MCGOVERN INSTITUTE

BRAINSCAN

WINTER 2020 — ISSUE 51

TOOLKIT

McGOVERN MUSINGS

Perspectives from our community

Targeting specific genes for precise editing with CRISPR technology has dramatically revolutionized genetic research. Twenty years ago, the discovery of RNA interference (RNAi) allowed scientists to use short RNAs to silence specific genes. That technology eventually led to a therapeutic agent, patisiran, which recently gained FDA approval for treatment of a lethal amyloid disease.

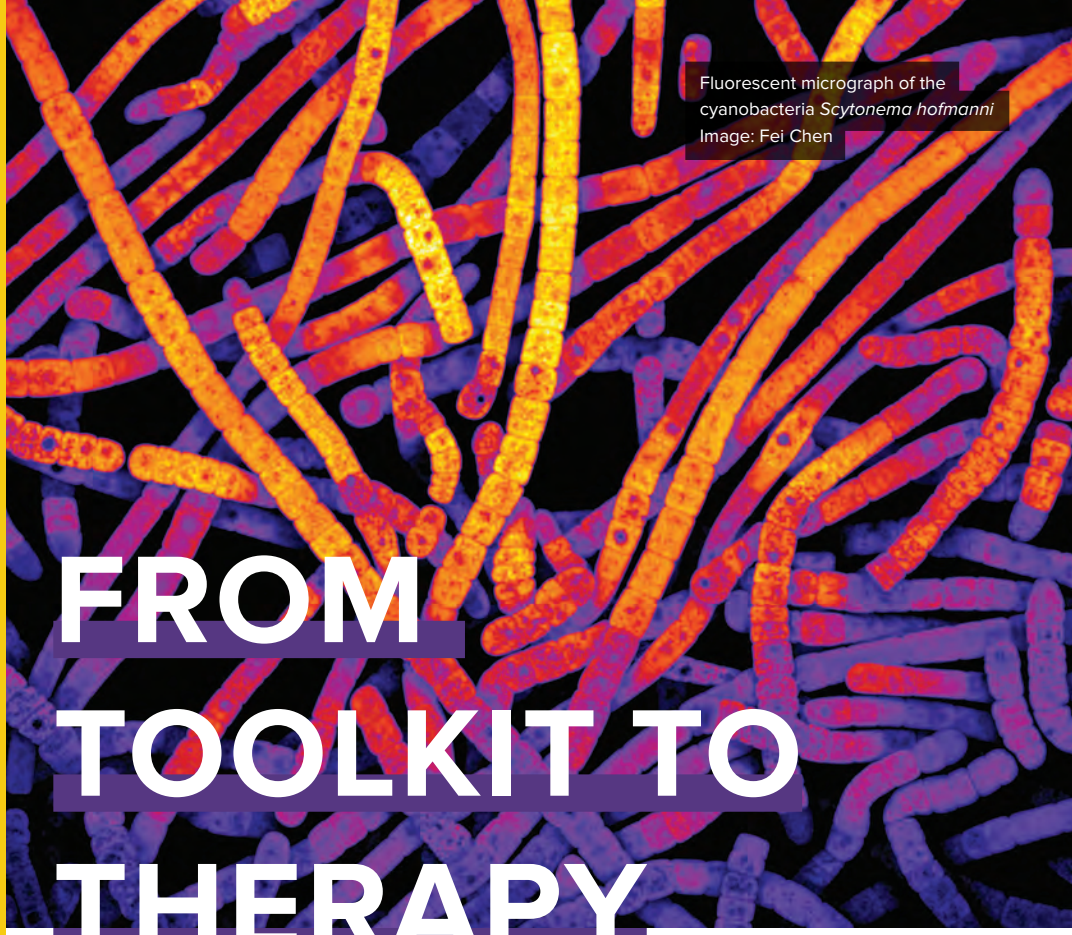
With CRISPR, every day a new application or disease insight appears. Perhaps even more than RNAi, CRISPR technology is a transformative advance, and without a doubt, treatments based on CRISPR technology will be similarly approved in the coming years for control of life-threatening conditions. I watch the creativity of the scientists leading this revolution with amazement.

PHILLIP A. SHARP

Founding Director, McGovern Institute
Institute Professor, MIT

BUILDING A TOOLKIT

The CRISPR revolution began with a discovery that the bacterial enzyme Cas9 could be used to edit DNA in human cells. Cas9 is now one of many tools in the growing CRISPR toolbox.



Fluorescent micrograph of the cyanobacteria *Scytonema hofmanni*
Image: Fei Chen

FROM TOOLKIT TO THERAPY

McGovern researchers are expanding the CRISPR toolbox toward gene therapy for neurons.

Think of the human body as a community of cells with specialized roles. Each cell carries the same blueprint, an array of genes comprising the genome, but different cell types have unique functions—immune cells fight invading bacteria, while neurons transmit information.

But when something goes awry, the specialization of these cells becomes a challenge for treatment. For example, neurons lack active cell repair systems required for promising gene editing techniques like CRISPR.

Can current gene editing tools be modified to work in neurons? Can we reach neurons without impacting healthy cells nearby? McGovern Institute researchers are trying to answer these questions by developing gene editing tools and delivery systems that can target—and repair—faulty brain cells.

EXPANDING THE TOOLKIT

Natural CRISPR systems help bacteria fend off would-be attackers. Our first glimpse of the impact of such systems was the use of CRISPR-Cas9 to edit human cells.

"Harnessing Cas9 was a major game-changer in the life sciences," explains Feng Zhang, an investigator at the McGovern Institute and the James and Patricia Poitras Professor of Neuroscience at MIT. "But Cas9 is just one flavor of one kind of bacterial defense system—there is a treasure trove of natural systems that may have enormous potential, just waiting to be unlocked."

By finding and optimizing new molecular tools, the Zhang lab and others have developed CRISPR tools that can now potentially target neurons and fix diverse mutation types, bringing gene therapy within reach.

PRECISE IN SPACE AND TIME

A single letter change to a gene can be devastating. These genes may function only briefly during development, so a temporary "fix" during this window could be beneficial. For such cases, the Zhang lab and others have engineered tools that target short-lived RNAs. These molecules act as messengers, carrying information from DNA to be converted into functional factors in the cell.

"RNA editing is powerful from an ethical and safety standpoint," explains Soumya Kannan, a graduate student in the Zhang lab working on these tools (see back cover). "By targeting RNA molecules, which are only present for a short time, we can avoid permanent changes to the genetic material, and we can make these changes in any type of cell."

Zhang's team has developed twin RNA-editing tools, REPAIR and RESCUE, which can fix single RNA bases by bringing together a base editor with the CRISPR protein Cas13. These RNA-editing tools can be used in neurons because they do not rely on cellular machinery to make the targeted changes. They also have the potential to tackle a wide array of diseases in other tissue types.

CAST ADDITION

If a gene is severely disrupted, more radical help may be needed: insertion of a normal gene. For this situation, Zhang's lab recently identified CRISPR-associated transposases (CASTs) from cyanobacteria. CASTs combine Cas12k, which is targeted by a guide RNA to a precise genome location, with an enzyme that can insert gene-sized pieces of DNA. Transposases were originally identified as enzymes that help rogue genes "jump" from one place to another in the genome. CAST uses a similar activity to insert entire genes self-sufficiently without help from the target cell so, like REPAIR and RESCUE, it can potentially be used in neurons.

"Our initial work was to fully characterize how this new system works, and test whether it can actually insert genes," explains Alim Ladha, a graduate fellow in the Tan-Yang Center for Autism Research, who worked on CAST with Jonathan Strecker, a postdoctoral fellow in the Zhang lab.

The goal is now to use CAST to precisely target neurons and other specific cell types affected by disease.

TOWARD DELIVERY

As the gene-editing toolbox expands, McGovern labs are working on precise delivery systems.

Adeno-associated virus (AAV) is an FDA-approved virus for delivering genes, but has limited room to carry the necessary cargo — CRISPR machinery plus templates — to fix genes. To tackle this problem, McGovern Investigators Guoping Feng and Feng Zhang are working on reducing the cargo needed for therapy.

In addition, the Zhang, Gootenberg and Abudayyeh labs are working on methods to precisely deliver the therapeutic packages to neurons, such as new tissue-specific viruses that can carry bigger payloads. Finally, entirely new modalities for delivery are being explored in the effort to develop gene therapy to a point where it can be safely delivered to patients.

"Cas9 has been a very useful tool for the life sciences," says Zhang. "And it'll be exciting to see continued progress with the broadening toolkit and delivery systems, as we make further progress toward safe gene therapies."

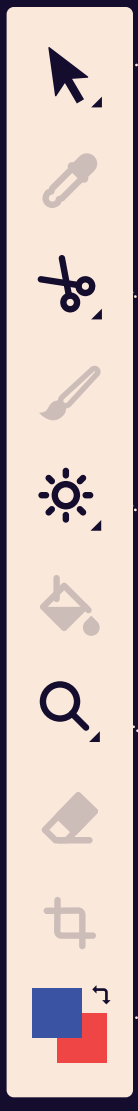
"With traditional CRISPR you can make simple changes, similar to changing a few letters or words in a Word document. The new system can 'copy and paste' entire genes."

– ALIM LADHA, ZHANG LAB

THE CRISPR TOOLBOX

They cut. They edit. They repair.

The tools of the CRISPR gene editing toolkit are revolutionizing neuroscience research, and McGovern scientists are creating some of the most powerful tools in the kit. Here is how they work.



TOOLS

MOVE

FUNCTION Precisely and efficiently insert large DNA sequences into a genome

APPLICATIONS Target genetic mutations (e.g. SHANK3) linked to brain disorders (e.g. autism); immunotherapy

CUT

FUNCTION Permanently and selectively delete/inactivate genes from a cell's DNA

APPLICATIONS Test gene functions, disable mutant genes, target infectious agents

EXPRESS

FUNCTION Precisely manipulate gene expression

APPLICATIONS Identify and repair genes linked to disease (e.g. muscular dystrophy, diabetes)

SEARCH

FUNCTION Detect trace amounts of DNA or RNA

APPLICATIONS Detect pathogens (e.g. viral/bacterial outbreaks) and cancer-related mutations

EDIT

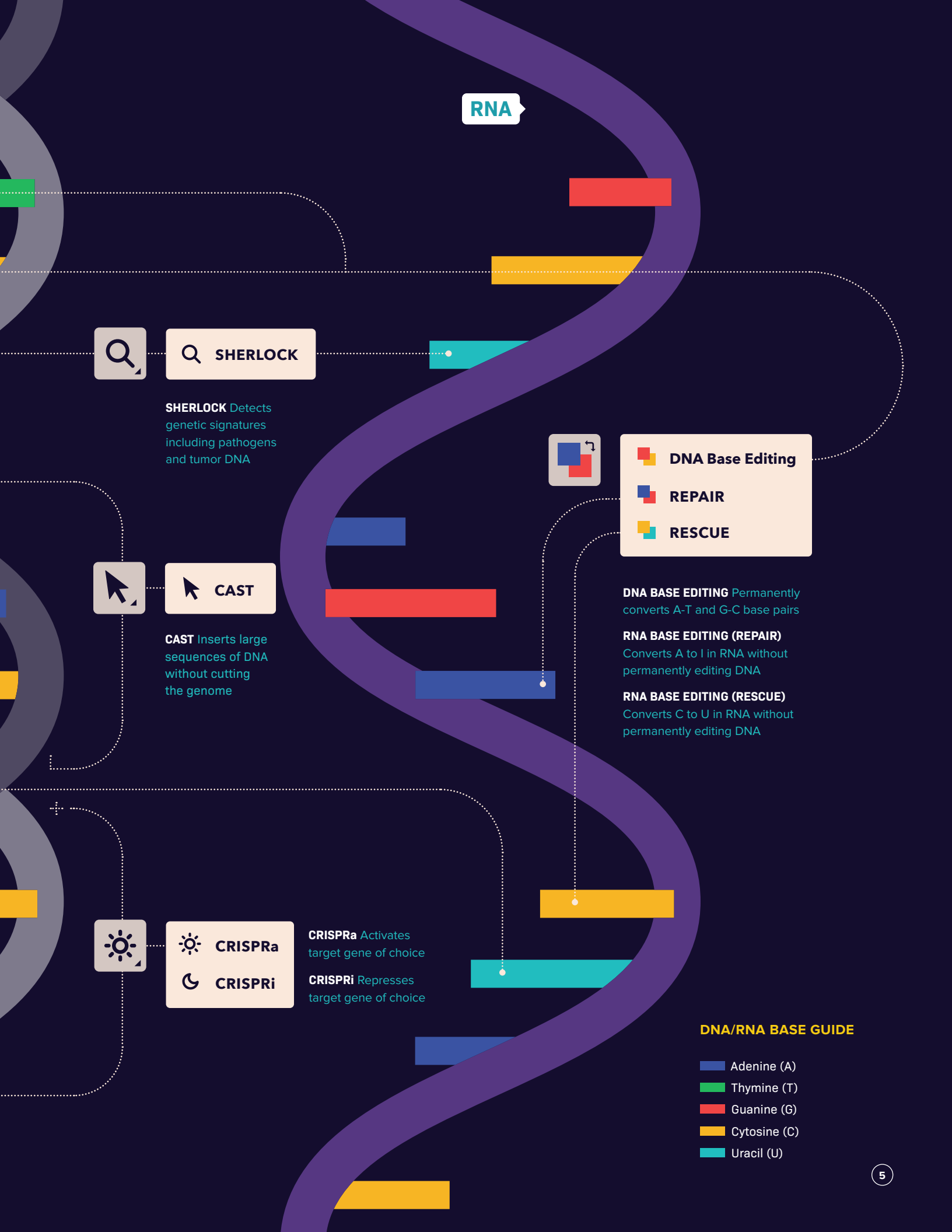
FUNCTION Efficiently and selectively change bases in DNA or RNA

APPLICATIONS Target single letter changes linked to disease (e.g. Rett syndrome, Alzheimer's disease)

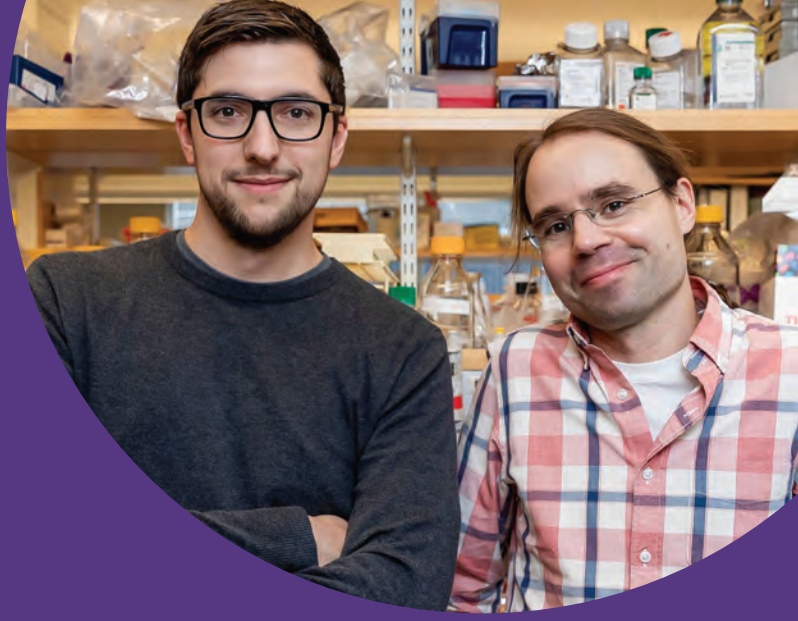
Cas9 First CRISPR tool to precisely cut DNA of living human cells

Cas12 Accesses and cuts different regions of DNA than Cas9

Cas13 First CRISPR tool to precisely cut RNA


RNA
SHERLOCK
SHERLOCK Detects genetic signatures including pathogens and tumor DNA
DNA Base Editing
REPAIR
RESCUE
DNA BASE EDITING Permanently converts A-T and G-C base pairs
RNA BASE EDITING (REPAIR) Converts A to I in RNA without permanently editing DNA
RNA BASE EDITING (RESCUE) Converts C to U in RNA without permanently editing DNA
CAST
CAST Inserts large sequences of DNA without cutting the genome
CRISPRa
CRISPRi
CRISPRa Activates target gene of choice
CRISPRi Represses target gene of choice
DNA/RNA BASE GUIDE
Adenine (A)
Thymine (T)
Guanine (G)
Cytosine (C)
Uracil (U)

Q & A: Two CRISPR Scientists on the Future of Gene Editing



Jonathan Wilde (left) and Martin Wienisch (right) of the Feng lab look into the crystal ball to predict the future of CRISPR tech.

WHERE WILL CRISPR BE IN 5 YEARS?

Jonathan: We'll definitely have more efficient, more precise, and safer editing tools. An immediate impact on human health may be closer than we think through more nutritious and resilient crops. Also, I think we will have more viable tools available for repairing disease-causing mutations in the brain, which is something that the field is really lacking right now.

Martin: And we can use these technologies with new disease models to help us understand brain disorders such as Huntington's disease.

Jonathan: There are also incredible tools being discovered in nature: exotic CRISPR systems from newly discovered bacteria and viruses. We could use these to attack disease-causing bacteria.

Martin: We would then be using CRISPR systems for the reason they evolved. Also improved gene drives, CRISPR-systems that can wipe out disease-carrying organisms such as mosquitoes, could impact human health in that time frame.

WHAT WILL MOVE GENE THERAPY FORWARD?

Martin: A breakthrough on delivery. That's when therapy will exponentially move forward. Therapy will be tailored to different diseases and disorders, depending on relevant cell types or the location of mutations for example.

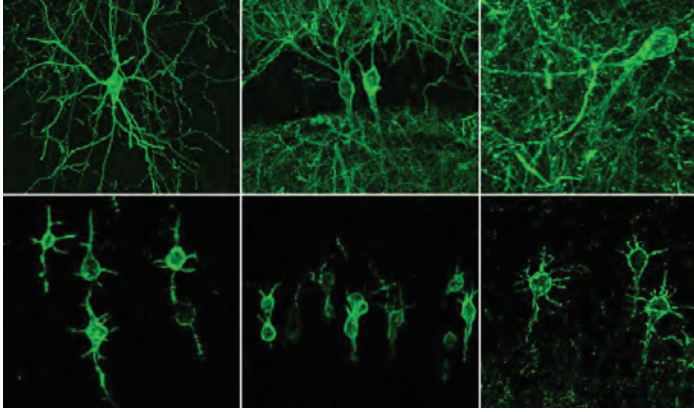
Jonathan: Also panning biodiversity even faster: we've only looked at one small part of the tree of life for tools. Sequencing and computational advances can help: a future where we collect and analyze genomes in the wild using portable sequencers and laptops can only quicken the pace of new discoveries.

Do you have questions about CRISPR and its future?

mcgovern.mit.edu/ask-the-brain

"When we started working on Cas9, its natural function was already known. Now we are discovering completely novel microbial systems, studying their natural biology, and engineering them for use in human cells."

—RHIANNON MACRAE, SCIENTIFIC ADVISOR, ZHANG LAB



COGNITIVE NEUROSCIENCE

Pitch Perception

A new study out of the **McDermott** lab has found that perception of musical pitch varies across cultures and that people interpret musical notes differently, depending on the types of music they have been exposed to in their lifetime. This kind of broad cultural study allows researchers to tease out different elements of perception that cannot be seen when examining only a single, homogenous group.

Benefits of Sleep

In a study of MIT students, **John Gabrieli** and colleagues found a strong relationship between students' grades and sleep habits. What time students went to bed, the amount of sleep they got, and the consistency of their sleep habits played a significant role in academic performance, while physical exercise, surprisingly, did not.

COMPUTATIONAL NEUROSCIENCE

Mental Computations

Using a novel combination of task design, data analysis, and modeling, **Mehrdad Jazayeri** and colleagues now provide compelling evidence that the core elements of an internal model also control purely mental processes. Understanding the building blocks that control such mental processes could help to paint a better picture of disruptions in mental disorders.

CELLULAR & MOLECULAR NEUROSCIENCE

Seeing Circuits

Using a fluorescent probe that lights up when brain cells are electrically active, **Ed Boyden** and colleagues have shown that they can image the activity of many neurons at once, in the brains of mice. This technique, which can be performed using a simple light microscope, could allow neuroscientists to visualize the activity of brain circuits with unprecedented clarity and link them to specific behaviors.

Fluorescent probe reveals electrical activity in neurons (top row); new variant of probe reveals activity specifically in cell bodies (bottom row). Image: Ed Boyden

SYSTEMS NEUROSCIENCE

Noise Reduction

People with autism often experience hypersensitivity to noise and other sensory input. **Michael Halassa** and **Guoping Feng** have now identified two brain circuits that help tune out distracting sensory information, and they have found a way to reverse noise hypersensitivity in mice by boosting the activity of those circuits.

In Memoriam

With a heavy heart we announce the recent passing of Marshall Tulin (MIT Class of 1946) of Santa Barbara, California, a longtime friend and generous supporter of schizophrenia research at the McGovern Institute. We are most fortunate that Marshall's generosity and enthusiasm for cutting-edge research into the biological basis of mental illness will continue on in perpetuity through the endowed Michael, Leah and Marshall P. (1946) Tulin Fund.

“Our work is to shrink RNA editing systems, making them easier to deliver to target cells.”

– SOUMYA KANNAN, ZHANG LAB

Before CRISPR gene-editing tools can be used to treat brain disorders, scientists must find safe ways to deliver the tools to the brain. One promising method involves harnessing viruses that are benign, and replacing non-essential genetic cargo with therapeutic CRISPR tools. But there is limited room for additional tools in a vector already stuffed with essential gear.

Soumya Kannan is addressing this capacity problem in Feng Zhang’s lab with fellow graduate student Han Altae-Tran, by developing smaller CRISPR tools that can be more easily packaged into viral vectors for delivery. She is focused on RNA editors, members of the Cas13 family that can fix small mutations in RNA without making changes to the genome itself.

Learn more about this research:
mcgovern.mit.edu/RNAediting

McGOVERN INSTITUTE

Massachusetts Institute of Technology
77 Massachusetts Avenue 46-3160
Cambridge, MA 02139
mcgovern.mit.edu

GIVE at mcgovern.mit.edu/giving

EDITOR Julie Pryor
WRITER Sabbi Lall
DIRECTOR OF DEVELOPMENT Kara Flyg
PHOTOGRAPHERS Caitlin Cunningham, Justin Knight
COVER ART Magoz
DESIGN Opus Design