hinch, first author and a post-graduate student at Oxford University's Wellcome Trust Centre for Human Genetics.

Myers added: "More than half of African-Americans carry a version of the biological machinery for recombination that is different from that of Europeans. As a result, African-Americans experience recombination where it almost never occurs in Europeans."

The findings were published in the July 21 edition of *Nature*. An independent study using a similar strategy to build a genetic map in African-Americans—led by University of California, Los Angeles, scientists Daniel Wegmann, Nelson Freimer and John Novembre—will be published in *Nature Genetics*.

RECOMBINATION HOTSPOTS

Scientists have only recently begun to explore the genetic differences between individuals and populations, as well as the role those differences play in human health. The first draft of the human genome, completed a decade ago, was only a starting point for understanding the genetic origins of disease.

As researchers begin to parse those differences, a crucial tool has emerged: the genetic map, which in this case is based on where recombination has occurred across the genome. Recombination, together with mutation, accounts for all of the genetic, and thus physical, variety seen within species. But whereas mutation refers to the errors introduced into single locations within genomes

when cells divide, recombination refers to the process by which huge chunks of chromosomes are stitched together during sexual reproduction.

This stitching process only occurs at specific locations. In a prior landmark set of papers, Myers and his colleagues identified a DNA code, or motif, that attracted part of the recombination machinery, a gene called PRDM9. Knowing this motif, a string of 13 DNA letters, researchers could zero in on locations where recombination typically occurred—"recombination hotspots."

"When recombination goes wrong, it can lead to mutations causing congenital diseases—for example, Charcot-Marie-Tooth disease or certain anemias," said Myers. "We found the same 13-base motif marking many of these disease-mutation sites."

Explained Reich: "Places in the genome where there are recombination hotspots can thus also be disease hotspots. Charting recombination hotspots can identify places in the genome that have an especially high chance of causing disease."

The researchers discovered that the 13 base-pair motif responsible for many hotspots in Europeans accounts for only two-thirds as much recombination in African-Americans. They connected the remaining third to a new motif of 17 base pairs, which is recognized by a version of the recombinational machinery that occurs almost exclusively in people of African ancestry.

MAPPING DISEASE GENES

These findings are expected to help researchers understand the roots of congenital conditions occurring more often in African-Americans due to mutations at hotspots that are more common in this population. The findings should also aid in the discovery of disease genes in all populations, thanks to researchers' ability to map these genes more precisely.

The new map is so accurate because African-American individuals often have a mixture of African and European ancestry from the last 200 years. David Reich and Simon Myers are experts in analyzing genetic data to reconstruct the mosaic of regions of African and European genetic ancestry in DNA of African-Americans. By applying a computer program they had written previously, Anjali Hinch identified places in the genomes where the African and European ancestry switches in almost 30,000 people, detecting about 70 switches per person. These areas correspond to recombination events in the last few hundred years. The researchers identified more than two million recombination events, which they used to build the map.

The study was possible because of collaboration from 81 co-authors using DNA samples from five large studies of common diseases, such as heart disease and cancer.

Said James Wilson, a professor at the University of Mississippi Medical Center who coordinated the collaboration, "All the co-authors worked together in an incredibly collegial way to put together the enormous set of samples and high quality genetic data that made this study a success."

—David Cameron

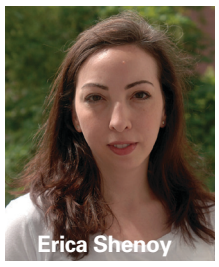
Find a link to the recombination map at focushms.com. To learn more, students may contact David Reich at reich@genetics.med.harvard.edu.

JORG GREUEL/GETTY IMAGES

Antibiotics and the Calculus of Risk

In medicine, as in life, nothing is guaranteed

ANGELA ALBERTI



Erica Shenoy

My patient's admission to the hospital was elective, certainly nothing emergent. Yet within days he was transferred to the Intensive Care Unit.

How did we get here?

I met Mr. B early in my first year of fellowship. He was recovering from a crushed ankle, which had been repaired with screws. The consult was to become a fairly typical introduction to a long-term relationship: a patient with a joint replacement or fracture repair that had become infected.

Treatment offers two options: Remove the hardware, treat the infection with antibiotics and eventually replace the implants; or leave the hardware in place, "wash out" the infected area in the operating room, and expect the patient to remain on antibiotics for months, or perhaps for life. Hardware can make clearing infection nearly impossible, so the first option is preferred. But when the hardware is hard to reach or necessary for stability, removal may be impossible.

These foreign bodies become magnets for bacteria, which can quickly form a biofilm that resists eradication. After a period of initial intense IV antibiotics, patients transition to oral antibiotics to suppress the recurrence of infection. But antibiotics are often poorly tolerated. A recent study found that, each year, close to 150,000 emergency room visits are prompted by adverse reactions to antibiotics.

Mr. B had developed an infection with multiple bacteria. At first, the hardware stabilizing his ankle had to stay in place, and we treated him with IV antibiotics. Within days he developed a full body rash, and his skin blistered and peeled. His reaction to the antibiotic was one of the worst that I've seen, though unlike some he did not end up in the burn ICU.

Switched to a powerful oral antibiotic, Mr. B tolerated it well. After six weeks, we switched him to another, to be taken for at least a year. During that year he was diagnosed with an unrelated progressive lung disease. His ankle remained stable. By year's end we were discussing the comparative risks of continuing antibiotics and of stopping them. He decided to stop.

Within a week or so, swelling and pain developed in Mr. B's ankle. In clinic, he wore a look of resignation. He was now on home oxygen and in a wheelchair, his lung condition accelerating.

A small part of his incision had opened and started to drain. Sitting with his wife and daughter, he asked what I thought was happening. When I said that I feared the infection had invaded bone, he did not seem surprised. X-rays showed that several screws holding his ankle together had broken; the bone was not healing. Together, his surgeons and we decided to admit him to remove the hardware.

But Mr. B was found to be in heart failure, and the OR was out of the question. The specter loomed of a potential superimposed pneumonia. Admitted to the ICU, he agreed to a short time on the ventilator if the team felt it might pull him through, but he preferred home to prolonged ventilation. The resident talked him and his family through these tough choices, introducing the concept of hospice.

Then Mr. B pressed me: Which antibiotics should he take for his ankle? He still had some pills at home (as ever, the practical type). I wanted to apologize. How did we go from swelling and pain to near death in a week? Could I—could anybody—have predicted this?

The truth is that nothing in medicine is risk-free. In some cases, the risk/benefit ratio is clear—without antibiotics, the patient will die from sepsis or lose a limb. In other cases, this ratio is uncertain. Could I tell Mr. B for sure that if he stopped the antibiotic, the infection would come back? I could not.

The infection might in fact return despite the antibiotic; moreover, he could have liver or kidney toxicity. And then there was the risk of *C. difficile* colitis, a serious, sometimes fatal complication of antibiotic use. I provide such warnings to my patients not for medico-legal reasons, but because I have seen these complications, and more, occur. Each time, I reflect on whether the risk was worth it.

Mr. B is now close to leaving the hospital. A couple more days of antibiotics will determine whether treating what may be pneumonia leaves him well enough to be among those he loves—and to spend his last days with them at home.

Erica Seiguer Shenoy, MD–PhD '07, is a fellow in infectious disease at Massachusetts General Hospital and Brigham and Women's Hospital. The opinions expressed are not necessarily those of Harvard Medical School, its affiliated institutions or Harvard University.

Overweight

Continued from page 1


Elsie Taveras

is co-director of the Department of Population Medicine's Obesity Prevention Program at Harvard Pilgrim Health Care.

Q: Are children now overweight at birth?
A: It doesn't look like birth weight has been increasing. In fact, it looks as if birth weight has remained pretty stable. Children are pretty much starting, on average, at a healthy weight.

Q: Do children who are overweight as infants or toddlers typically lose the extra weight over time?
A: I think the evidence really does support that this excess weight gain early in life is associated with obesity in later childhood and even adulthood.

Q: What are the demographics of early childhood obesity? Is the problem pretty pervasive across the United States?
A: What interests me most about the trends, especially the more recent trends, is that even though we seem to have reached a plateau in obesity prevalence, the prevalence is still high—and disproportionately high among some subgroups in the United States, including African-American children and Latino children. And you can already see what those early-life differences in obesity are going to mean for children as they get older. They're more at risk for cardiovascular disease, for diabetes, for a whole host of issues that used to be adult problems.

Q: Is there growing acknowledgement that these kinds of long-lasting and severe health problems are associated with early childhood obesity?
A: Absolutely. The Institute of Medicine report was commissioned by the Robert Wood Johnson Foundation after a pretty clear recognition that, for quite some time, our national efforts for obesity prevention have somewhat excluded children under 6.

Q: What were some of the major recommendations?
A: There were several. The charge for the committee was to propose, based on evidence, where policies could be developed in areas and settings where children spend the most time. So a lot of the recommendations are targeted to professionals who work with children: health care professionals, child care professionals, education professionals. One of the first recommendations was that pediatricians and other primary health care professionals who measure children should use the appropriate standards for tracking growth in children; the report gave some suggestions on how to detect a child who is gaining excessive weight.

—Alyssa Kneller

HMS PODCAST

Listen to the full conversation with Taveras at focushms.com.

Cell-in-Cell

Continued from page 1

for its host to divide. What happens next researchers are only beginning to understand, and their discoveries are shedding new light on aggressive cancers.

Entosis, the process by which wandering cells invade host cells to form cell-in-cell structures, was first described in 2007 by a team led by Joan Brugge, chair of Cell Biology at HMS, and former research fellow Michael Overholtzer, now at Memorial Sloan-Kettering Cancer Center. While this process generally contributes to tumor suppression by eliminating rogue cancer cells, this spring, the group reported a new role for entosis: Entosed cells arrest host cells midway during cell division and generate abnormal chromosome content, a condition called aneuploidy that is a marker of aggression in human cancers.

Cancer metastasizes when tumor cells break free from their primary location, invade local tissue, spread through the lymph or blood, and set up homesteads in distant tissues. Since most cells depend on signals from the extracellular matrix within their normal niches, normal cells are unable to survive floating free in the bloodstream. The same is true for cancer cells during metastasis.

While studying how detached mammary epithelial cells undergo cell death in the Brugge lab, Overholtzer stumbled on the phenomenon of entosis, observing that many detached cells actively invade other cells and take refuge in vacuolar structures within their host cells. He also discovered that the fate of most internalized cells was death following entosis, which he distinguished from genetically programmed cell death, or apoptosis. To survive metastasis, a tumor cell must avoid nutrient deprivation and metabolic impairment due to loss of signals in their normal niches, as well as death by apoptosis or entosis.

Although biologists had reported cell-in-cell structures as far back as 1864, Brugge credits Overholtzer's "keen observational skills" and curiosity for several new discoveries: that matrix-detached cells burrow into host cells; that there is a new, lysosomal death program mediated by entosis; and that cell-in-cell structures formed fortuitously bear significant physiological consequences.

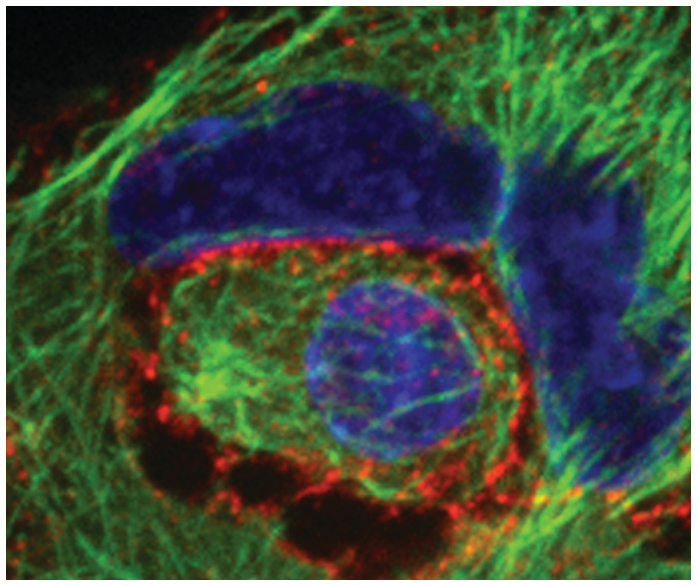
RED FLAG FOR AGGRESSION

Observing that entosis occurs in human tumors in vivo and that most entosed cells were killed inside their hosts, Brugge and Overholzer proposed in 2007 that entosis suppressed tumors by eliminating wandering tumor cells. But their latest findings, published in the March 2011 issue of *Nature Cell Biology,* point to another, more sinister, role.

In considering the potential effects of entosis on host cells, the researchers made an unexpected discovery: Under certain conditions, entosed cells halted cell division, leaving their hosts aneuploid—stuck with an abnormal number of chromosomes.

After duplicating its chromosomes through mitosis, a cell cleaves into two daughter cells. Using time-lapse microscopy in the Brugge lab, Overholtzer observed that some entosed cells, upon failing to divide properly, became binucleate. Following up on these observations, Matej Krajcovic, the lead author of the study from the Overholtzer lab, found that entosed cells physically blocked the plane of cell division, and that the resulting binucleate host cells generated highly aneuploid cell lineages. Some entosed cells caused even more havoc, disrupting two cycles of host-cell division and creating "Frankenstein" cells marked by additional chromosomal errors. The researchers concluded that entosis-driven aneuploidy could be tumor-promoting and might serve as a marker for certain cancers with high levels of genomic instability.

PHOTO COURTESY MICHAEL OVERHOLTZER



In entosis, one living cell enters another, often disrupting division of that host cell. In this photomicrograph, the host cell has two nuclei, stained in blue. Immunostaining reveals, in green, alpha-tubulin, a protein component of a cell's cytoskeleton; and, in red, E-cadherin, a protein important in cell adhesion.

Together, the two papers on entosis make a testable prediction, Overholtzer said. A tumor-cell population undergoing entosis at high rates would expand or grow slowly due to the constant death of some tumor cells; but in the long run, any remaining host cells would be endowed with aggressive characteristics due to aneuploidy, a known promoter of tumor progression.

"So the same process that could slow tumor growth might also contribute to promoting chromosomal changes ultimately associated with more aggressive cancers," Overholtzer said.

These findings have profound implications for aggressive human cancers, including breast cancers, in which the frequency of cell-in-cell structures correlates with disease severity. Because cell-in-cell structures are often reported in tumor cells extracted from fluid in the abdomen or lungs, the researchers hope to understand how and whether entosis in matrix-detached breast-tumor cells might exacerbate secondary cancers in the lungs.

Entosis research is a field in its infancy and a rich vein for inquiry, Brugge said. Molecular mechanisms behind the formation of cell-in-cell structures and the elimination of entosed cells remain poorly defined. Overholtzer's lab is now investigating what lets loose the lysosomal death pathway on an unsuspecting entosed cell, knowledge that could one day be used to kill tumor cells.

While chance may favor the prepared research fellow, landmark discoveries require concerted cooperation between a curious postdoc willing to explore uncharted territory and an adviser open to risk-taking. "I give Mike a lot of credit," Brugge said, "and he thanks me for allowing him to do it."

—Raji Edayathumangalam

For more information, students may contact Michael Overholtzer at overhom1@mskcc.org.