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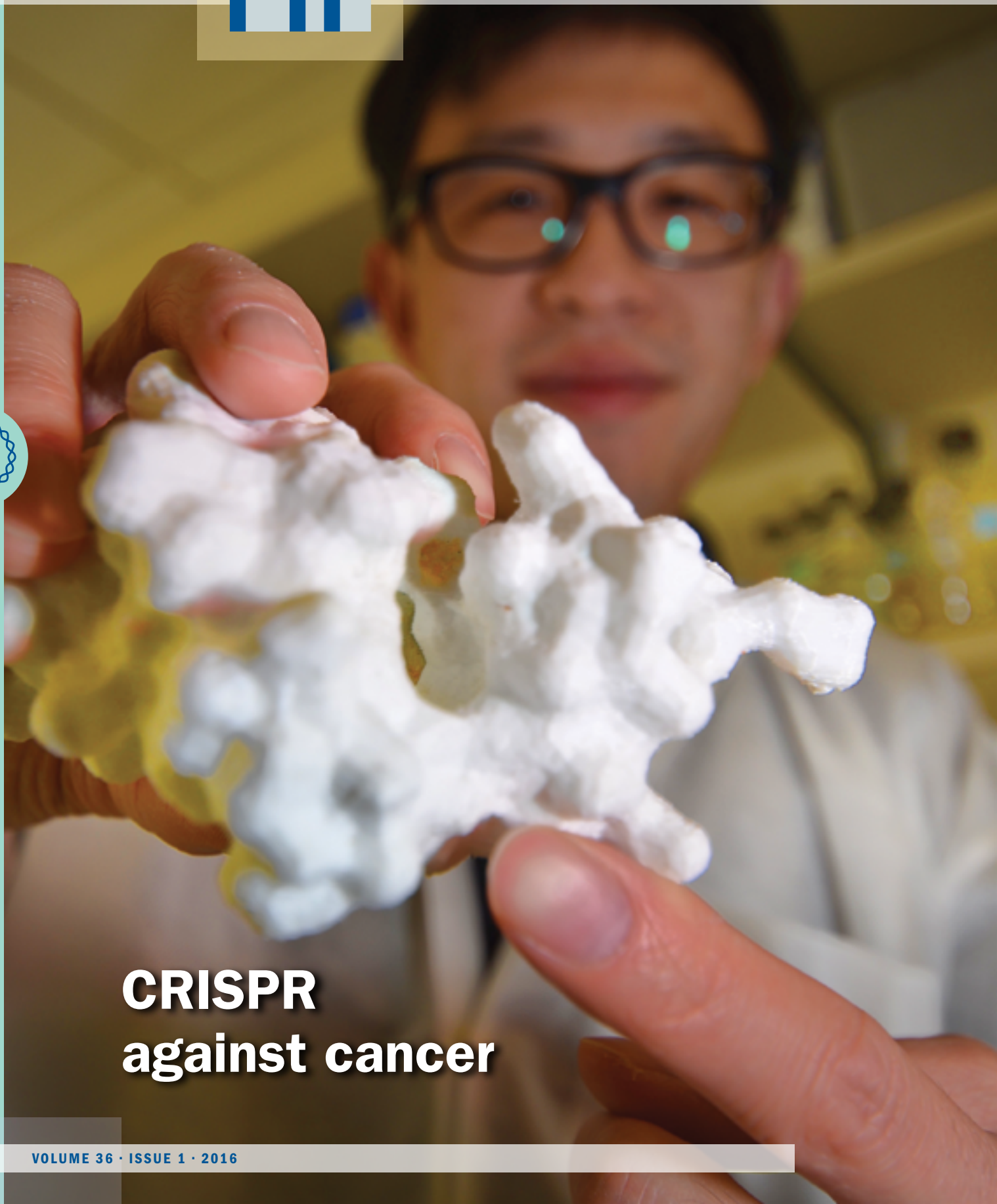

CRISPR against cancer

VOLUME 36 · ISSUE 1 · 2016

Cold Spring Harbor Laboratory

PRESIDENT'S MESSAGE

Congratulations to the faculty of Cold Spring Harbor Laboratory, who last year secured 46% of the National Institutes of Health (NIH) research grants they applied for—a success rate that far surpasses the national average of 17%! This achievement comes at a time of increasing competition for shrinking federal dollars that support basic research.

The decade-long downward trend in government funding is undermining our nation's scientific research infrastructure. Thus I also applaud the creation of the Science Philanthropy Alliance, whose 5-year goal is to boost philanthropic giving to basic research by $1 billion a year, an increase of 50%. Alliance members—the Gordon and Betty Moore Foundation; the Kavli Foundation; the Simons Foundation; the Alfred P. Sloan Foundation; the Howard Hughes Medical Institute; and the Research Corporation for Science Advancement—want other institutions and individuals to emulate their example in furthering basic research. Their effort complements rather than competes with our own fundraising campaigns.

The Alliance, which has selected CSHL as a key institution making smart investments in furthering basic research, benefits from the leadership of our nation's great philanthropists, including Marilyn Simons, Vice Chairman of our Board of Trustees, and Honorary Trustee Jim Simons. With their help, the Alliance is advising new, emerging and current philanthropists on how to effectively support basic research. Our own experience shows that funding innovative investigator-led ideas leads to powerful breakthroughs, and the Laboratory has several initiatives that leverage private funds to nurture fundamental discovery science. (Read more at www.cshl.edu/development)

We are grateful to Marilyn and Jim for their engagement in our science. We applaud, too, their monumental decision to join the "Giving Pledge" and donate the great majority of their wealth during their lifetimes. The Simons Foundation is one of the largest science grant-makers in the country, carrying on the American tradition of philanthropy supporting science. Speaking as a scientist and on behalf of my fellow researchers, we appreciate the foresight of the Simons Foundation and the Science Philanthropy Alliance.

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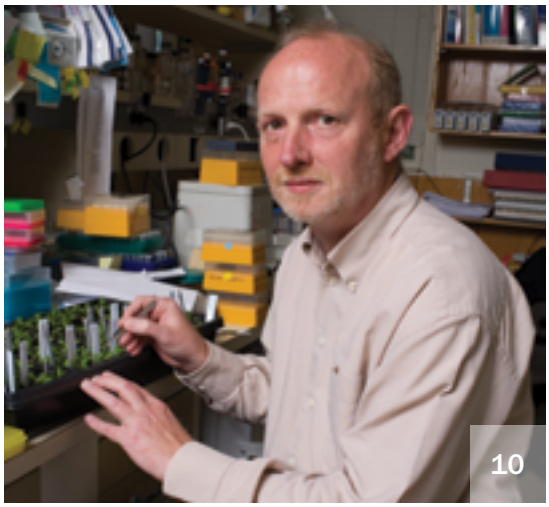

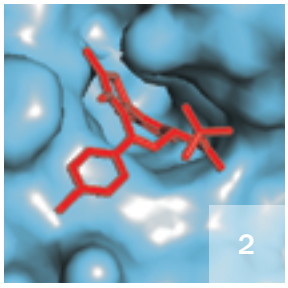

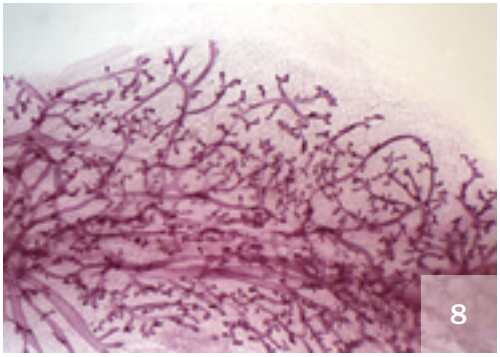

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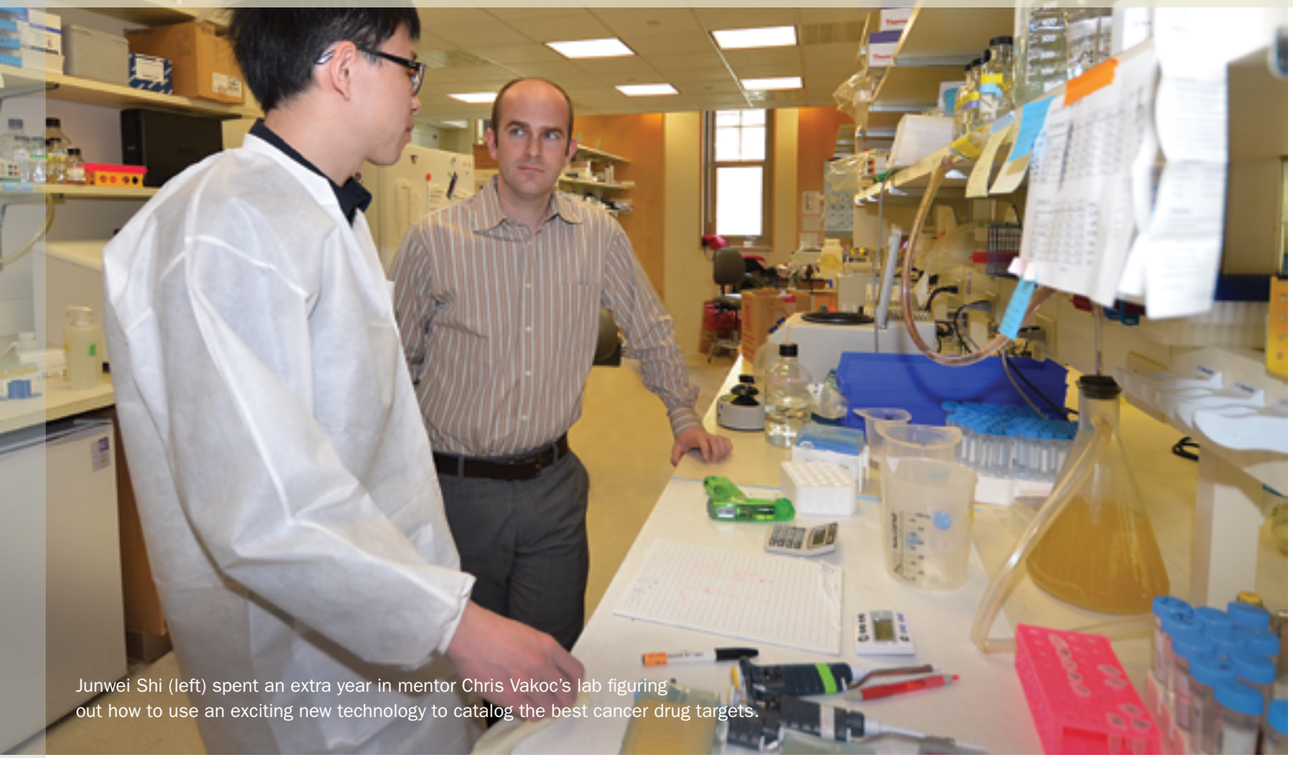
FEATURES

FACULTY & FRIENDS

On the cover and on the web:
Junwei Shi of the Vakoc lab points to a binding pocket on the surface of a protein called BRD4 that when blocked causes leukemia cells to die. Shi discovered a way to use the CRISPR gene editing tool to compile a comprehensive catalog of such binding sites, across cancer cell types.

Harnessing CRISPR to target cancer



Junwei Shi (left) spent an extra year in mentor Chris Vakoc's lab figuring out how to use an exciting new technology to catalog the best cancer drug targets.

As it is embraced enthusiastically by biological researchers in labs worldwide, the powerful new gene-editing technique called CRISPR has inspired grand visions of curbing malaria, curing genetically caused human illnesses, and even bringing extinct animals like the woolly mammoth back to life.

At Cold Spring Harbor Laboratory, the lab group headed by Associate Professor Christopher Vakoc, upon learning of CRISPR a few years ago, decided to take a practical approach, and in 2015 they published a method of using the technique to find new targets for cancer drugs. In so doing, they demonstrated that "The CRISPR Craze," whatever its long-term potential, has immediately powerful implications for human health.

CRISPR refers to a method of editing the DNA letters of genomes, as one would delete and add text with a word processor. Researchers have been modifying genomes for decades, albeit painstakingly. They have never had a method so simple, precise and inexpensive.

It's based on a simple DNA-cutting mechanism that bacteria use to defend themselves against viruses and other foreign invaders. CRISPR is a two-component search-and-destroy machine consisting of an enzyme called Cas9 that cuts DNA, and a short strand of RNA that it carries, like a searchlight, to spot specific DNA sequences in a genome. Upon reaching its target along the double helix, the guide RNA positions the Cas9 enzyme over the sequence, which the enzyme then snips out.

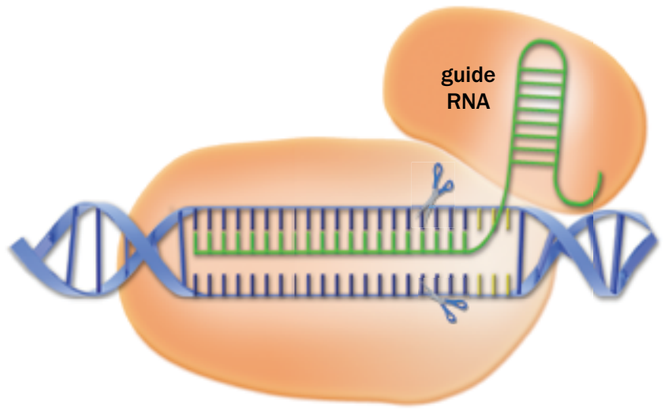
In 2012–13, scientists in California, Massachusetts and Sweden demonstrated that CRISPR can be used to cut and paste DNA in many living things, including human cells, even though Cas9 is not native to the human system.

An inspiring decision

Along with the rest of the research world, Junwei Shi, at the time a Ph.D. student in the Vakoc lab, read exciting reports about using CRISPR in human cells and started to think about adapting it for his own work. This happened to coincide with an important meeting in 2013 with his thesis committee. "They told me, 'It's time to finish your Ph.D. and move forward with your training!'" Shi recalls.

But, says Vakoc, "he actually begged them to stay." He wanted another year in Vakoc's lab to try CRISPR, even though he didn't need to do that work, as Vakoc explains. "Junwei already had published several important papers, one of which reported the discovery of a powerful leukemia drug target called BRD4 that's now the focus of a clinical trial. Even though he had completed all his requirements to graduate, he was driven to make more discoveries, even if it meant a delay in moving to the next career step—which we all found pretty inspiring."

That extra year began slowly, as Shi spent 3 months optimizing CRISPR and tackling the question of how to deliver it into human leukemia cells. Once he could use it to cut DNA anywhere in the human genome, the question, he says, became: "*where* do we want to cut along *specific* genes?" The question gets at why CRISPR has generated so much excitement: it is a reprogrammable cutting machine. The DNA scissors can be sent to any target in the genome, simply by providing the correct genomic "address" to the RNA guide strand carried by the Cas9 enzyme.



CRISPR-Cas9 is a reprogrammable DNA cutting machine that is being used to edit genomes in many organisms for research purposes. Its primary component, the Cas9 enzyme (orange), cuts genomic DNA (blue). The enzyme is directed with exquisite specificity to its target—essentially any sequence along the genome—by hitching it to a strand of "guide" RNA (green) whose sequence is complementary to that of the DNA target. Upon finding and pairing with it, Cas9 snips out the target segment. It can either be deleted or replaced with another DNA sequence (not shown here).

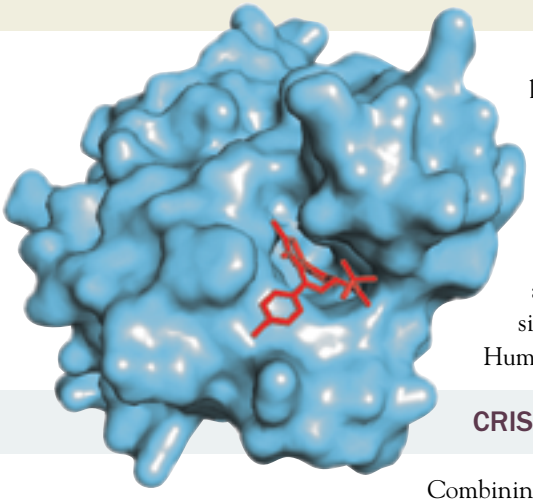
Emmanuelle Charpentier (left) and Jennifer Doudna, two of the pioneers in adapting CRISPR-Cas9 to genome editing, seen last year at a major Cold Spring Harbor meeting on CRISPR that drew over 400.

As it happened, CSHL had expertise in making the kind of short RNAs needed to program Cas9. Greg Hannon (now an adjunct professor), along with Leemor Joshua-Tor and other CSHL colleagues, made important discoveries in the early 2000s about the workings of another genome-regulating system called RNA interference, or RNAi. Hannon's team built large libraries of small RNA molecules which could be used in experiments to "knock down" expression of any of the 21,000 human genes, often in a single experiment. With CRISPR, one doesn't knock down, *i.e.*, reduce, expression of a gene as in RNAi but instead knocks out the gene or a part of it entirely, either deleting it or replacing it with a modified sequence.

But, as Shi asked: *where* to cut? He started with a gene the team knew well. He picked *BRD4*, which encodes a protein that leukemia cells cannot live without, as the team discovered in 2011. That finding led to the current clinical trial of a drug that inhibits BRD4 and has a powerful killing effect on cells of acute myeloid leukemia (AML) while not causing significant harm to healthy cells.

"Junwei tested CRISPR by targeting Cas9 to snip out different parts of the *BRD4* gene in human leukemia cells," says Vakoc. This led to a big discovery. "Junwei noticed that of the many places to cut the gene, there is only one kind of sequence to cut if you want to kill the leukemia cells efficiently. You cut the DNA that encodes the pockets on the surface of the BRD4 protein where our drug, JQ1, binds."

This provided a basis on which to apply CRISPR to leukemia and cells of other cancer types, on a massive scale: program CRISPR to target binding pocket motifs—DNA



3D model of the BRD4 protein, showing how JQ1 (red), a drug now in clinical trials, blocks a key binding pocket that leukemia cells depend upon to grow and spread.

letters that encode such features on proteins of interest. A comprehensive set of such motifs is known, thanks to extensive genome annotation work performed since completion of the Human Genome Project.

CRISPR scanning

Combining this knowledge with Shi's optimization of CRISPR and methods of producing massive numbers of small RNAs, the team arrived at a powerful way to discover new drug targets, not only in leukemia, but across all cancer cell types. Vakoc and Shi call their method CRISPR scanning. It's the fruit of Shi's "extra" year—which extended to 2 years before Shi this winter accepted a tenure-track assistant professorship in the cancer biology department at the University of Pennsylvania.

In a single experiment, the team uses thousands of CRISPR "scissors" to cut out different DNA regions in a given gene. Each Cas9 enzyme is loaded with a different guide RNA. When piggy-backed to an inactive virus, each enzyme and its guide RNA infects a single cancer cell, grown in a culture dish. "It's called multiplexing, and in one experiment, you can make thousands of different cuts in the genome and track the impact on cancer cell death," Vakoc says.

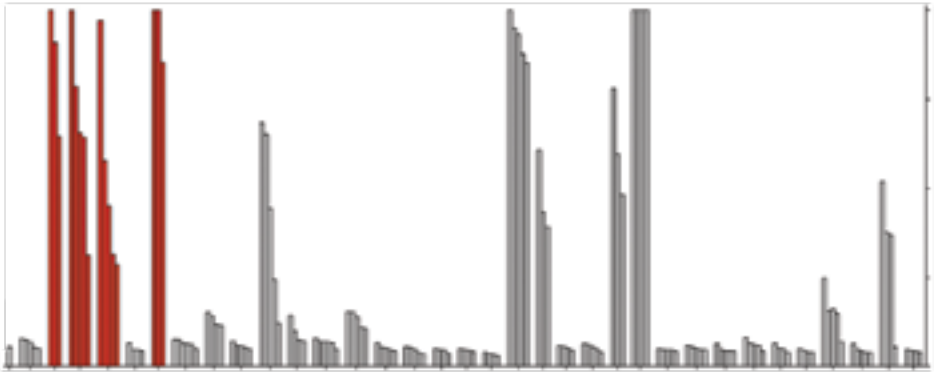
In effect, the scientists use CRISPR to mimic, in thousands of binding pockets at once, the impact of a drug blocking the pocket, just as JQ1 blocks a key pocket on BRD4. Most of these actions won't slow growth of the cancer cell. It's the few that do—pockets that, when blocked, cause cells to die—that the team is looking for. These needles in DNA haystacks are made visible on what the scientists call a Manhattan plot—a few "skyscrapers" of cell-killing activity along an otherwise flat horizon.

"This technology allows us to map every active surface of every protein that every cancer requires," says Vakoc. "There are other ways of finding proteins that cancer cells need. Our approach reveals not just the proteins but information about which surface features on them that we want drugs to hit in order to have dramatic killing effects."

The team used CRISPR to screen 192 gene-regulatory domains of interest in mouse AML cells, and found all six previously known drug targets as well as 19 previously unidentified binding pockets that the cells could not live without. These are immediate targets of interest in drug discovery—DNA addresses of binding sites for future drugs that could have powerful killing effects on cancer cells. Adding to the immediacy of the potential benefit, this screen tested only those targets that current pharmaceutical science already knows how to hit. They're "druggable targets," in the language of chemists.

Ahead of the competition, the Vakoc lab is able to use CRISPR to find these powerful targets in cancer thanks in part to the dedication of Junwei Shi. "His success," says Vakoc, "is a good example of what young researchers at the Lab can aspire to. You can come to Cold Spring Harbor and help advance a field."

Peter Tarr

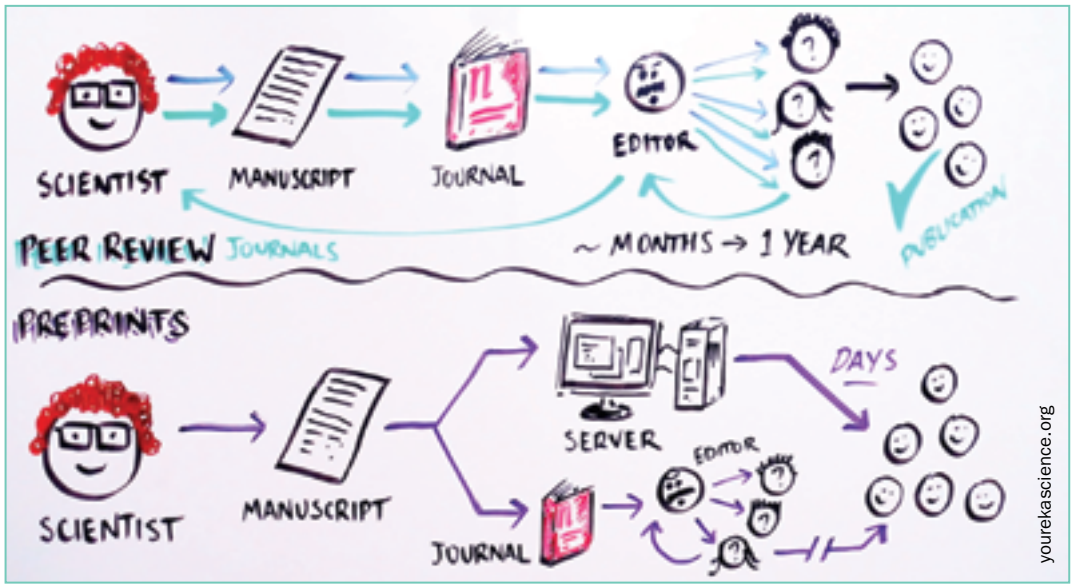


Shi and Vakoc hitched numerous RNA guide molecules to Cas9 enzymes in order to make literally thousands of cuts in leukemia cancer genes. This "Manhattan plot" registers the "skyscrapers" where particular binding pockets on the cancer cells, when blocked or inactivated, have powerful killing effects on the cancer. Red peaks are previously known targets. Grey peaks are among 19 powerful new targets discovered in a single experiment.

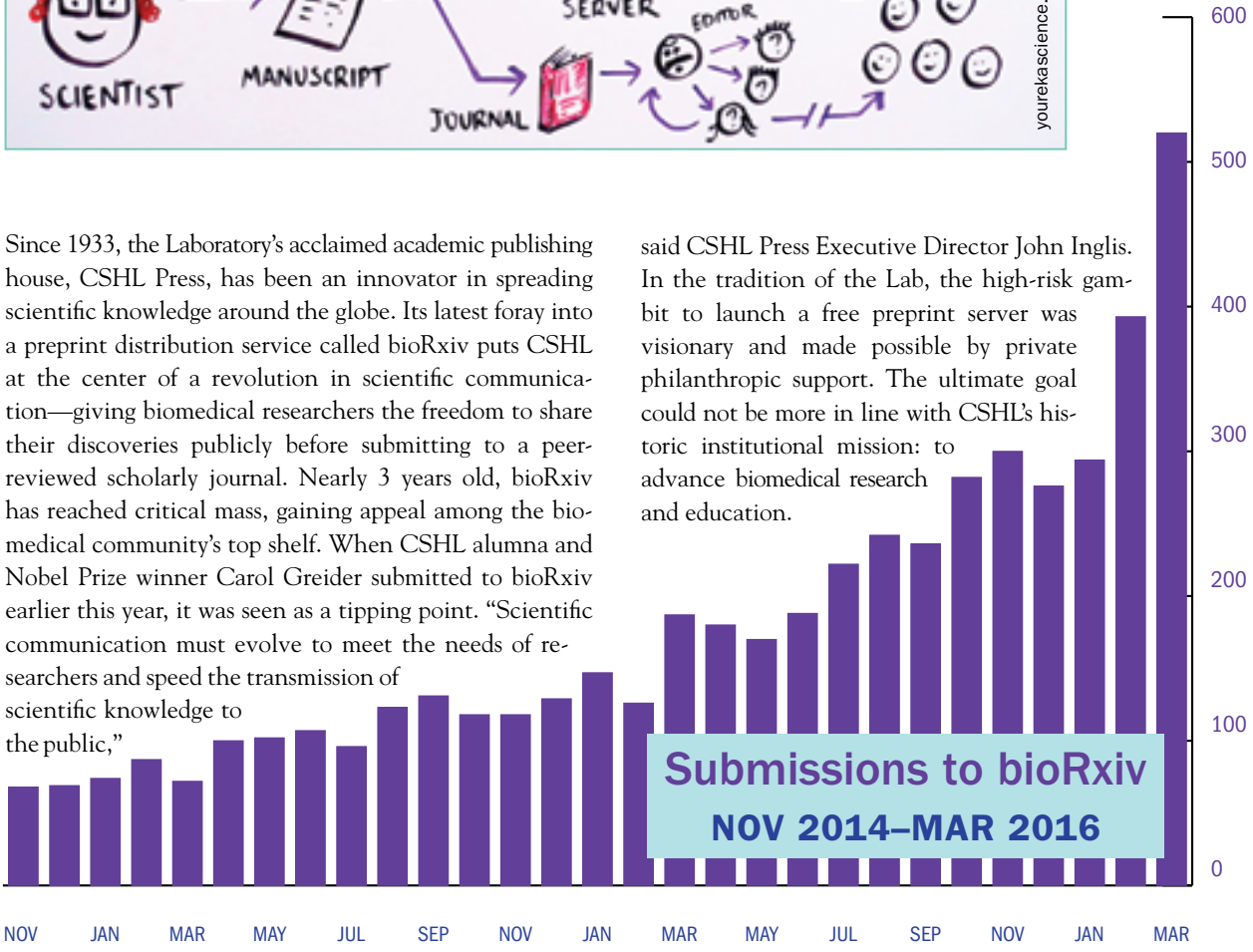
High risk, high reward

bioRxiv beta

THE PREPRINT SERVER FOR BIOLOGY



Since 1933, the Laboratory's acclaimed academic publishing house, CSHL Press, has been an innovator in spreading scientific knowledge around the globe. Its latest foray into a preprint distribution service called bioRxiv puts CSHL at the center of a revolution in scientific communication—giving biomedical researchers the freedom to share their discoveries publicly before submitting to a peer-reviewed scholarly journal. Nearly 3 years old, bioRxiv has reached critical mass, gaining appeal among the biomedical community's top shelf. When CSHL alumna and Nobel Prize winner Carol Greider submitted to bioRxiv earlier this year, it was seen as a tipping point. "Scientific communication must evolve to meet the needs of researchers and speed the transmission of scientific knowledge to the public," said CSHL Press Executive Director John Inglis. In the tradition of the Lab, the high-risk gambit to launch a free preprint server was visionary and made possible by private philanthropic support. The ultimate goal could not be more in line with CSHL's historic institutional mission: to advance biomedical research and education.



Submissions to bioRxiv
NOV 2014–MAR 2016

Watson School 2016 Ph.D.s

Robert G. Aboukhalil

McGill University

National Institutes of Health Predoctoral Trainee

Starr Centennial Scholar

Over the last few years, I have greatly enjoyed working with Mike Wigler and Mickey Atwal at the intersection of data science and cancer genomics. Computational biology is a growing and exciting field, where vast amounts of data are generated every day, and where many biological and computational insights remain to be gleaned from these data. I hope to be part of this adventure for many years to come.

Arkarup Banerjee

University of Delhi

Tata Institute of Fundamental Research

Crick-Clay Fellow

Goldberg-Lindsay Fellow

In my research on the brain's olfactory system in the laboratory of Florin Albeanu, I've had a vivid experience of how the initial fluttering of an idea, after being churned over and over again, slowly begins to take shape and eventually transforms itself into something more tangible. I have matured as a scientist and I feel confident to embark on future scientific journeys.

Colleen Mara Crowley-Carlston

Harvard University

National Science Foundation Graduate Research Fellow

John and Amy Phelan Student

As a clinical molecular genetics fellow at the University of Utah I have the best job in the world. Every day in the lab or clinic I help families, some of whom have spent over a decade on diagnostic odysseys, figure out the genetic conditions affecting their loved ones. This was the kind of impactful work I had always dreamed of doing, and thanks to the education I received at the Watson School in the lab of Christopher Hammell, I was able to realize that dream.

Silvia Fenoglio

University of Turin

Elisabeth Sloan Livingston Student

I set out in my graduate research with the ambitious goal of learning about the function of genes during tumor growth in order to uncover cancer vulnerabilities. I now know how to tackle this problem in preclinical models of pancreatic cancer, and importantly I acquired a whole panel of technical and intellectual skills in the Hannon and Sordella labs that I can apply to any career and direction life will steer me to.

Tyler Harley Garvin

University of Southern California

National Institutes of Health Predoctoral Trainee

John and Amy Phelan Student

Mike Schatz, my advisor, connected me to the cutting edge of sequencing and genomics technologies and taught me so much about computational biology and how to develop my ideas in a way that I could write about and present them confidently. My experience in Mike's lab and my interactions within the CSHL community now enable me to step out of my Ph.D. and be ready for what comes next.

Anja Fides Hohmann

University of Cambridge

Boehringer Ingelheim Fonds Fellow

Leslie C. Quick, Jr. Fellow

Starr Centennial Scholar

Over the last few years, I have greatly enjoyed working in Christopher Vakoc's lab, where I evaluated chromatin regulators as drug targets in leukemia. Translation of our findings to the clinic is a major motivation, and I was fortunate to gain insight into the pharmaceutical drug development process as part of my Ph.D. experience. As I now prepare to move on, I take with me many fond memories of my time at CSHL.

Lisa S. Krug

Skidmore College

National Institutes of Health Predoctoral Trainee

My thesis project in Josh Dubnau's lab focused on testing the role of transposons—"jumping genes"—in neurodegenerative diseases. The peculiarities of studying transposons, as well as the way Josh conducts research and the relative novelty of the study of transposons in the brain, permitted me to do very exploratory and creative science, an intellectually stimulating gift for which I will be forever grateful.

Winship Herr Teaching Award

"When our students do great and amazing things, as they often do, there's some part of that greatness that we attribute to their teachers."

Selected by the students, the award has recognized professors for excellence and creativity in teaching.

2016 Mickey Atwal: Quantitative Biology
2015 Bo Li: Scientific Reasoning & Logic - Neuroscience
2014 Zach Lippman: Genetics
2013 Mickey Atwal: Quantitative Biology
2012 Mike Schatz: Quantitative Biology
2011 Mike Schatz: Quantitative Biology
2010 Josh Dubnau: Genetics
2009 Greg Hannon: Scientific Reasoning & Logic - Study Section
2008 Glenn Turner: Scientific Reasoning & Logic - Neuroscience
2007 Josh Dubnau: Genetics
2006 David Spector: Cell Structure & Function

One experiment

Women consistently report that nursing is easier after a first pregnancy. Some remarkable genomic work led by Professor Greg Hannon provides a biological reason for this effect and shows more broadly how bodily experiences can prepare us to respond to future stimuli.

The razor-thin slice of breast tissue pictured here is sampled from a mouse 6 days after becoming pregnant. Delicate branching structures support the ducts through which milk will flow. Spurred by estrogen and progesterone, breast cells proliferate and the ductal network becomes more profuse. After nursing, the tissue "involutes"—returns to its less dense pre-pregnancy state. Yet in subsequent pregnancies, breast tissue expands much more rapidly at the first hormonal signal, and becomes so densely packed with ducts that a light shining from below can barely pass through a slice like the one shown here.

"It's as if the gland already knows these hormones," says Camila dos Santos, now a CSHL Assistant Professor. As a Hannon lab postdoc, dos Santos devised a technique that helped the team reveal the secret to what is in fact a life-long biological memory of an earlier pregnancy. In one experiment, dos Santos used a marker she'd discovered to classify six types of breast cells. She then prepared maps revealing all the positions along the genome where methyl molecules "marked" the double helix in each cell type, before and after a first pregnancy.

The team's discovery: during a first pregnancy, these epigenetic marks are wiped away from many genomic positions. Those positions become occupied by a gene-regulating transcription factor called Stat5a. Should hormones signal another pregnancy, breast cells already know to activate adjacent genes—which regulate cell proliferation, ductal growth and lactation. After a first pregnancy, therefore, breast cells are primed for nursing. Dos Santos now seeks to unravel the mystery of how pregnancy-induced changes, perhaps including these, protect some women from breast cancer.

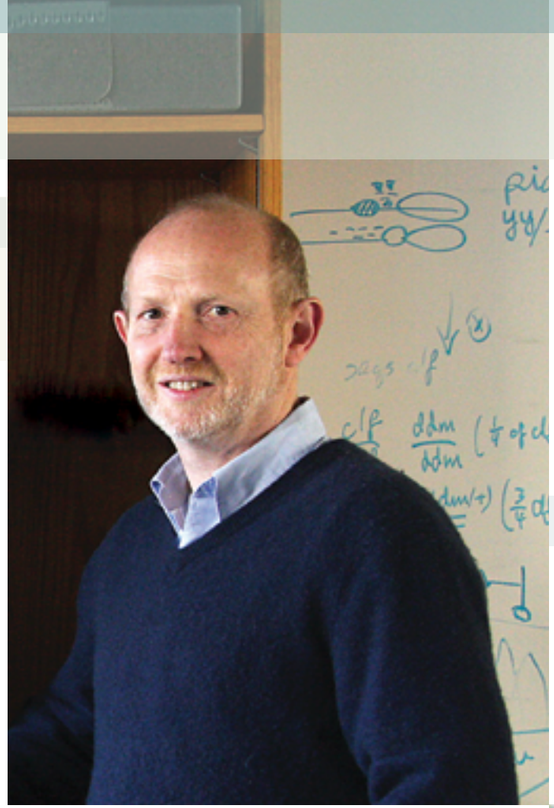
Peter Tarr

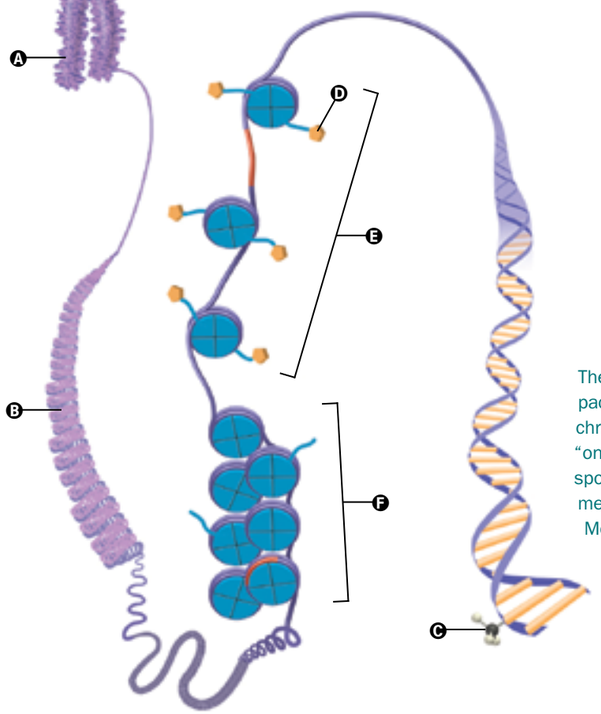
RESEARCH PROFILE

Robert Martienssen

In the 63 years since Watson and Crick discovered the double helix, we've all learned that the genome is "the blueprint for life." For the last 25 years, CSHL's Rob Martienssen, as much as any other scientist, has enabled us to understand that while this is true, having the blueprint—whether for single-celled yeast, plants or people—is only the first step in understanding what makes genes act the way they do.

Martienssen, a CSHL Professor, Fellow of the Royal Society, and Investigator of the Howard Hughes Medical Institute and Gordon and Betty Moore Foundation, is a plant geneticist who has made trailblazing discoveries in the field of epigenetics. That term, proceeding from the prefix "epi," directs our attention to a realm of action that occurs literally "on" or "on top of" the genome.





Think of the double helix as a long, twisting ladder, to which chemical tags of various kinds can attach. When a methyl group (chemical formula CH_3) binds at one of the ladder's "rungs"—*i.e.*, a DNA base—it can change the activity of the underlying gene, typically silencing it. Why? The protruding methyl group prevents specialized machines from attaching at that genome position and copying the DNA—the first step in gene expression.

Martienssen and collaborators have been among the leaders in assembling a comprehensive account of how several overlapping layers of epigenetic signals function, interacting in complex ways to regulate gene activity.

The DNA of living things is compressed into inconceivably small packages called chromatin (B), which is itself bundled to form chromosomes (A). Epigenetic signals are chemical modifications "on top of" DNA itself, or the histone proteins around which DNA is spooled to form chromatin bundles. The drawing at left shows how methyl groups (CH_3) can attach directly to the double helix (C). Methylation and other epigenetic marks, collectively called epigenetic factors, can also attach to tiny "tails" on histone proteins (D). Their presence or absence helps determine whether the DNA within the bundle is "open" and thus accessible to DNA copying machinery; or "closed" and inaccessible. Genes within DNA in open chromatin areas (E) can be expressed; in closed areas (F), they are silenced.

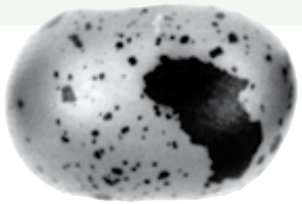
This integrated account is of great relevance in efforts to coax plants to higher yields or adapt to changing environmental conditions. It's also important in efforts to understand human diseases, from neurodegeneration to cancer. More broadly, it tells us something profound about life. Just like genetic mechanisms, epigenetic mechanisms that regulate genes are a product of evolution operating over hundreds of millions of years. Keeping genomes intact, and, in Martienssen's words, ensuring "the immortality of the germline," they are an important part of what makes life so remarkably robust and enduring.

Moving beyond Mendel's Laws

In the 1980s, as an undergrad at Cambridge in his native England, Martienssen received a classical training in genetics. To his inquiring mind, "anything that *contravened* formal genetics was interesting." Indeed, Mendel's "laws" couldn't account for plenty of things—for instance, the way a plant or animal's genes respond to the environment. Events that occur during the life of an organism, such as stress, can significantly alter gene activity and function, and these changes can be passed down to progeny.

Martienssen and McClintock in the greenhouse, *ca.* 1990.

The scientist who was Martienssen's greatest inspiration and influence did advance theories on such phenomena. Her name was Barbara McClintock, and in a way that he could not have anticipated, his discoveries have proven the existence of epigenetic mechanisms she predicted decades earlier. He had the thrill of working at McClintock's side in his first 2 years at CSHL.



McClintock linked spots on corn kernels to jumping genes.

Martienssen's earliest research extended McClintock's most famous observation. Working "in Barbara's favorite organism, maize," the newest CSHL faculty member demonstrated what happened when a chunk of DNA literally jumped into a new position in the genome. Back in the 1940s McClintock had described "jumping genes," or as scientists would later call them, transposable elements (TEs). It took 40 years for the importance of this discovery to be recognized, in the form of a Nobel Prize.

Having observed curious patterns of spots in corn kernels, McClintock had famously traced them to the impact of a TE jumping into a gene that controlled kernel color. In his first published paper, Martienssen showed how a different transposon generated similar patterns of stripes in leaves of the maize plant.

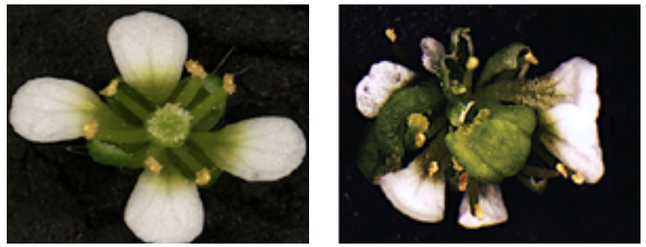
He succeeded in identifying the gene into which the transposon had jumped; more important, he discovered what was in effect an epigenetic on-off switch. When the transposon was unmethylated, the gene was silent; when methylated, the gene came back "on." This suggested something broader that Martienssen and others would flesh out more fully: *methylation keeps transposons in check*—prevents them from hopping at will from genomic place to place, interrupting genes, and inevitably, threatening the viability of the organism. Epigenetic control, it became clear, was a kind of genome defense mechanism.

In future years, Martienssen and others would discover that various epigenetic mechanisms were interrelated. His experimental approach was subtractive. "I'm a geneticist," he explains, "and so I tend to take things away or look for things that are missing and find out what goes wrong." For instance, believing methylation to be important in the suppression of TEs, he sought further evidence by finding mustard plants (*Arabidopsis thaliana*) in which methylation was notably absent. He traced this oddity to a mutated gene called *DDM1*.

DDM1 showed Martienssen something no one expected, and for a long time, few believed: that methylation and another gene-regulating process called chromatin remodeling "were actually two sides of the same coin."

Chromatin is the packaging used by cells to bundle their genomes; it consists of an organism's DNA, spooled around proteins called histones. When chromatin is very tightly bundled, the DNA it contains is inaccessible to the cell's gene-expressing machinery. Only DNA in loosely packed chromatin can be expressed. Chromatin remodeling factors help determine what genes can be expressed at a given moment in time. Martienssen's discovery that methylation can affect chromatin's form shed light on a poorly understood process.

The lab's epigenetic experiments focused on a structural location in chromosomes, the centromere, where DNA sequences were known to repeat over and over again. These "centromeric repeats" were considered "junk DNA" by some, but Martienssen, thinking of McClintock, suspected otherwise. They were a signature of TEs.



Loss of methylation in mustard plants results in massive activation of TEs; the impact is devastating.

In 2004, a Watson School graduate working in Martienssen's lab named Zachary Lippman (now a CSHL Associate Professor) was first author on a paper Martienssen considers one of his most important. By this time, another project that Martienssen had worked on with CSHL's master gene sequencer, Dick McCombie, and others, was complete. They had assembled the first complete plant genome, that of the mustard plant *Arabidopsis*.

Having the sequence in hand enabled Martienssen and Lippman to design experiments showing that areas of repeat DNA in and around centromeres and in a few other dense, knob-like regions in the *Arabidopsis* genome—composed of tightly packed chromatin—were full of TEs. Importantly, their DNA was peppered with methylation marks, accounting for their silencing. But in plants in which *DDM1* was mutated—plants that lacked methylation—the team showed how TEs sprang to life, harming genome integrity and eventually rendering the plant sterile.

This and related work validated a momentous theory McClintock had ventured decades before, without benefit of the modern tools of molecular biology. She deduced that TEs, by jumping into or next to genes, could actually shape evolution. Martienssen's group now could show a specific example, a TE that jumped next to a gene in *Arabidopsis* that controls the time to flowering. Since the TE was methylated, the timing gene was effectively *controlled* by the TE, which thus determined the plant's developmental fate. Says Martienssen: "The way in which that gene was controlled epigenetically was exactly what Barbara predicted when she called TEs 'controlling elements.' That's how she discovered epigenetics—because TEs can control genes."

This same line of research brought Martienssen's work into conjunction with that of CSHL's Greg Hannon, then investigating a recently discovered mechanism called RNA interference (RNAi). Like epigenetic mechanisms, RNAi was native to cells and could silence gene expression. In mutant *Arabidopsis* plants, and then in fission yeast, which have very simple genomes, Martienssen's team identified a protein that would later be named Argonaute—a key component of the RNAi gene silencing machinery.

In 2002, the journal *Science*, citing "electrifying discoveries" by Martienssen and several others, called advances in understanding RNAi and epigenetics the "Breakthrough of the Year." Martienssen had the satisfaction of seeing his research on methylation and chromatin remodeling converge with new experiments on RNAi in yeast. By subtracting the RNAi machinery from yeast cells, he showed that tightly packed chromatin (the TE-rich portion, which biologists call heterochromatin) could no longer form properly at the centromere of chromosomes, causing cell division to go awry.

This work revealed how small RNAs generated by repeat DNA sequences in heterochromatin are needed to guide chromatin modification. Today, says Martienssen, it's understood that "all these mechanisms—RNAi, chromatin modification, DNA methylation—help to silence heterochromatin. They depend on each other. If you take any one of them out you tend to disrupt the other two. Somehow it all works to keep the genome intact, and to prevent TEs from damaging anything."

In recent years, the lab has tackled another big mystery. With every generation there is a complete erasure of epigenetic marks in germline cells, sperm and eggs, in many plants and animals. This "starting afresh" would seem to provide a brief period during "reprogramming" for transposons to run amok. The lab's work on pollen has revealed an astonishing reprogramming trick that skirts potential disaster. Individual pollen grains consist of two cell types: emergent sperm cells and the nurse cells that support them. A nurse cell, bearing the same genome as the corresponding sperm cell, "de-represses" its TEs by removing epigenetic marks, and then makes small RNAs (called easiRNAs) to mark the vacated genome positions. These small RNAs are later taken up by the sperm cell, in which they guide methylation marks to the proper DNA sequences, ensuring that TEs in the male germline will remain silenced genome-wide.

Solving environmental problems

Martienssen is sanguine about future work in the lab. "As we learn more and more about plant biology, I am confident we can come to grips with some really big problems," he says, among them boosting global food production and slowing climate change. There are practical limits to new variation that plant breeders can generate in staple crops, he notes. One way of rising above approaching thresholds is to discover better ways to clone prized varieties, while avoiding pitfalls like those seen in oil palm [see box below]. To clone a prized plant is to reproduce it in the absence of fertilization, *i.e.*, without having it reproduce sexually—an act that inevitably involves "shuffling the genome" and wiping the genetic slate clean. The lab is engaged in adapting a process of asexual reproduction called apomixis, which occurs naturally in some species, such as dandelion. It involves tricking a prized variety of a sexually reproducing plant, say an important staple like rice or wheat, to clone its own seeds and thereby ensure that the next generation retains all of the superior characteristics of the parent.

A second forward-looking project pivots on the fact that "plants are the fundamental arbiters of climate change." As Martienssen notes, "Plants fix carbon—they take in carbon dioxide and they expel oxygen. Animals do the reverse." A first step is to produce renewable, carbon-neutral biofuels—fuels made from living rather than fossilized plants. "In fossil fuels, we take carbon that plants successfully took out of the atmosphere eons ago and reintroduce it!" The ultimate solution, however, is to grow a low-maintenance, highly adaptable, carbon-loving plant like duckweed in vast quantities, and then to sequester the plants so that the carbon they remove from the air remains fixed in perpetuity.

Peter Tarr

The secret of bad *karma*

The fruit of 25 years of research is well represented in this image of the ruined fruit of an oil palm clone, the "victim," improbably enough, of bad *karma*. In a paper that brings a career's worth of epigenetics insight to bear on a costly problem in tropical agriculture, Martienssen and colleagues showed in a 2015 *Nature* cover story that loss of methylation by a single transposable element (TE) embedded in the oil palm genome has caused the loss of hundreds of millions in ruined crops. When high-yielding trees are cloned, they're grown in culture, from detached leaves. The team traced the problem to a gene called *mantled*, "which, in a way McClintock would have appreciated, happens to be under the control of an adjacent TE," called *karma*. The result: ruined "mantled" fruit—the result of an oil palm leaf, detached from its meristem, having lost the ability to manufacture small RNAs to guide *karma* methylation. Such ill-fated clones can now be spotted in advance, saving breeders the effort of raising trees that yield worthless fruit.

Double Helix Day 2016

DNA Damage, Repair & Mutation

Monday, February 29 - Cold Spring Harbor Laboratory

Invited speakers:

James Watson | Cold Spring Harbor Laboratory | *Discovering the double helix: Going for gold!*

Leona Samson | Massachusetts Institute of Technology | *The pros & cons of DNA repair*

Thomas Kunkel | National Institute of Environmental Health Sciences | *Generating & repairing mismatches during eukaryotic DNA replication*

James Haber | Brandeis University | *How cells fix their broken chromosomes*

Serena Nik-Zainal | Wellcome Trust Sanger Institute, UK | *The genome: An archaeological record of mutational processes*

Organized by:

David Stewart | Executive Director, Cold Spring Harbor Laboratory Meetings & Courses Program

Bruce Stillman | Executive Director & CEO, Cold Spring Harbor Laboratory

Endowed by James Watson, this special annual celebration ("Double Helix Day") is intended to coincide with the actual date that he and Francis Crick discovered the double helix structure of deoxyribonucleic acid (February 28th 1953) in Cambridge, England. Each year, a theme related to DNA science is explored through a series of review-style talks aimed at a broad scientific audience. The theme of how cells repair damaged DNA and how mutations arise was selected as the theme of the 2016 celebration.

Faculty & Friends

Regeneron President elected CSHL Trustee

Dr. George D. Yancopoulos, Chief Scientific Officer of Regeneron Pharmaceuticals, Inc. and President of Regeneron Laboratories, has joined the CSHL Board of Trustees. "CSHL is applying basic research-driven discoveries in the clinic for the benefit of patients and Dr. Yancopoulos offers the unique perspective of a scientist, clinician and industry leader," said CSHL Chairman Jamie C. Nicholls. He joined Regeneron in 1989 as its Scientific Founder and is a

principal inventor and developer of the company's four FDA-approved drugs. Dr. Yancopoulos received an M.D. and Ph.D. in Biochemistry and Molecular Biophysics from Columbia University and has been a familiar face on the CSHL campus, having taught the Cloning course for many summers in the 1980s. Welcome (back) George!

CSHL neuroscience faculty on IARPA brain map

The Intelligence Advanced Research Projects Activity (IARPA)—part of the Office of the Director of National Intelligence—awarded a $21 million contract to discover the brain's learning rules and synaptic circuit design. Anthony Zador's lab is contributing a technique called BOINC (Barcoding of Individual Neuron Connections) to allow for a complete map of the precise location, shape and connections of all neurons to be generated. Zador's CSHL colleagues Alexei Koulakov and Je Lee are also part of this effort, spanning six institutions: The Wyss Institute at Harvard, MIT, Columbia University, Carnegie Mellon University and The Johns Hopkins University.

For his research, Zador was named one of 2015's "Top Global Thinkers" by the journal *Foreign Policy*. Previous honorees have spanned the gamut of achievement, from Pope Francis to Elon Musk. Zador and other honorees "have demonstrated extraordinary innovation, passion, creativity, and thirst, and have translated their ideas into action, impacting millions worldwide," wrote editors of the journal.

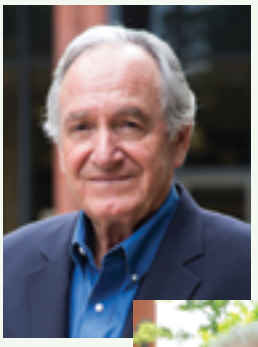
Fellow Jason Sheltzer wins Early Independence Award

Freshly-minted MIT Ph.D. Jason Sheltzer was recruited last summer as a CSHL Fellow to pursue cancer research, and he is off to a great start with a prestigious award from the National Institutes of Health. In 2015, the NIH awarded only 16 Early Independence Awards to outstanding early-career scientists, encouraging them to take risks and explore potentially ground-breaking concepts. At CSHL, Jason is studying genes that may make a difference between tumors that are benign and those that are cancerous and likely to spread to other parts of the body. Analyzing data from cancer patient survival studies, he is using the latest genome-editing technology—CRISPR (see page 2)—to establish the molecular links between these genes and cancer prognosis. Jason was also named to the 2015 *Forbes* magazine "30 under 30 in Science" list.

Cocktails & Chromosomes—join us!

We've taken CSHL science to an unlikely place: the local pub! With Cocktails & Chromosomes, the Lab is participating in a phenomenon called the "science café." Across the globe, non-scientists are gathering in bars and coffee shops to interact with scientists interested in sharing their academic insights. Armed with just a microphone—no slides, and strict orders to leave scientific jargon at the door—our researchers are letting neighbors in on the "WOW!" of their work.

Two of our investigators, Steve Shea and Tony Zador, have already been on stage at Finley's in Huntington, NY. Coming up are cancer researcher Chris Vakoc and plant biologist Zach Lippman. Keep up with all CSHL public events at cshl.edu, where you can sign up for our monthly email newsletter.



WSBS honorary degrees to a senior statesman and a scientific scholar

On the occasion of its 13th Commencement Convocation, the Watson School of Biological Sciences presented honorary degrees to Senator Tom Harkin and Dr. John Tooze.

Senator Harkin served for 10 years in the U.S. House of Representatives and five terms in the U.S. Senate, where his signature legislative achievement was the Americans with Disabilities Act. As chairman of the Senate panel that funds medical research, he was a champion of research on cardiovascular disease, cancer, Alzheimer's and other diseases. After succeeding Ted Kennedy as the Chairman of the Senate Health, Education, Labor and Pensions Committee in 2009, Senator Harkin retired from the Senate in 2015.

Dr. John Tooze is credited with invaluable contributions to the scientific enterprise. He served for 20 years as the Executive Secretary of the European Molecular Biology Organization (EMBO) and oversaw scientific infrastructure at the Imperial Cancer Research Fund/Cancer Research UK in London and at Rockefeller University in New York City. Dr. Tooze has had notable influence in scientific publishing as Deputy Editor of *Nature* and founding editor of the *The EMBO Journal*. CSHL Press published his influential *The Molecular Biology of Tumor Viruses* in 1974. Dr. Tooze has collaborated with Jim Watson and others on books and textbooks on topics in DNA, recombinant DNA and protein structure.

Join our Drive for 125

Impact the future with a legacy gift to Cold Spring Harbor Laboratory

"I hope my scientific legacy—and I know my financial legacy, through long-term support of pioneering research at CSHL—will benefit humankind and our fragile planet on which all life depends. Giving back is far more rewarding than receiving.

I had the very good fortune in the 1970s of 1) doing my Ph.D. research at CSHL under the mentorship of Jim Watson and Ray Gesteland, 2) collaborating with Rich Roberts, Rich Gelinas and Louise Chow on the discovery of split genes and RNA splicing, and 3) being inspired and encouraged by Barbara McClintock to follow my scientific instincts despite their conflicts with accepted dogma and choirs of naysayers."

~Daniel Klessig, Ph.D.

To discuss making a gift to CSHL, contact Diane Fagiola at 516-367-8471 or email fagiola@cshl.edu

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Dan, a new Helix Society member and former CSHL scientist, is currently a professor at Cornell University's Boyce Thompson Institute.

Cold Spring Harbor Laboratory
Cold Spring Harbor, NY 11724

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