# THE SEARCH

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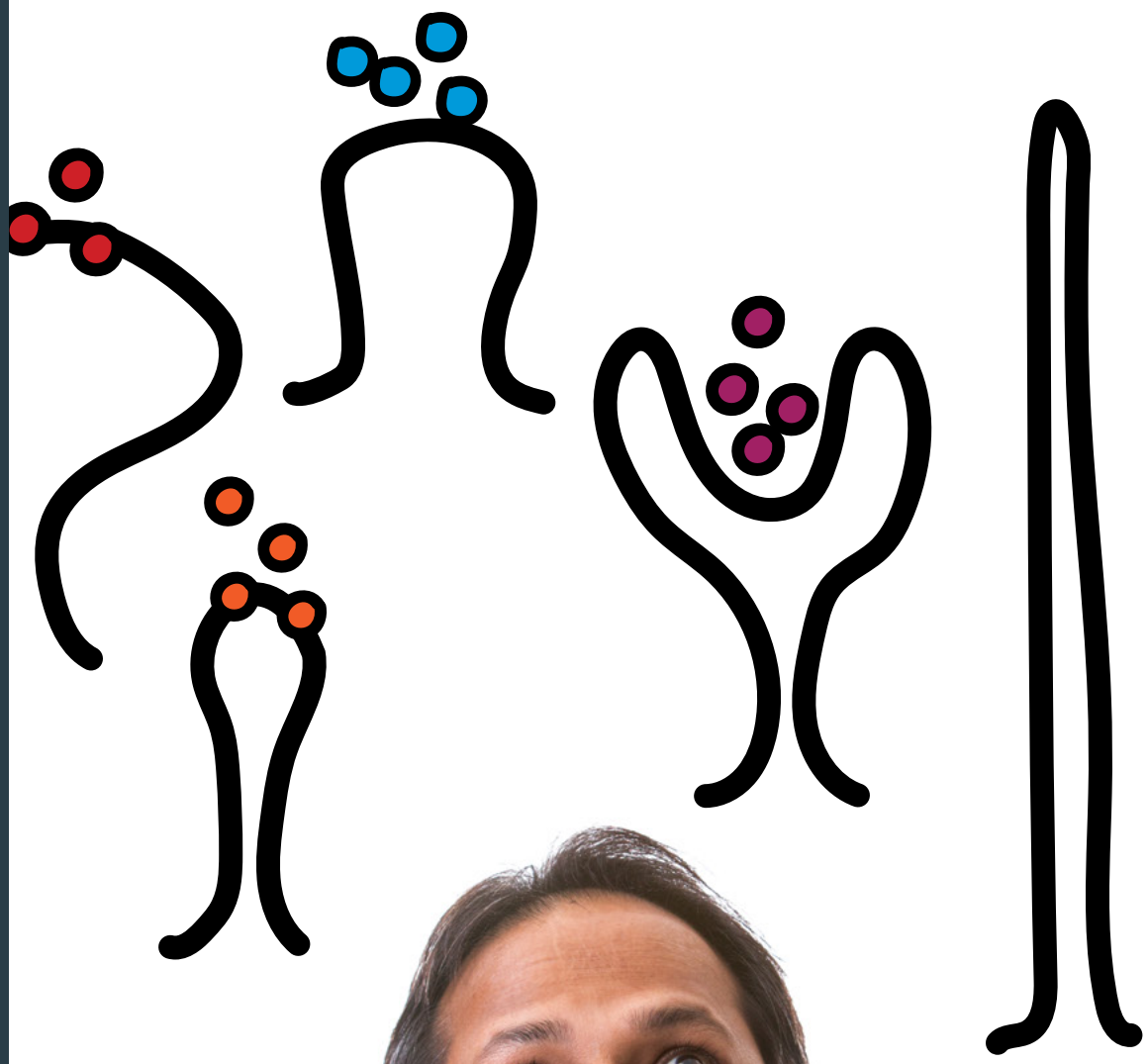
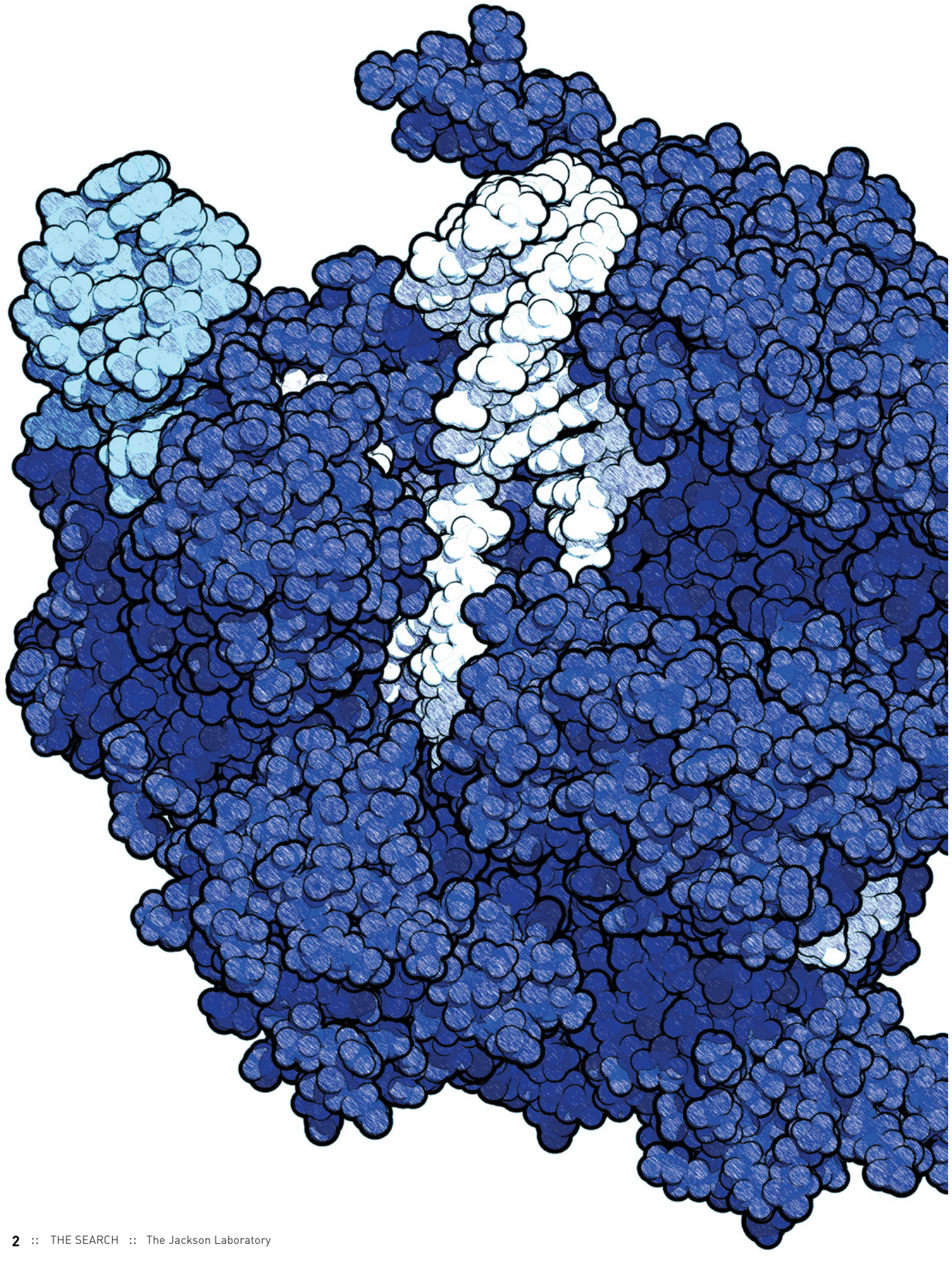


- Why is cancer so difficult to cure?
- Turning science into cures
- Exploring the mysteries of the brain
- The gene detective
- Leaving a lasting imprint
- The 3-D genome



# THE SEARCH

A PUBLICATION OF **THE JACKSON LABORATORY**

**ON THE COVER**
By blending classical and cutting-edge genetic approaches, Vivek Kumar, Ph.D., is unveiling the genes at work within the brain to control complex behaviors, such as anxiety and addiction. *Illustration by Karen Davis, photography by Marie Chao.*

**LEFT**
Pictured is the CRISPR-Cas9 gene editing complex from Streptococcus pyogenes. The Cas9 nuclease protein uses a guide RNA sequence to cut DNA at a complementary site. Atoms are shown as colored spheres. Cas9 protein: blue; RNA: light blue; DNA fragments: white. *Illustration adapted by Danielle Meier.*

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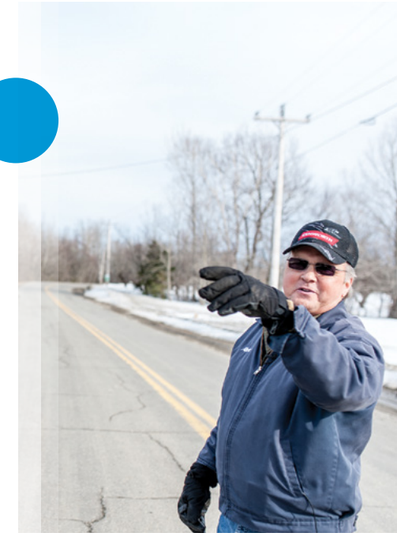

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We discover precise genomic solutions for disease and empower the global biomedical community in our shared quest to improve human health.

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The Jackson Laboratory
*Leading the search for tomorrow's cures*


# President's message

## ACCELERATING DISCOVERY. DRIVING INNOVATION. CREATING HOPE.

At JAX, we are the masters of complex genetics and functional genomics. We are harnessing the power of new technologies to understand health and disease, and to lead the way to new treatments and cures.

Last year was a banner year for The Jackson Laboratory. Our successes — impactful scientific discoveries resulting in increased research grant support, in moving a therapeutic molecule through pre-clinical development, and in increased philanthropy — put us in a better position than ever to lead the accelerating progress of precision medicine into 2016 and beyond.

The story of Al Raymond, featured in this issue, underscores the power and potential of the work that we do — as well as its urgency. Ultimately, what motivates us is not just the satisfaction of making discoveries in the lab; it is the joy of turning that science into real advances that will impact human lives. Challenges lie ahead. As another article in this issue explains, there are many reasons we don't yet have a cure for cancer. Yet because of our success, I believe that JAX is in a remarkable position to lead the search for treatments and cures — not just for cancer, but for the many diseases and conditions that are rooted in our genes.

We could not do this without your support. Thanks to you, our partners in discovery, we are accelerating research progress, bringing new knowledge and innovations to the broader scientific and biomedical community, and creating new hope for people like Al — people for whom devastating disease is not a scientific challenge, but a deeply personal one.

Thank you for helping JAX rise to meet that challenge.

Edison Liu, M.D.

President and CEO, The Jackson Laboratory



# news&notes

## GROWING OUR WORLD-CLASS TEAM

### PHYSICIAN-SCIENTIST IN CARDIOLOGY J. TRAVIS HINSON, M.D., JOINS JAX AND UCONN HEALTH

J. Travis Hinson, M.D., a board-certified cardiologist and researcher interested in bringing an interdisciplinary approach to the study of heart muscle diseases, has joined the faculties of The Jackson Laboratory for Genomic Medicine and UConn Health.

Hinson comes from Brigham and Women's Hospital in Boston, a Harvard Medical School teaching affiliate, where he was an instructor in cardiovascular medicine and an associate physician. He treated patients with inherited forms of cardiovascular diseases, especially hypertrophic and dilated cardiomyopathies, diseases of the heart muscle that can lead to heart failure and ultimately death. Using genome editing technology, he engineered heart-like structures — "small beating tissues" — with cells containing specific genetic mutations. Hinson hopes that results of these studies may translate to new therapeutic opportunities for his patients.

## PARTNERING TO ADVANCE HEALTH

### CANCER CENTERS AT JAX AND BIDMC LAUNCH PILOT CANCER GENOMICS GRANT PROGRAM

As part of a comprehensive relationship to advance cancer research and accelerate personalized genomic medicine, The Cancer Center at Beth Israel Deaconess Medical Center (BIDMC) and The Jackson Laboratory have launched seven joint research projects to study a variety of cancer types, including multiple myeloma, lung, breast, prostate and brain.

The projects bring together 28 scientists — 14 from each institution — to lead investigations ranging from basic science to the development of clinical therapies.

A number of these projects will take advantage of the two groups' sophisticated platforms to model human cancers and test therapeutic outcomes. The pioneering JAX mouse model, PDX (patient-derived xenograft), provides a platform for studying the genomic profiles of individual cancers through molecular diagnostic testing. BIDMC's "Mouse Hospital" uses genetically altered mice to replicate human cancers and enables investigators to conduct human clinical trials in parallel with animal studies.

## FUNDING MAKES OUR WORK POSSIBLE

### JAX SPINOUT CYTEIR THERAPEUTICS ANNOUNCES $5.5M SERIES A FINANCING ROUND

Cambridge-based biopharmaceutical company Cyteir Therapeutics announced the close of a Series A financing totaling $5.5 million. Cyteir, a spinout of The Jackson Laboratory, is leveraging its expertise in DNA repair and genome instability to develop new targeted therapeutics for a range of human diseases, including cancer and autoimmune disorders.

The financing round included participation from a syndicate of private investors and Celgene Corporation. In addition, Cyteir Therapeutics obtained an exclusive license to key technologies and patents from JAX.

Cyteir Therapeutics was founded by JAX Adjunct Professor Kevin Mills, Ph.D., together with Tim Romberger, who led the company as interim CEO through the Series A financing, and JAX Chair Emeritus David Shaw.

## JAX LANDS $8.2M FEDERAL GRANT FOR RESEARCH IN EGG AND SPERM FORMATION

A Jackson Laboratory research team headed by Professor Kenneth Paigen, Ph.D., has received a five-year, $8.2 million grant to research early steps in the formation of sperm and eggs.

This multidisciplinary research team includes a molecular biologist, Research Scientist Petko Petkov, Ph.D.; a cytogeneticist, Senior Research Scientist Mary Ann Handel, Ph.D.; and a computational biologist, Assistant Professor Gregory Carter, Ph.D.

The team will focus on a key protein, known as PRDM9, that members of the team discovered several years ago controls the initiation of genetic recombination, the exchange of parts between pairs of chromosomes that is essential for the successful production of sperm or eggs.

## JAX RECEIVES $3.7M FEDERAL GRANT TO DEVELOP A NEW SYSTEM FOR UNDERSTANDING THE 3-D GENOME

A five-year, $3.7 million grant was awarded to a team led by Professor Yijun Ruan, Ph.D., of The Jackson Laboratory for Genomic Medicine, to fund research into how the human genome is organized in the nucleus of the cell.

Composed of DNA, the genome in a single cell would stretch to more than six feet in length if extended. Instead it is intricately looped and physically packed into microscopic nuclei. This three-dimensional (3-D) structure is thought to play many important roles in genome regulation and function.

Ruan is the Florine Deschenes Roux Chair professor and director of genomic sciences at The Jackson Laboratory. An international leader in genome structure research, he is developing a new system for understanding 3-D genome organization and regulation.

The grant is part of the 4-D Nucleome program, supported by the National Institutes of Health's Common Fund to investigate nuclear organization in space and over time (the fourth dimension, or 4-D).

## $500K LIBRA FOUNDATION GIFT TO FUND HIGH-IMPACT THERAPIES AND TECHNOLOGIES INITIATIVE

A $500,000 gift from the Libra Foundation of Portland, Maine, to The Jackson Laboratory will support technological and therapeutic innovations with exceptional potential to improve health care and biomedical science.

The JAX High-Impact Therapies and Technologies (HITTs) initiative is aimed at launching promising new projects into the next phase of development, speeding the delivery of new therapies to help patients and new technologies to advance biomedical research.

JAX already has several projects that are at a stage where an investment could launch them into the next stage of development. By focusing support on projects ready to make the leap to the clinic or to the market, the HITTs fund leverages the power of philanthropy to achieve sustainability and maximize impact — on the patient, on science and on society.

**Stay up to date with the latest JAX news. Visit *www.jax.org/news*.**





# Alumnus Robert Lanza

*"To be at The Jackson Laboratory with thoughtful, distinguished people… If I hadn't had that experience and those interactions, I would never have had the confidence to pursue my interest in science."*

– Robert Lanza, 1973 JAX Summer Student

Robert Lanza, Ph.D., was not raised in a home that nurtured his scientific interests. So when he landed as an awkward but ambitious teenager at The Jackson Laboratory's Summer Student Program in 1973, it was like a light coming on in a shadowy room. Now, more than 40 years later, Lanza himself is a bright light — and a bit of a bioscience rock star — using stem cell therapy to lead the way into the emerging field of regenerative medicine.

Lanza's career has been a remarkable success by any measure. He developed methods to make embryonic stem cells without destroying embryos and his team was the first to clone an endangered species — a type of wild ox known as a guar. He is a leader in the growing field of regenerative medicine and his pioneering work has led to effective treatments for autoimmune conditions such as multiple sclerosis, Crohn's disease and lupus in laboratory animal models.

Lanza has received many high-profile awards and recognitions for his work, including being named to *TIME* magazine's 2014 list of the "100 Most Influential People in the World." In 2010, he received the NIH Director's Award and — along with genomicist Craig Venter, Ph.D., philanthropist Bill Gates and President Barack Obama — recognition as a "mover and shaker" in shaping the future of biotech from the journal *BioWorld*.

Learn more about Lanza and other JAX alumni at *www.jax.org/alumni*.

STORY BY MEG HASKELL
PHOTOGRAPHY BY SEAN HARRIS

# Why is **cancer** so difficult to **cure**

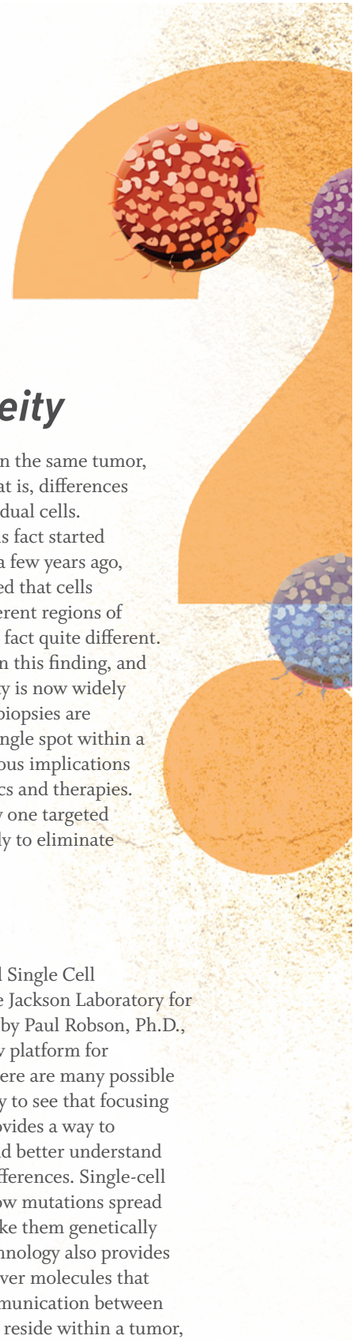
STORY BY MARK WANNER
ILLUSTRATION BY KAREN DAVIS

Recent advances in cancer research have revealed much more about just how complex cancer is and how difficult it is to completely cure.

It can be sobering to confront the challenges ahead. Day by day researchers better understand why my cancer is not your cancer, and one-size-fits-all therapies seem less and less realistic.

So is the cancer research picture bleak? Far from it! Targeted therapies will yield huge gains, with fewer severe side effects, compared to the sledgehammer chemotherapeutic and radiation approaches — which kill almost all quickly dividing cells — currently serving as the standard of care. It just helps to be realistic about progress and accept that success is unlikely to yield "cures" for cancer, at least not in the near future. Nonetheless, it is likely to provide far better prognoses for a large number of cancer patients.

For a better handle on where we are now, what challenges remain to be overcome, and what progress may be around the corner, here are a few key biological concepts, how they relate to cancer, and research at the National Cancer Institute-designated Jackson Laboratory Cancer Center that is increasing our understanding of each concept and, in some cases, is identifying new therapeutic targets.



## *Heterogeneity*

Cancer cells, even within the same tumor, are heterogeneous — that is, differences exist between the individual cells. The consequences of this fact started coming into focus only a few years ago, when researchers showed that cells collected from four different regions of the same tumor were in fact quite different. Further studies have rein this finding, and cancer cell heterogeneity is now widely recognized. Given that biopsies are typically taken from a single spot within a tumor, this fact has serious implications for improving diagnostics and therapies. It also indicates that any one targeted therapy is highly unlikely to eliminate all cancer cells by itself.

## *RESEARCH AT JAX*

The recently established Single Cell Genomics Center at The Jackson Laboratory for Genomic Medicine, led by Paul Robson, Ph.D., provides a powerful new platform for investigating cancer. There are many possible applications, but it's easy to see that focusing on one cell at a time provides a way to identify, characterize and better understand the effects of cellular differences. Single-cell sequencing can track how mutations spread through tumors and make them genetically heterogeneous. The technology also provides the opportunity to uncover molecules that control cell-to-cell communication between the many cell types that reside within a tumor, identifying potential drug targets. And it has the potential to reveal cell types that are rare or otherwise difficult to study but are important to cancer growth and survival.

## Evolution

Fast-growing cancers depend on a fine balance between DNA damage and repair, but genetic changes add up over time, and the result is like evolution at warp speed, where growth-promoting mutations lead to even more rapid expansion. This contributes to the heterogeneity discussed above. It also means the cancer you find today may differ from the one you try to treat in the weeks and months to come. With modern sequencing and analysis, it's now possible to track cancer cell evolution and begin to predict the changes before they occur. Nonetheless, it's much harder to hit a moving target than a stationary one, and even a highly effective, precisely targeted combination of therapies may not succeed if enough cancer cells survive initial treatment and further evolve.

### RESEARCH AT JAX

Adjunct Professor Kevin Mills, Ph.D., has identified a natural system in immune cells that provides a promising therapy target that also short circuits cancer cell evolution. In immune cells, a protein known as AID creates widespread DNA damage to generate the antibody response needed to fight off a diverse array of bacteria and other pathogens. Another system repairs the DNA, including "off-target" damage in other areas of the genome. AID is usually not active in other cells, but in some cancers — Mills estimates 40 percent — it becomes overactive and causes widespread DNA damage and rapid cancer cell evolution. These AID-positive cancers, in turn, become "addicted" to the repair mechanism, depending on it to survive. Mills discovered that blocking repair in these cancers not only leads to catastrophic levels of DNA damage and death, it also prevents the cancers from evolving and recurring. At Cyteir Therapeutics, a company he co-founded based on his research at JAX, he is now working with compounds that block DNA repair in AID-positive cancer cells, killing them without harming other tissues. Mills hopes that they will benefit cancer patients within the next decade.

## Structural variation

Structural variants (SVs) include duplications, deletions, inversions and insertions of stretches of DNA, changes in the genome that don't change the sequence per se but can have significant consequences. While most SVs are hard to detect and details about them are just beginning to emerge, the role of a particular structural variant in cancer has been known for a very long time. Researchers discovered the famous Philadelphia chromosome, which gives rise to chronic myeloid leukemia (CML), in 1960. SVs add to the list of genetic changes that can tip the balance toward cancerous growth through overexpression of duplicated oncogenic (cancer-causing) genes, underexpression of deleted cancer suppressor genes and other insertions/translocations giving rise to oncogenic proteins.

### RESEARCH AT JAX

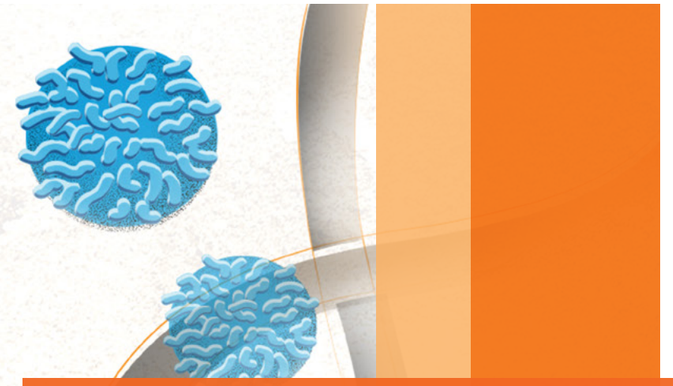
Although it can be relatively easy to find structural variants that involve long segments of DNA, such as the Philadelphia chromosome, many elude standard sequencing and analysis methods. JAX Genomic Medicine's Scientific Director and Professor Charles Lee, Ph.D., helped pioneer structural variant research and discovered that such variants are common in healthy people. Lee's recent work in gastric cancer, which currently has a poor prognosis, revealed that a significant percentage of patient tumors had additional copies of a gene, *BCL2L1*, that prevents cells from self-destructing. Thus, even in conditions that would normally initiate the self-destruct process, a cell will continue to grow and divide and be very susceptible to turning cancerous. Lee also found a drug that inhibits *BCL2L1* function in cancer cells. It allowed the self-destruct process to reactivate, leading to cell death, making it a promising new therapeutic target for gastric cancer.

## Immune system evasion

Cancer cells, although different in many ways from other cells in the body, are known to evade our immune system or suppress key elements of the usual immune response. In some cases aggressive cytotoxic (killer) T cells — the immune cells that locate and kill invading pathogens — actually infiltrate tumors. For some reason, however, they soon halt their attack through a combination of cell-to-cell signaling and an influx of T regulator cells, a different type of immune cells that suppress the immune response. Other research found that a chemical compound is sometimes added to cancer cell DNA and suppresses the activity of certain genes, making the cells much less likely to be targeted by the immune system. By controlling the activity of these genes, cancer is able to hide in plain sight within the body and avoid an immune response.

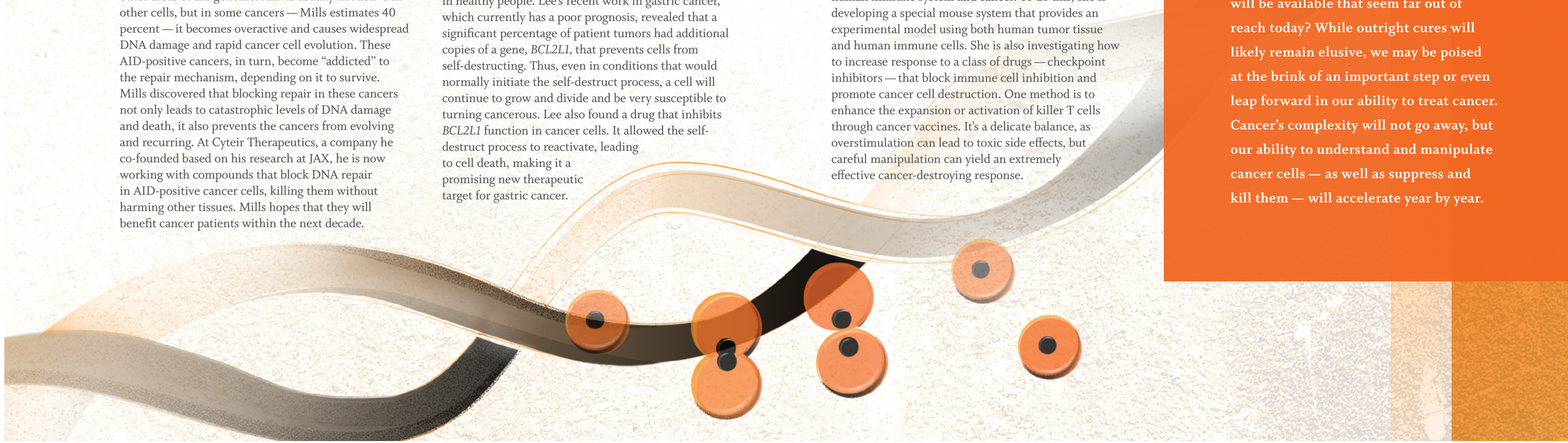
### RESEARCH AT JAX

Cancer immunotherapy, using the body's own immune system to target and destroy cancer cells, is one of the most exciting fields in biomedical research. The excitement is merited, and there have been spectacular successes in human patients, albeit in small, preliminary studies. And many challenges remain. JAX Professor Karolina Palucka, M.D., Ph.D., is working to better understand the complex interplay between the human immune system and cancer. To do this, she is developing a special mouse system that provides an experimental model using both human tumor tissue and human immune cells. She is also investigating how to increase response to a class of drugs — checkpoint inhibitors — that block immune cell inhibition and promote cancer cell destruction. One method is to enhance the expansion or activation of killer T cells through cancer vaccines. It's a delicate balance, as overstimulation can lead to toxic side effects, but careful manipulation can yield an extremely effective cancer-destroying response.



## *Cancer remains a difficult disease to treat,*

**but the emerging therapies are increasingly effective. As we pass the mid-decade point, it is interesting to speculate what we will be able to do when we stand on the threshold of 2020. What therapies will be available that seem far out of reach today? While outright cures will likely remain elusive, we may be poised at the brink of an important step or even leap forward in our ability to treat cancer. Cancer's complexity will not go away, but our ability to understand and manipulate cancer cells — as well as suppress and kill them — will accelerate year by year.**



# Turning science into cures

STORY BY BARRY TEATER
PHOTOGRAPHY BY SEAN HARRIS AND MARIE CHAO

On a spring day in 2007 Albert Raymond was working atop his 18-wheel tractor trailer, securing a stack of pulp logs bound for a paper mill, when he suddenly felt clammy and turned ashen. His coworkers thought he was having a heart attack. It turned out to be his first day of a battle with cancer that continues, nearly a decade later.

Mr. Raymond, a self-employed truck driver and grandfather of six from Kingman, Maine, went to his doctor, who detected an abnormally high white blood cell count. The results were a potential early warning sign for chronic lymphocytic leukemia, or CLL, a cancer of the white blood cells.

"When I heard the word cancer I kept saying to myself I hope that's not me, I hope that's not me," Mr. Raymond recalls, shaking his head with downcast eyes.

> *"Your spleen should be about the size of your fist. His was the size of a football."*
>
> – BELINDA RAYMOND

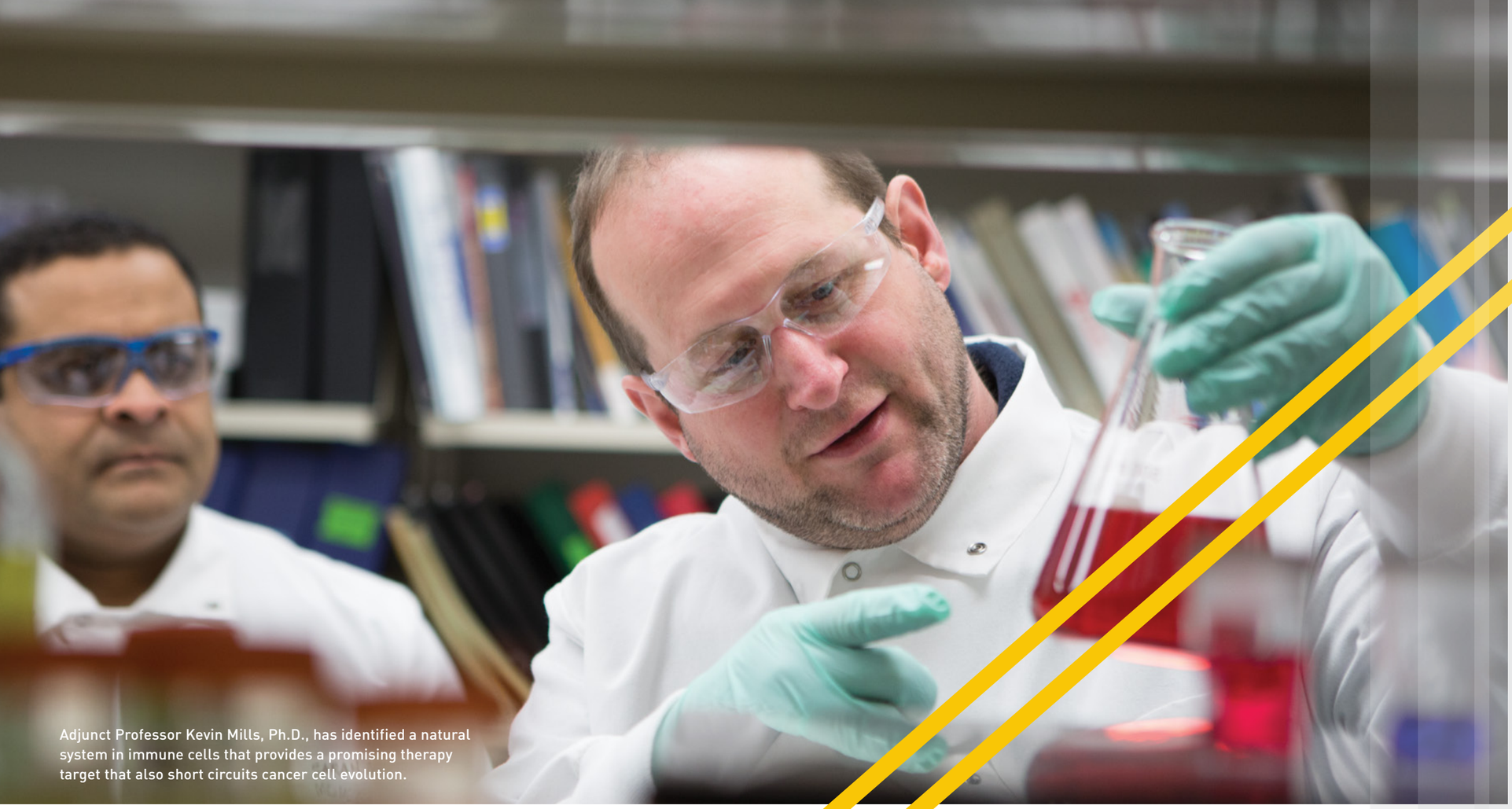
Because Mr. Raymond had no other symptoms of CLL, such as weight loss or involvement of organs or lymph nodes, his doctor advised a "watchful waiting" strategy. For the next five years he went for regular tests to monitor his blood cell counts.

"Albert was what they called a yo-yo," says his wife, Belinda Raymond. "His numbers would go up and then they would drop right back down."

After five years, tests revealed a spike in his white blood cell count and an enlarged spleen, the organ that filters the blood and plays a role in the immune system.

"Your spleen should be about the size of your fist," says Mrs. Raymond. "His was the size of a football. So it was making him very sluggish and very tired, and he was losing weight."

Mr. Raymond began receiving chemotherapy at Cancer Care of Maine in Brewer. He soldiered through the usual side effects and even felt well



Adjunct Professor Kevin Mills, Ph.D., has identified a natural system in immune cells that provides a promising therapy target that also short circuits cancer cell evolution.

enough to return to work for a few months. Eventually he showed no signs of CLL.

That good news was tempered, however, by another sobering revelation. After he began feeling tired again, a secondary cancer, called myelodysplastic syndromes (MDS), was diagnosed.

MDS is a group of diverse bone marrow disorders with one thing in common: the bone marrow doesn't produce enough healthy blood cells. Ironically, Mr. Raymond's MDS was likely caused by one of the chemo drugs he had taken for CLL.

Mr. Raymond was referred to the Dana-Farber Cancer Institute in Boston, where his new oncologist told him that he would need more chemotherapy and a bone marrow stem cell transplant, or else he would succumb to MDS. Fortunately, his sister, Estella, tested as a perfect match for donating stem cells.

Mr. Raymond spent a month in the hospital undergoing the procedure in February 2014. He survived the grueling regimen but paid a stiff price for its side effects and complications, including nausea, diarrhea, iron toxicity, dehydration and high blood sugar. In the next months there were more hospital stays, blood transfusions and new medicines.

"His energy and all of his vitality were just being washed away," recalls Mrs. Raymond.

Mr. Raymond's immune system, decimated by chemo and then re-seeded by the infusion of stem cells, was highly vulnerable to infection, so he avoided public contact.

"It's scary," he says, "because when they wipe out your immune system, you can catch, at the snap of your finger, anything that's going around. So you've got to be really cautious."

For months he wore a mask and gloves to protect himself from germs. He quit his job and avoided stores and restaurants. He refrained from hugging his four daughters and six grandchildren.

"Anything you consider normal that you would do in a day, he does not do unless it's within his home," says Mrs. Raymond. "You give up your work, you give up your family and your friends. Everything about it is life altering."

## *New hope at JAX*

Mr. Raymond's cancer odyssey resonates throughout The Jackson Laboratory, where scientists have investigated the genetic origins of cancer for the last eight decades, and are developing new and novel approaches to its treatment.

Among the 50 or so scientists working today in JAX's National Cancer Institute-designated Cancer Center is Adjunct Professor Kevin Mills, Ph.D., who studies DNA damage and repair. Mills' lab is aiming to develop targeted therapies for cancers such as CLL.

"Kevin Mills wants to find a way to attack the cancer without attacking the patient, so that patients like Al can look forward to better treatment options, improved outcomes and longer, healthier lives," says Edison Liu, M.D., president and CEO of JAX.

## *Exploiting cancer's weakness*

Chronic lymphocytic leukemia is the most common type of leukemia in adults. It affects B-cell lymphocytes, cells of the immune system that originate in the bone marrow, develop in the lymph nodes and normally fight infection by producing antibodies. In CLL, B cells grow uncontrollably and accumulate in the bone marrow and blood, crowding out healthy blood cells.

One of the defining features of CLL, as with many other cancers, is its inability to copy its genome accurately during cell division.

"That is really also the Achilles' heel," Mills says. "The very thing that makes CLL a cancer is also a weakness that we can exploit."

As the genetic copying errors pile up in each successive generation of cells, the accumulation "creates breaks and mutations and nicks all through the genome, all through the chromosomes," Mills explains. "Because of that, cancer cells become critically addicted

to DNA repair. They need to have really souped-up DNA-repair mechanisms in order to take care of that damage, in order to survive.

"What my lab has discovered is that if we can take away some of their DNA-repair capacity… we can actually cause a CLL cell to essentially mutate itself to death. And so, we have been focused on developing new therapeutic and treatment strategies that leverage and take advantage of this biology."

The importance of DNA repair was underscored recently when three scientists — Tomas Lindahl, Paul Modrich and Aziz Sancar — were jointly awarded the 2015 Nobel Prize in Chemistry for their advances in the field.

"At JAX, we not only understand the mechanism of DNA repair; we are unlocking its potential to serve as the basis of new approaches to cancer treatment," says Liu.

Targeting cancer's over-reliance on DNA repair is a promising alternative to chemotherapy, Mills says. He cites the leukemia drugs Gleevec and Imbruvica as therapies that successfully exploit biological weaknesses of cancer, yielding better results and less-punishing side effects.

"The problem with these drugs — while they're substantially better than chemotherapy — is the cancer cell almost always evolves resistance (to them)," Mills says. "So what we really need is yet another approach in our therapeutic toolbox — drugs that can prevent or forestall the acquisition of therapy resistance.

"If we can succeed, we can develop new therapies that are far more effective, far more long lasting and have dramatically fewer side effects than chemotherapy," he says.

"Right now, we measure five-year survival rates. But if we can attack tumor evolution at its roots, we can begin to think about 10- or 15- or 25-year survival rates."

To see a short film and learn more about Al's story, visit *www.jax.org/CLL*.

## *Slow road to recovery*

Those extended survival rates would be a godsend to cancer patients like Al Raymond, who remains largely confined to his home as he recovers from his disease and its debilitating treatments.

"I think Al's story is a powerful one because it really illustrates the toll that cancer and cancer treatment can take on an individual and can take on a family," Mills says. "Our mission is to improve cancer treatment so that Al's story doesn't have to be realized by future cancer patients.

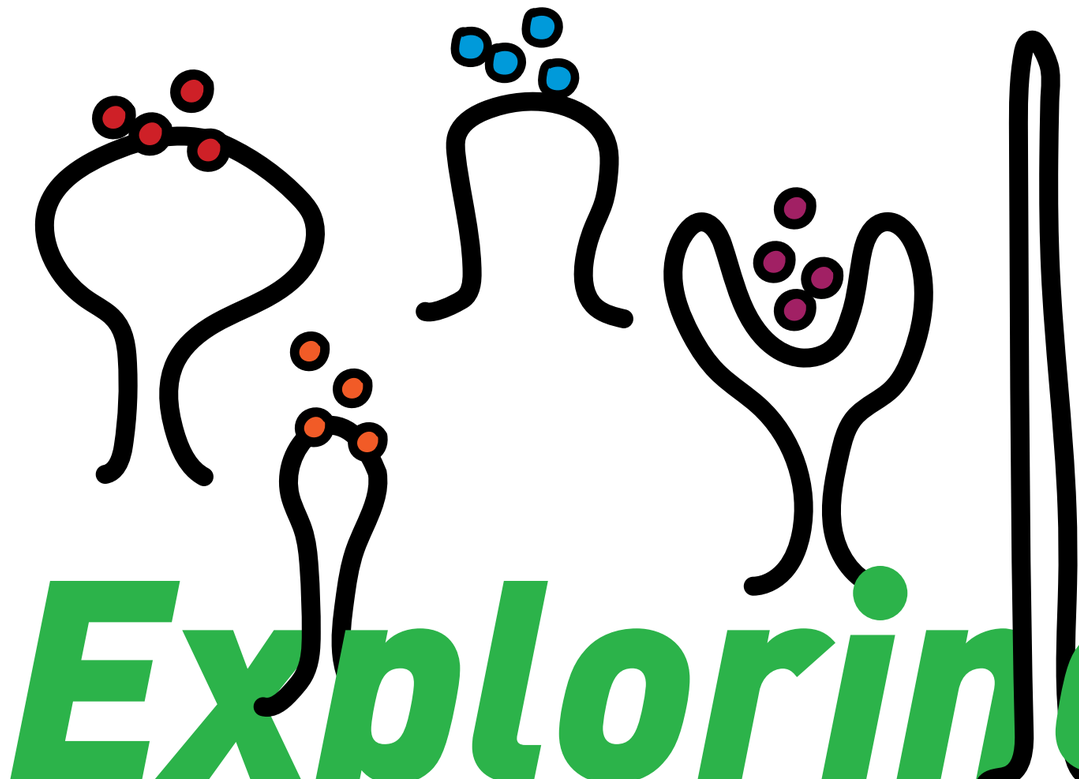
"There's no reason that a cancer diagnosis should be a devastating diagnosis. And there's no reason that we shouldn't be able to treat cancer in a way that preserves the health of the patient and doesn't leave them with long-term, lingering after effects."

Two years after his stem cell transplant, Mr. Raymond still avoids stores and other public places, fearful that he will catch a germ that could overwhelm his weak immune system.

"I live in a small bubble," he says, but it's "getting a little bit bigger as I progress, as I get better."

Eventually he hopes to go back to his truck-driving job and the freedom of the road, if only part time.

"Albert will tell you, he'll say, 'Every day is a good day,' because he is here," says Mrs. Raymond. "Now we just strive to get to where we actually can get back to what most people take for granted as normal living. We just want to get there from here."



# Exploring the mysteries of the brain

*By blending classical and cutting-edge genetic approaches, Vivek Kumar is unveiling the genes at work within the brain to control complex behaviors, such as anxiety and addiction.*

STORY BY NICOLE DAVIS, PH.D.
PHOTOGRAPHY BY MARIE CHAO
ILLUSTRATION BY KAREN DAVIS

The brain is the most complex system in the body. It is home to billions of neurons, which mingle with other cells, making trillions of minuscule connections. This delicate circuitry forms the ultimate command center, controlling our thoughts, actions and emotions. Deciphering its structure and its major outputs — chief among them, behavior — is one of the most important and perplexing frontiers in modern science.

Assistant Professor Vivek Kumar, Ph.D., is one of the fearless explorers of this uncharted world. He joined The Jackson Laboratory (JAX) in early 2015, launching a research group devoted to some of the most complex, yet profound, questions in neurobiology: What is the biological basis of complex behaviors, such as addiction, anxiety and depression? What genes are involved? And how do mutations — changes in the genetic code that can alter how and when these and other genes act — alter the cellular form and function of the brain?

These are difficult questions to ask and answer, but Kumar is hardly one to wither when the going gets tough. "He's not afraid to take on a new challenge — to change and learn something completely new," says Joseph Takahashi, Ph.D., a professor and chair of the department of neuroscience at the University of Texas Southwestern Medical Center, and one of Kumar's mentors. "You have to be able to move into different fields and technologies to really make new discoveries, and Vivek is able to do that."

## Once-in-a-lifetime opportunity

After earning his undergraduate degree from the University of Chicago, Kumar headed west for graduate school. At the University of California, San Diego, he worked in the laboratory of Michael Rosenfeld, Ph.D., where he spent six years dissecting a key protein involved in transcription — a fundamental activity cells use to convert (or "transcribe") the information within DNA into a more nimble chemical form, made of the related genetic material, RNA.

"My graduate career focused on hard-core biochemistry and structural biology approaches, and [as I considered my next step], I felt I was really lacking a genetic component," says Kumar.

He learned that Takahashi, then at Northwestern University, had received a major grant to apply genetic approaches in mice to unlock the basis of complex behaviors, such as learning, anxiety, impulsivity and addiction. In the 1990s, Takahashi pioneered the discovery of a critical gene in the mouse, called *Clock*, which is now recognized as the molecular mastermind of circadian rhythms — biological processes, such as sleep, that fluctuate on a 24-hour cycle.

Takahashi's find was remarkable for two reasons. First, *Clock* was the first gene to be linked with circadian rhythms in mammals, opening up the field of circadian biology. Second, it was identified through a classic "forward genetics" approach, in which disease models are created (using methods that can perturb the genome, such as chemicals or irradiation), analyzed for unusual biological traits (called phenotypes), and then meticulously studied at the DNA level to pinpoint the culprit gene mutation.

Historically, such forward genetic screens have been a mainstay of research in the fruit fly, *Drosophila melanogaster*. The notion of applying them in mice — and to an area as complex as behavior — was seen as a bold move. Bolstered by *Clock*'s discovery, Takahashi subsequently launched a five-year, large-scale, forward genetics screen in mice to uncover genes that affect other complex behaviors.

While interviewing Kumar for a postdoctoral position, Takahashi told him that if he came to the lab, he could lead the portion of the screen devoted to cocaine response. "I have to be honest; I didn't know that much about the screen at the time, and I certainly had never worked in mice," says Kumar. "But it was one of those once-in-a-lifetime opportunities."

Kumar was hooked.

## Serendipity

As he settled into Takahashi's lab, Kumar set to work on his screen. The goal was to identify mice with unusual responses to a dose of cocaine.

Ideally, he would uncover mutant mice whose behavior diverged significantly from that norm — so-called six standard deviation mutants, like *Clock*. These extreme outliers are not only easier to discern, but they also lend themselves more readily to genetic analysis.

To step up his genetic mapping game, Kumar came to JAX, spending a week in Bar Harbor as a student in a course on systems genetics taught by Gary Churchill, Ph.D.

"The knowledge I gained through his course gave me the tools to go back to the lab and start thinking about ways of mapping my mutant mice," says Kumar.

Although Kumar's newfound knowledge was a boon, it did not change one of the stark realities of statistical genetics approaches. The portions of the genome that emerge through the mapping process tend to be quite large, leaving a sizeable amount of genomic real estate that must be searched to find the offending genetic change. In the absence of known genes that can serve as likely suspects and thus help narrow the search, the task can be a major challenge.

But thanks to the power of next-generation technologies for decoding DNA, this once-huge hurdle — whittling down 50 million or so genetic letters to a single discrete typo — has now been reduced to a speed bump.

As Kumar prepared to map his stable of mutant mice, 10 of them in total, he searched for a suitable mouse strain to mate with them. (These so-called mapping crosses are a key first step in the mapping process.) He examined several different strains, including the well-known strain, C57BL/6 (called "Black 6" for short), which comes in two seemingly identical varieties: C57BL/6N and C57BL/6J.

Through this process, he stumbled upon a startling discovery. "I found that the Black 6N had a different cocaine response than the Black 6J, and that was really surprising because these were considered to be essentially the same stock of mice."

First established by C.C. Little, the founder of The Jackson Laboratory, the Black 6J have been maintained in Bar Harbor for decades. In 1951, a colony was shipped to the National Institutes of Health, establishing a second source of the mice, known as Black 6N. Although researchers often use these mice interchangeably, Kumar's finding hinted at an important difference — one that led him directly to a gene that is mutated exclusively in the N subline and causes a diminished cocaine response.

In a paper published in *Science* in late 2013, Kumar reported that mice carrying mutations in this gene, called *Cyfip2*, have abnormal neuronal structure in a key part of the brain known for its role in behaviors, including addiction. These neurons have fewer hair-like projects (called dendritic spines), which are important sites of neurochemical signaling.

The serendipity of this *Cyfip2* find is not lost on Kumar. "In one case, we were creating mutations using a chemical, and in the other, there were 50 years of genetic drift. The end result was the same — a single nucleotide change causes a large behavioral difference."

## Peering into the treasure chest

Now, as he establishes his own laboratory at JAX, Kumar is extending his work on *Cyfip2*. One major project is to unravel the biology of *Cyfip2* and understand its potential role in addiction, using specialized behavioral tests. On a broader level, his lab will focus on how the circuit that regulates motivated behavior is constructed and maintained at the molecular level, using *Cyfip2* as an entry point. His group will also explore how changes in neuronal shape (known as structural plasticity) affect drug responses. Through these experiments, Kumar and his team can determine whether mutations in *Cyfip2* are connected with voluntary cocaine intake.

He also has his collection of mutant mice from the screen in Takahashi's lab. Kumar has already genetically mapped some of them, and now the precise gene mutations must be found. The behavioral defects in these mice span not just addictive behaviors, but also anxiety and hyperactivity, offering biological inroads into a range of complex disorders in humans — from addiction to depression to attention deficit and hyperactivity disorder (ADHD).

"Not many people are willing to take this approach. And because Vivek has a bank of mutant mice that he's already found, it's like a little treasure chest — he can go in there and keep finding new genes," says Takahashi.

Perhaps more than anything else, Kumar views his mutant mice as a means to decipher a fundamental part of the brain, called the mesolimbic pathway. This area connects multiple neuroanatomical regions and is driven by the chemical signal, dopamine. Also known as the reward pathway, it plays a crucial role in impulse control and motivation.

"This circuitry is really a survival mechanism," explains Kumar. "Every day, an animal has to make a decision whether it is going to stay safe in its nest or go out and explore — find food, a new mate, a better place to live. It is this delicate balance that the [mesolimbic] circuitry regulates and that drugs of abuse hijack."

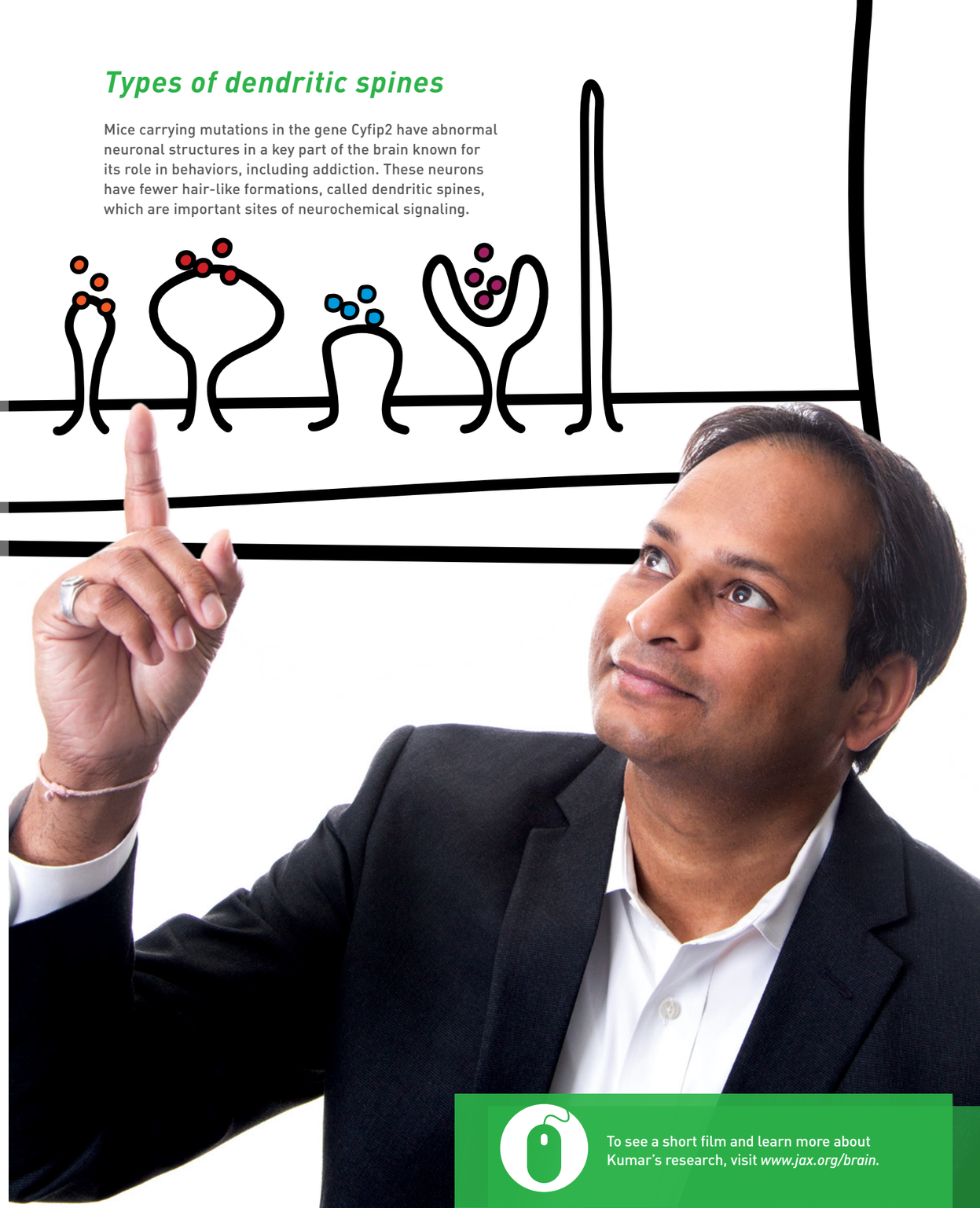
He also hopes to steer his laboratory in another important direction: developing a new generation of mouse neurobehavioral assays. Many of the existing tests of mouse behavior are highly artificial and do not adequately mimic an animal's natural activities. That has significant implications for the genes that can be identified through forward genetic screens, which rely on these behavioral tests as the primary means to detect mutants. "Going forward, I think we'll need a melding of the minds from folks from computation, engineering and genetics," says Kumar. "This field, known as computational ethology, is in its infancy and holds great promise."

With innovative new tests that are more robust and more scalable, a new universe of genes could be revealed. Kumar believes JAX is the perfect place for him to pursue this work.

"JAX has such a great history," says Kumar. "I'm really thankful to have the opportunity to contribute to this organization, and I look forward to what we together — my lab and JAX as a whole — can accomplish."

### Types of dendritic spines

Mice carrying mutations in the gene Cyfip2 have abnormal neuronal structures in a key part of the brain known for its role in behaviors, including addiction. These neurons have fewer hair-like formations, called dendritic spines, which are important sites of neurochemical signaling.



To see a short film and learn more about Kumar's research, visit *www.jax.org/brain*.

# The gene detective

STORY BY NICOLE DAVIS, PH.D.
PHOTOGRAPHY BY MARIE CHAO

For decades, scientists have searched our DNA for clues about the origins of disease, seeking to find the culprit genes and the key changes within those genes that can disrupt normal biology. In the early days of gene sleuthing, long before the Human Genome Project and the advent of high-throughput tools for DNA sequencing and analysis, this work was slow and somewhat hit-or-miss. It also tended to focus on single-gene diseases like cystic fibrosis or Huntington's disease. Although such conditions are rare, they stem from just one aberrant gene, and are therefore more readily deciphered.

Fast forward about 30 years, and now, researchers at The Jackson Laboratory (JAX) and across the world are able to set their sights on a range of devastating maladies, including diseases that are not only more common but also more genetically complex. These so-called "complex diseases," including type 2 diabetes, cancer, Alzheimer's disease and many others, are the work of multiple genes — tens or even hundreds — that collude to increase a person's risk of illness.

This increased complexity brings new challenges, and requires robust methods to sift through ever-increasing quantities of data — encompassing not just individual genes but entire genomes — to reveal telltale patterns and connections that point to the genes involved in disease. For the past 25 years, JAX Professor Carol Bult, Ph.D., has been at the forefront of these data-mining efforts. A computational biologist who joined JAX in 1997 and who was appointed to the Knowlton Family Chair, a newly established endowed professorship, in 2015, Bult is wielding the powers of quantitative science to crack open big biological problems.

"It is very much like detective work," Bult says. "If you are trying to solve a crime, you look at some of the usual suspects, but you don't just focus on one individual — you look at who they associate with as well. You follow those associations, and you start building a picture not about an individual, but about a network of individuals that might be in collusion."

Bult is deeply passionate about these pursuits, but what drives her is not one particular disease, but rather the desire to apply her expertise in meaningful ways. "[As a scientific community], we've invested all of these resources in generating large data sets, and you generate the data,

By harnessing the most advanced data-mining tools, Carol Bult is leading the hunt for suspect genes that contribute to a common, deadly birth defect.

To see a short film and learn more about Bult's research, visit *www.jax.org/detective.*

you publish one paper, and then you move on to the next [problem]. I think it's a lost opportunity not to take advantage of all the information that is out there, that has been published from different data sets, and to get new information out of it."

Over the course of her career, Bult has worked on a variety of questions in human biology. One in particular involves a common and often deadly birth defect. Known as congenital diaphragmatic hernia (CDH), the disorder is characterized by a hole in the diaphragm, the muscular sheet that separates the abdominal and chest cavities. Because of this hole, the liver and even the stomach can move up into the chest and impinge on the lungs, restricting their growth and compromising function. Although the condition can be surgically repaired, the mortality rate remains quite high, around 50 percent. Those who do survive have long-term health problems.

Although scientists have recognized that CDH has genetic roots, the disorder involves a multitude of genes. Together with collaborators at the Massachusetts General Hospital in Boston, Mass., Bult and her team have been scouring databases filled with genome-scale data sets, spanning both human and mice, to reconstruct the intricate networks of genes that conspire to cause CDH.

"We're using this kind of detective work approach to identify as many of the genes contributing to this defect as we can," explains Bult. "We identify a suspect, and then we look in human populations to see if any of the affected individuals have a variation in the gene, and then we go to the mouse and re-create that variation in the mouse, and then ask, in the mouse, does this cause the disease or not?"

If the suspect indeed causes disease, then it becomes "perpetrator number one," forming the first link in a vast genetic "crime ring" that underlies CDH. Then, Bult and her colleagues begin again — using "guilt by association" to identify other genes and gene variations that track along with the first, and then exploring how those work in the mouse. Through this iterative process, Bult and her team can reveal the entire network that underlies CDH. In fact, they have already identified a surprising new suspect gene, called PBX-1.

This find, together with the other suspects Bult is collecting, forms a critical knowledge base that sheds a bright light on the biology of CDH. Indeed, great power flows from this genome-based view of human disease. It is the foundation for a new era of medicine, known as genomic or precision medicine.

"When we say genomic medicine, we mean in every single case the possibility of knowing every single component contributing to that disease," says JAX President and CEO Edison Liu, M.D.

By assembling a comprehensive genomic picture of CDH, Bult and her colleagues are not only improving the biological understanding of the disorder, but also accelerating efforts aimed at finding more effective methods for diagnosis, treatment, and prevention. This kind of translational work, connecting basic science with clinical medicine, is precisely the goal of JAX's newest facility, The Jackson Laboratory for Genomic Medicine in Farmington, Conn.

"For 85 years, JAX has been the premier institution for using the mouse as a model for human diseases, and it's allowed us to delve deeply into the biology of specific genes, and how those genes interact with one another," says Charles Lee, Ph.D., scientific director of JAX Genomic Medicine. "Now, we can take the technologies that we have, the knowledge that we're building, the research that we're doing, and directly bring it back to the patient."

For Bult, this is the opportunity of a lifetime. "The fact that I'm getting to work at a place that is setting the stage for the future of precision medicine, the application of those basic concepts into patient care, that's not something I could have anticipated," she says. "It's an extraordinarily rewarding and exciting time."

# LEAVING A LASTING *imprint*

STORY BY BARRY TEATER
ILLUSTRATION BY DANIELLE MEIER
PHOTOGRAPHY BY ARTURO PEDROZA
AND PROVIDED BY THE
PAUSTENBACH FAMILY

## Risk-assessment pioneer Dennis J. Paustenbach pledges a $1 million planned gift to JAX

As a board-certified toxicologist and industrial hygienist, Dennis J. Paustenbach, Ph.D., deals with complicated science every day.

"He's constantly solving these very complex intellectual problems," observes David Roux, a longtime friend of Paustenbach.

It's no surprise then that Paustenbach is passionate about The Jackson Laboratory, an organization dedicated to the complex science of understanding and solving genetic diseases.

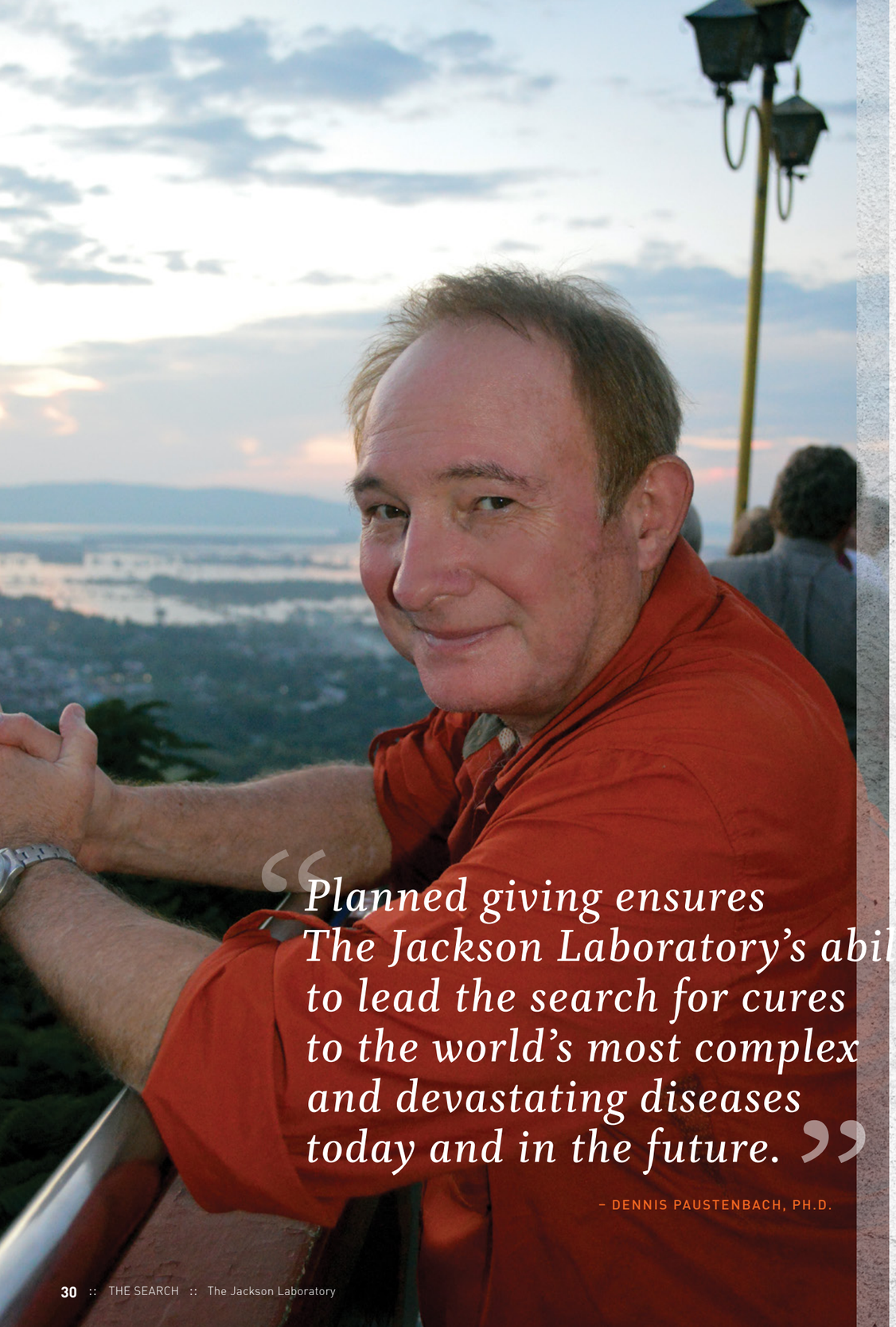
His nearly 30 years of career success had positioned Paustenbach financially to begin looking for a worthy cause to support in his later years. Roux, a JAX trustee, introduced him to the Laboratory in 2013 and encouraged him to join the Board of Trustees.

"I knew that he would be a diligent, committed and highly engaged board member and that he'd model the kind of behavior that we value so much and that is so important to the Laboratory's future," recalls Roux, a major JAX donor and now chair of the board. "He is generous with his time and his financial resources."

After getting to know the Laboratory, Paustenbach concluded that JAX had an outstanding cancer center and unparalleled expertise in mouse genetics and mouse models of human disease.

"JAX can use the mouse to give us insights into diseases within a fraction of the time that other institutions might get to the same place," he says. "What is really special about JAX is its ability to use mouse genetics and immunology to get an upper hand on cancer."

Paustenbach recently made a $1 million planned gift to the Laboratory to support this type of research. The gift, to be funded from his estate, will establish an endowment called the Paustenbach Family Cancer and Immunology Research Fund.






“This seemed like a highly efficient, worthwhile cause that was unique,” Paustenbach says. “Because the Laboratory’s mission-centric, nonprofit business of making research models available to the global community pays for the organization’s administration and overhead, 100 percent of every gift goes directly to supporting precision-medicine research.”

Paustenbach compares this to gifts he has made to other institutions where only 25 percent of the monies are used by the research faculty, with the balance used to cover overhead, pay students or buy equipment. “JAX can avoid this,” he says, “because it can use the equipment, facilities and staff from its mouse operations to advance the classic research side of the house.”

Roux says Paustenbach’s gift underscores the importance of planned giving, a type of charitable contribution that provides donors with the opportunity to make a future gift to an organization through a range of vehicles that suit diverse financial situations and objectives.

Paustenbach says planned giving “ensures The Jackson Laboratory’s ability to lead the search for cures to the world’s most complex and devastating diseases today and in the future. I take great pleasure from knowing that my family and I are part of this important legacy.”

## Midwestern roots

As the son of German immigrants growing up in rural Pennsylvania and Ohio in the 1950s and 60s, Paustenbach had no idea he would become a pioneer in environmental and health risk assessment — the field didn’t even exist yet. He simply knew that he wanted a better job than the factory jobs his friends were taking fresh out of high school.

”When you’re raised by blue-collar parents, everything is about getting a good job,” he says. “Because we were lower middle class, and certainly not a scholarly family, early on I had lots of aspirations about getting a fine education, seeing the world, learning as much as I could about other cultures, and figuring out how the world really worked.”

Paustenbach has realized those dreams and today is a real “Renaissance man” with wide interests, says Roux. Paustenbach has traveled to more than 60 countries, has amassed a museum-quality collection of American folk art and antiques, and is an avid fly-fisherman, golfer and skier, as well as a scientist, entrepreneur and philanthropist.

Paustenbach says he owes much of his success to a good early education at Catholic schools, which instilled discipline and a love of learning.

Those traits led him to start playing chess in the fifth grade, “and I got really good at it,” he says. He played in weekend tournaments throughout the Midwest and the East Coast and became nationally competitive until he turned 21 and began focusing on his career.

He studied chemical engineering at Rose-Hulman Institute of Technology in Terre Haute, Ind. His first job out of college was as a process engineer for Eli Lilly in Indianapolis, where he designed better ways to manufacture pharmaceuticals.

After two years at Lilly he decided he needed more education, so he earned a master’s degree in industrial hygiene at the University of Michigan. And three years later, he enrolled at Purdue University. While earning a Ph.D. in environmental toxicology there, he taught more than 300 students over four years as an adjunct professor and won a “best teacher” award.

## A serendipitous career

As he was finishing his doctorate in 1982, the new field of risk assessment was emerging, and it integrated the very disciplines he had studied. Paustenbach moved to Connecticut to work for a chemical company, assessing health hazards in the workplace. The job took him frequently to Washington, D.C., where he interacted with federal environmental agencies and gained expertise in how chemicals were regulated.

He received a phone call one day asking him to interview for a job with Syntex, a pharmaceutical company in California’s Silicon Valley. “Somehow they’d heard about my skillsets in solving complicated environmental problems,” he explains.

Syntex had rented a building to a small firm at their facility located near Times Beach, Mo., that had been previously used in the manufacture of hexachlorophene, an antibacterial agent used in household disinfectants. Large amounts of dioxin, a toxic byproduct of hexachlorophene, had

been accidentally mixed with motor oil and sprayed on roads and land throughout the town, creating a huge liability. The Times Beach site became the country's most visible toxic waste site, forcing the town's entire population to re-settle elsewhere.

Paustenbach worked on the project for the next three years, earning more valuable experience and further raising his stature in the risk-assessment profession.

"It was probably the best illustration of what I did well, which is integrate eight or 10 scientific fields to solve environmental problems — basically understand the facts and then try to selectively draw on this vast amount of data from many fields to identify an efficient resolution to the problem," Paustenbach says. "And that's what I've been doing ever since."

## The birth of ChemRisk

While at Syntex he began moonlighting on weekends for Hewlett Packard and IBM under the name ChemRisk in 1985.

Two years later an engineering consulting firm, McLaren-Hart, asked him to start its occupational health division. He ended up forming a joint venture with the company, also called ChemRisk, and shaped it into a 100-person consulting firm in six years.

He sold the firm to his partner and in 1997 took a job at Exponent, a larger consulting firm in Silicon Valley. He started up Exponent's health sciences group and grew it to about 150 employees before leaving to re-start ChemRisk on his own in 2003.

Paustenbach merged the company in 2012 with Cardno, a professional infrastructure and environmental services company, to form Cardno ChemRisk, a division of Australia-based Cardno (a publicly traded firm). As president of the division, headquartered in San Francisco, he oversees about 110 scientists and staff who serve clients in various industries around the world.

During his career Paustenbach has directed the scientific aspects of toxic tort cases and provided expert witness testimony in about 400 depositions and three dozen trials concerning the health effects of chemicals in sediments, air, soil, consumer products, groundwater and the workplace. He has also mentored dozens of young Ph.D. scientists whom he has taught or hired.

"It's fascinating and challenging work," he says. "That's why I don't retire and why I am wildly excited about what I do every day."

## Promoting shared research

Paustenbach says there is no single case that brings him the most pride. Instead, it was his influence in promoting the sharing of privately conducted scientific research that satisfies him most.

For the first three decades of the occupational and environmental health consulting industry, he says private firms held their scientific information close to the vest, believing it gave them a competitive advantage while shielding them from legal liability. "They had their reasons," he says, "but the rationale for sharing and the transparency, in my mind, overweighed the potential downsides."

Paustenbach hopes he is remembered as "the person who got the non-university community and, in particular consulting firms and many Fortune 500 firms, to publish and share what they used to call proprietary information related to the environment and the workplace."

In 1983 he convinced the board of directors of Syntex to let him publish work about the Times Beach case, "which was unheard of," he says. Since then thousands of scientific and technical papers by private companies have been published so the public can read them and other researchers can build on them.

"Without them," he says, "the environmental sciences would be 10 years further behind in its quest for answers." ChemRisk alone, he says, has published more than 600 papers on environmental and occupational hazards of one sort or another.

"That's definitely what I'm most proud of," he says. "It has changed everything."



# The 3-D genome

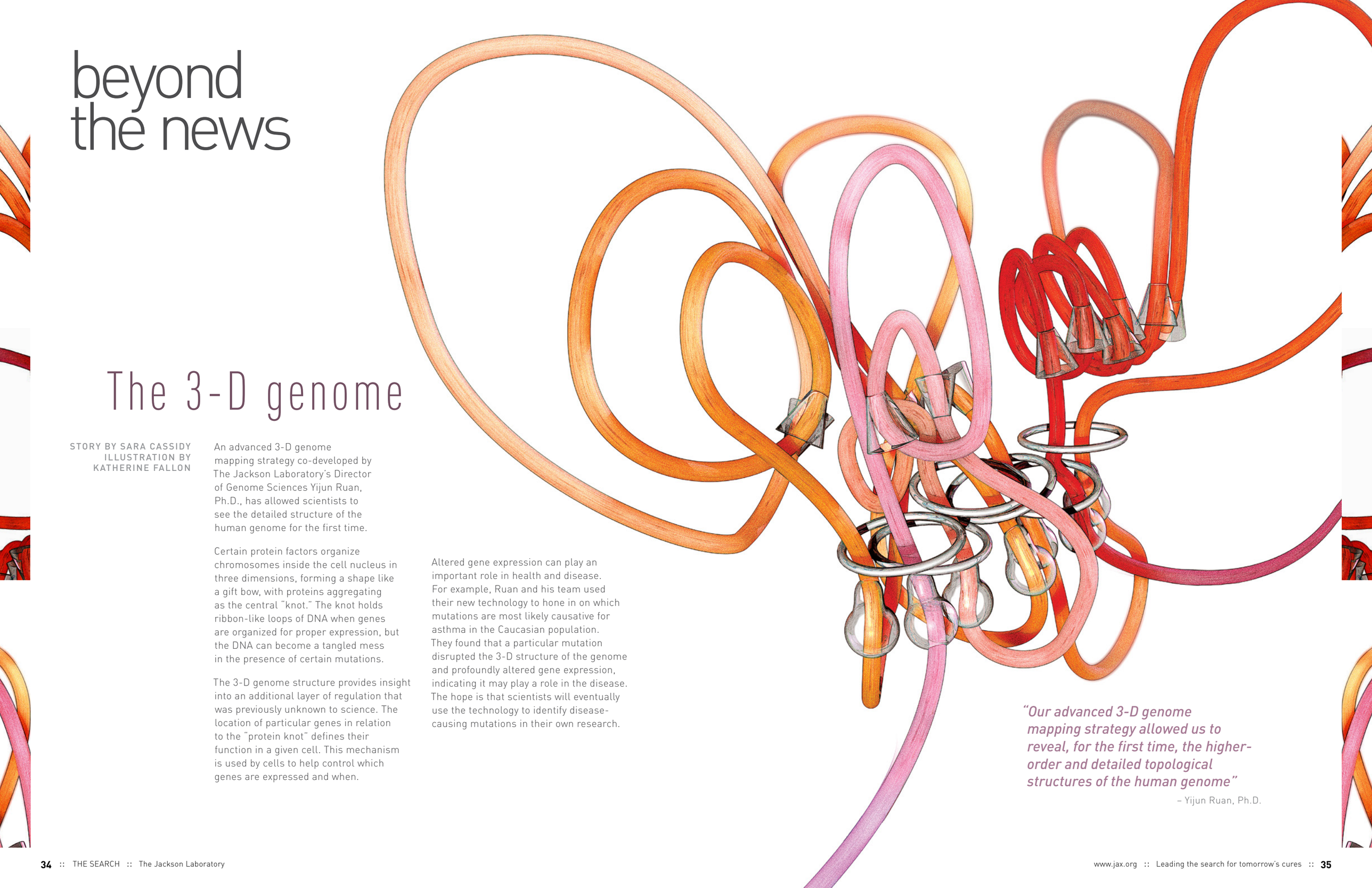
STORY BY SARA CASSIDY
ILLUSTRATION BY
KATHERINE FALLON

An advanced 3-D genome mapping strategy co-developed by The Jackson Laboratory's Director of Genome Sciences Yijun Ruan, Ph.D., has allowed scientists to see the detailed structure of the human genome for the first time.

Certain protein factors organize chromosomes inside the cell nucleus in three dimensions, forming a shape like a gift bow, with proteins aggregating as the central "knot." The knot holds ribbon-like loops of DNA when genes are organized for proper expression, but the DNA can become a tangled mess in the presence of certain mutations.

The 3-D genome structure provides insight into an additional layer of regulation that was previously unknown to science. The location of particular genes in relation to the "protein knot" defines their function in a given cell. This mechanism is used by cells to help control which genes are expressed and when.

Altered gene expression can play an important role in health and disease. For example, Ruan and his team used their new technology to hone in on which mutations are most likely causative for asthma in the Caucasian population. They found that a particular mutation disrupted the 3-D structure of the genome and profoundly altered gene expression, indicating it may play a role in the disease. The hope is that scientists will eventually use the technology to identify disease-causing mutations in their own research.



*"Our advanced 3-D genome mapping strategy allowed us to reveal, for the first time, the higher-order and detailed topological structures of the human genome"*

– Yijun Ruan, Ph.D.

The Jackson Laboratory

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Bar Harbor, ME 04609-1523

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**More than 130 people attended the Genomic Workforce Consortium Bioscience Career Forum in December at The Jackson Laboratory for Genomic Medicine in Farmington, Conn. Attendees interacted with bioscience leaders about a wide range of topics including skill acquisition and training; career building and networking; and how to secure a job in the bioscience industry.**
*Photograph by Marie Chao*