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Feng Zhang

Pioneer of CRISPR genome editing

FROM THE **DIRECTOR**

In this issue we feature Feng Zhang and his groundbreaking work on the development of CRISPR, a genome-editing technology that has vastly increased our ability to alter the DNA of living cells, including those of humans. It is no exaggeration to say that CRISPR has revolutionized biomedical research, and many believe it will lead to major new therapies for human disease. Given that CRISPR has also generated substantial commercial interest, I should note that MIT and the Broad Institute (where Feng holds a joint appointment) are co-owners of patents on key CRISPR technologies developed by Feng. As Feng himself has always acknowledged, his genome-editing work builds upon more than two decades of basic research on the biology of CRISPR as a microbial immune system, and my colleague Eric Lander, director of the Broad Institute, has emphasized this point in a fascinating history of CRISPR's origins, recently published in *Cell*. The CRISPR story represents the work of many people, and any attempt to apportion credit is inevitably controversial. But it is an inspiring story of discovery and invention, and I encourage anyone interested in the subject to read Eric's review. Patents and prizes aside, we can all take encouragement from the speed of progress and the enormous potential that CRISPR holds.

Bob Desimone, Director
Doris and Don Berkey Professor
of Neuroscience

On the cover:
Structure of the CRISPR-Cas9 DNA-cutting complex.
Image: Feng Zhang and Steve Dixon

Photo: Justin Knight

Feng Zhang has launched a biotechnology revolution through his pioneering development of CRISPR genome-editing tools.

Feng Zhang was not yet 30 when he arrived at MIT in 2011 to set up his own research group at the McGovern Institute and the Broad Institute of MIT and Harvard. In a previous interview with *Brain Scan* in the fall of 2012, he spoke with reserve, a quiet up-and-comer working on microbial genome editing tools called TAL-effectors.

A few months later, in January 2013, he dropped a bombshell. He published a paper in *Science* demonstrating a new genome editing method called CRISPR. Zhang's paper, along with a parallel paper from Harvard geneticist George

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Church, showed for the first time that a bacterial enzyme called Cas9 could be used to change DNA sequences in human cells, far more easily than any previous method. Suddenly, it was possible to find and edit genes in the genome almost as simply as text in a Word document.

This feat was immediately recognized as a major breakthrough, and it unleashed a torrent of activity that continues to this day. The technology has now been used to engineer the genomes of mice, rats, flies, worms, zebrafish, dogs, and many other species, including human stem cells. Nearly 2000 papers on CRISPR have appeared since Zhang's original publication, and several companies have been launched to commercialize the technology, attracting hundreds of millions of dollars in investments. CRISPR has generated a whirlwind of media attention and Zhang himself was profiled in the *New Yorker* and in the newly launched publication *STAT*, which described him as "the most transformative biologist of his generation."

The CRISPR technology has been so influential that it was named by *Science* as the top breakthrough of 2015.

It is no exaggeration to say that Zhang's work has started a biotechnology revolution. But Zhang himself, who prefers the lab to the limelight, has remained focused, publishing over 40 papers since the seminal report in *Science*. In October, he surprised peers at a scientific meeting by presenting a second, alternative CRISPR tool that provides new functionalities compared to the earlier technology. The room rippled with excitement at the speed of his progress.

In addition to developing and sharing new tools, Zhang uses them in his own lab to study many diseases, including autism, schizophrenia, and Alzheimer's disease. "We don't just want to test tools, we want to use these tools in real-world applications," Zhang says. "Understanding the limitations of the technology fuels our ideas for further development."

AMI IMAGES/SCIENCE PHOTO LIBRARY / Broad Institute

CRISPR was originally identified in bacteria, in which it provides protection against attack by viruses.

Photo: Justin Knight

Le Cong (left) and Fei Ann Ran were among the first to join Zhang's lab, and were co-authors on the first CRISPR genome-editing paper.

Trend Setter

Zhang's interest in genome editing traces back to his childhood. In 1993, at age 11, he moved with his mother, a computer engineer, from China to Des Moines, Iowa. He spoke only a little English, but within a year he had mastered the language and enrolled in a special molecular biology class offered by his middle school. As part of the class, he watched the movie *Jurassic Park*, in which dinosaurs are resurrected using the power of fantastical biological engineering. He realized that biology might also be a programmable system, similar the computers his mother coded.

Zhang was hooked. Through his school, he found an opportunity to volunteer in a local gene therapy lab, where he engineered human melanoma cells to glow by inserting a fluorescent green jellyfish protein, and then showed that the protein could protect the cells from DNA damage by absorbing UV light. The experience taught him that biological tools had great potential to fight disease.

Following a degree in chemistry and physics from Harvard, a PhD in neuroscience from Stanford and a prestigious Harvard junior fellowship, he had just arrived at MIT and the Broad Institute and was starting to build his own group in 2011, when CRISPR first appeared on his radar.

Zhang read a paper from a Canadian lab showing that CRISPR (which had been studied by many labs since its first discovery in 1987) uses short pieces of RNA to find a specific DNA sequence, and a single enzyme, now called Cas9, to cleave them. "This paper got me really excited," Zhang says. "We were working on genome editing with TAL effectors, where the DNA sequence is recognized by a protein. But if we could use RNA instead, it would be much simpler to design because the code is much simpler."

Indeed the matching of RNA to DNA is as simple as a child's decoder-ring, substituting one letter for another. Zhang's excitement grew as he read every paper he could find about CRISPR and began to envisage the possibilities. He recalls sitting at a kitchen counter and sketching a roadmap for development: First, understand the biology and improve the technology; second, develop applications; and third, share the tools widely with other researchers.

For step one, he enlisted the help of his earliest lab members, including then-graduate students Le Cong and Fei Ann Ran. The team focused on a key goal: altering the bacterial CRISPR system so that it would work in human cells.

When they began, it was far from clear whether this goal was possible. The human genome contains 3 billion letters, equivalent to six feet of DNA, packed into every cell nucleus, so locating a single target site is like finding a needle in a haystack. Yet, Zhang and his team were able to engineer CRISPR to make precisely targeted changes with remarkable efficiency, as described in the pivotal 2013 *Science* paper, on which Cong and Ran were joint first authors. "I've been lucky to have amazing postdocs, students and colleagues," says Zhang. "I feel like a kid in a candy store here at MIT."

Use Cases

Step two of Zhang's road map is to develop practical applications, particularly in the field of brain disease. Over the past few years, many studies have identified genes linked to autism, schizophrenia, bipolar disorder, and other disorders. Zhang wants to use CRISPR to speed the systematic study of these genes, to understand how they influence disease processes and how these effects might be reversed.

One example is autism, which Zhang's lab is studying using human neurons engineered with CRISPR to carry mutations linked to the disorder. By studying these neurons in culture, he hopes to identify differences that may contribute to the development of autism and which could be potentially targeted in future therapies.

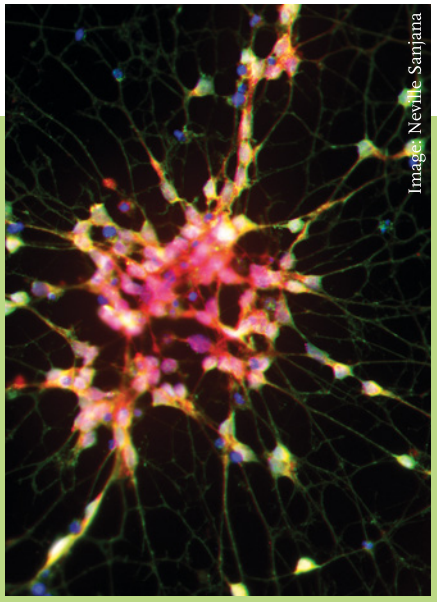


Image: Neville Sanjana

The Zhang lab is using CRISPR to study the effects of autism mutations in human neurons.

Zhang's lab is also using CRISPR to study genes that may cause or protect against Alzheimer's disease and to study the development of drug resistance in cancer. Another focus is on the creation of animal models of brain disorders. For example, he is collaborating with Mriganka Sur of MIT's Picower Institute to create mouse models of Rett Syndrome and with McGovern Investigator Guoping Feng to create models of autism and schizophrenia.

An important challenge for research and therapy is to deliver the CRISPR reagents to the relevant cells within the body. One way to do this is with viral vectors used for gene therapy, but the Cas9 enzyme is large and difficult to deliver. "We wanted to make it more compact," Zhang says. "So we thought, maybe there are other, smaller alternatives out there in nature too."

Zhang and his collaborators scanned the genomes of other bacterial species, searching for other proteins and systems that might be considered. One that appeared particularly attractive was a protein called Cpf1, and Zhang, along with his collaborator Eugene Koonin, announced last October that they had successfully harnessed Cpf1 for genome editing. Soon after this, Zhang, Koonin and Konstantin Severinov identified three additional CRISPR enzymes that could also be used for genome editing.

Sharing the Knowledge

As outlined in step three of his roadmap, Zhang has made it a priority to share his methods widely, and his CRISPR reagents have already been distributed over 27,000 times, at nominal cost, via a sharing service called AddGene. "New technologies need to be made accessible," he says. "It's very important to make sure what you build is easy to use and openly available."

Biotech investors were also quick to recognize the potential of CRISPR, and several new companies have been launched to develop CRISPR for human gene therapy and other practical applications. With four other scientists, Zhang co-founded Editas Medicine, which has licensed his CRISPR-Cas9 patents from Broad and MIT and which hopes to develop CRISPR-based treatments for human genetic disease. An early target is retinal blindness—for which the company expects to begin human trials in 2017—and treatments for more complex disorders could follow. "It would be a dream to be able to treat diseases like ALS (amyotrophic lateral sclerosis), Rett syndrome, or Parkinson's," says Zhang. "We think CRISPR could provide a completely novel way to treat these devastating and untreatable diseases."

Step Wise

The implications of CRISPR for science and medicine are exciting, but the notion of easy genome editing has also raised concerns about the prospect of "designer babies," and unforeseen effects if engineered genes spread into the environment. CRISPR has already been used to engineer food crops, research animals and even genetically altered pets.

Well aware of the potential concerns, Zhang is among many scientists working with the National Academies of Sciences and Medicine to consider guidelines for the ethical use of genome editing *(see page 6)*. But he also remains inspired by the possibilities, especially for brain research. CRISPR has the potential to help scientists understand how brain diseases rob people of social connections, happiness and memories, and it could, someday, guide the way to new therapies. "My hope is that CRISPR will live up to its promise by being used responsibly," he says. ■

Photo: Justin Knight

Graduate student Bernd Zetsche has studied the DNA-cutting enzyme Cpf1, which has a number of potential advantages as a tool for genome editing.

Photo: Justin Knight

Since his arrival at MIT and the Broad Institute in 2011, Zhang's lab has now grown to more than 30 people.

Ed Boyden Wins Breakthrough Prize


Photo: Mark Zuckerberg

Boyden with other Breakthrough laureates and prize sponsors at the Palo Alto home of Priscilla Chan and Facebook founder Mark Zuckerberg (front, second and third from left).


Photo: Breakthrough Prize

Ed Boyden accepts the 2016 Breakthrough Prize in Life Sciences at a ceremony at NASA's Ames Research Center in Mountain View, California.

Ed Boyden has won a 2016 Breakthrough Prize in Life Sciences for his role in the development of optogenetics, a technique for controlling brain activity with light. Karl Deisseroth, a Stanford University professor who pioneered the technique with Boyden, was also honored with a prize. The life sciences prize is given for "transformative advances toward understanding living systems and extending human life."

Boyden received the $3 million award at an Oscar-style event on November 8th that was televised live on the National Geographic Channel. Producer/actor/director Seth MacFarlane hosted the ceremony and awards were presented by celebrities including actors Russell Crowe, Hilary Swank and Kate Hudson.

Following the ceremony, Boyden and the other prizewinners were invited for lunch at the home of Facebook founder Mark Zuckerberg.

Visit our website to watch Ed Boyden explain optogenetics in a short video produced for the Breakthrough Prize ceremony. ■

Feng Zhang Speaks at Genome Editing Summit

Feng Zhang was among the speakers at the International Summit on Human Genome Editing, held in Washington DC from Dec 1-3, 2015.

The meeting, which attracted wide media coverage, was convened by the US National Academies of Sciences and Medicine, the Royal Society and the Chinese Academy of Sciences, to discuss the ethical and societal challenges raised by new genome-editing methods, in particular the CRISPR method which Zhang has pioneered. The main concern is the prospect of human germline editing, which could create genetic changes that are transmitted to future generations. Similar methods are already routine in model organisms such as mice, and a study in China last year showed that they could potentially be applied to human embryos (although the Chinese research involved non-viable embryos that could not be implanted).

The three-day summit brought together leading international experts in genetics, clinical medicine and bioethics, with the goal of developing consensus recommendations for responsible use of this rapidly evolving technology.

One key recommendation from the meeting was that any attempt at human germline therapy would be irresponsible given the current state of knowledge, and should not be considered until much more is known about the potential risks and benefits. ■

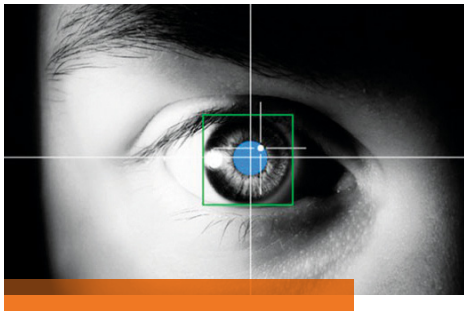

Photo: NASA/Pam Risdon

Feng Zhang speaks at the International Summit on Human Genome Editing in Washington DC.

Nancy Kanwisher and colleagues have identified a neural population in the human auditory cortex that responds selectively to music. The researchers, led by postdoc Sam Norman-Haignere and also including BCS faculty member Josh McDermott, were able to identify selective responses to several different types of sounds including speech as well as music.

Caroline Robertson, a postdoctoral researcher in **Nancy Kanwisher**'s lab, has found a link between autism and reduced activity of the neurotransmitter GABA, whose job is to dampen neuron excitation. The researchers examined the perceptual phenomenon known as binocular rivalry, in which different images are shown to each eye, causing the perception to switch back and forth between the two images. In healthy individuals the strength of this effect is correlated with the level of GABA in the visual cortex, but in people with autism this correlation breaks down, implying that GABA signaling is somehow impaired. The findings suggest that drugs that boost the action of GABA might improve some of the symptoms of autism.

Although it is known that psychiatric disorders have a strong genetic component, untangling the web of genes contributing to each disease is a daunting task. Neuroscientists in **Guoping Feng**'s lab have now shed light on how a single gene can play a role in more than one disease. In a recent study, they revealed that two different mutations of the Shank3 gene produce distinct molecular and behavioral effects associated with autism and schizophrenia.



McGovern researchers are studying how the brain controls eye movements during visual search tasks.

Searching for a particular object within a cluttered scene requires that we hold in mind a mental image of the target object. **Robert Desimone** and colleagues have now identified a brain region within the prefrontal cortex that stores this type of visual representation during a search. The researchers also found that this region, known as ventral prearcuate cortex, sends signals to the parts of the brain that control eye movements, thus guiding the eyes to the target location.

Feng Zhang, along with collaborators at NIH and Wageningen University in the Netherlands, described a new CRISPR system that can be adapted for genome editing. The new system is based on a newly described DNA-cutting enzyme called Cpf1, which has a number of potential advantages over the more familiar Cas9-based system.

Image: Martin Pot / wikimedia

Juvenile zebra finches learn to imitate their father's song through trial and error.

Male zebra finches learn their songs by copying what they hear from their fathers. These songs develop early in life as juvenile birds experiment with mimicking the tutor's song – analogous to the babbling of human infants. **Michale Fee** and colleagues have now uncovered the brain activity that supports this learning process. Sequences of neural activity that encode the birds' first song syllable are duplicated and altered slightly, allowing the birds to produce several variations on the original syllable. Eventually, through trial and error, these syllables are strung together into the bird's signature song, which remains constant for life. ■

Photo: Sham Sthankiya

Caroline Robertson (left) and Nancy Kanwisher have found altered brain chemistry in people with autism.

AWARDS AND HONORS

Photo: Dominick Reuter

Rebecca Saxe studies the neural basis of social cognition.

Photo: Society for Neuroscience

Ed Boyden (right) receives the Young Investigator Award from SfN President Steven Hyman.

Rebecca Saxe has been promoted to full professor in the Department of Brain and Cognitive Sciences at MIT.

Bob Horvitz has been named a 2015 Fellow of the National Academy of Inventors. Horvitz was recognized for his "prolific spirit of innovation in creating or facilitating outstanding inventions that have made a tangible impact on quality of life, economic development, and the welfare of society."

Feng Zhang received an honorable mention in the *Boston Globe Magazine's* 2015 Bostonians of the Year list as "one of the world's most creative and influential biological engineers."

In addition to winning a Breakthrough Prize *(see page 6)*, **Ed Boyden** has received the Young Investigator Award from the Society for Neuroscience. The $15,000 award, which was presented at the society's annual meeting in Chicago, recognizes "outstanding achievements and contributions by a young neuroscientist."

Mehrdad Jazayeri has been awarded the Klingenstein-Simons Fellowship Award in the Neurosciences. The award funds young investigators engaged in basic or clinical research that may lead to a better understanding of neurological and psychiatric disorders. ■

The McGovern Institute for Brain Research at MIT is led by a team of world-renowned neuroscientists committed to meeting two great challenges of modern science: understanding how the brain works and discovering new ways to prevent or treat brain disorders. The McGovern Institute was established in 2000 by Patrick J. McGovern and Lore Harp McGovern, with the goal of improving human welfare, communication and understanding through their support for neuroscience research. The director is Robert Desimone, who is the Doris and Don Berkey Professor of Neuroscience at MIT and former head of intramural research at the National Institute of Mental Health.

Further information is available at: http://mcgovern.mit.edu

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