NEWS

‘CRISPR’ way to cut genes speeds advances in autism

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Less than three years ago, two landmark publications in *Science* gave researchers a quick and easy recipe for tinkering with genes[1,2]. The papers described a new tool — a modified enzyme called CRISPR-CAS9 — that allows researchers to reach into the genome and snip, or substitute, DNA sequences with unprecedented precision and efficiency.

In cells, researchers have used this new system to disarm the culprit in single-gene disorders ranging from Huntington’s disease to cystic fibrosis[3,4]. And in one study published last year, researchers inserted a mutation into human stem cells that could prevent HIV from sneaking into the cells[5].

In the case of complex genetic disorders such as autism, the tool hews the way toward clarifying the subtle effects of thousands of autism-linked mutations. Using the method, researchers can create animal models endowed with these genetic quirks much faster and more efficiently. Tweaking the genes of monkeys, something that was challenging with conventional techniques, is also now a real option.

“It’s a breakthrough technology, that’s for sure,” says **Guoping Feng**, professor of brain and cognitive sciences at the Massachusetts Institute of Technology. Feng is using the method to engineer marmosets that lack the autism candidate gene **SHANK3**.

In one example of its potential for autism research, in October researchers used CRISPR to examine the effect of mutations in MeCP2, a candidate gene, on circadian rhythms[6]. Some teams are trying to recreate autism-linked mutations in mice and in stem cells, and to mutate combinations of genes in fish.

Making mice:

Since the 1990s, researchers have been able to edit genes by fusing enzymes that cut DNA with those that bind to specific sequences. The first of these tools, zinc-finger nucleases, relied on enzymes that each bind to preset sequences of three DNA base pairs. In 2011, two independent teams described a DNA-binding protein from bacteria that infect plants as the basis for a more precise gene-editing system, **called TALENS**. Still, each of these methods requires researchers to first create a protein that specifically binds their target sequence.

CRISPR, by contrast, is fused to a protein called CAS9, which binds to DNA. Strands of RNA that can be designed for any desired DNA sequence guide CAS9 to its target. Designing a precise RNA to match a DNA sequence is considerably easier than creating the equivalent protein match.

Last year, one of CRISPR's inventors, **Feng Zhang** at the Massachusetts Institute of Technology, engineered a mouse that already expresses CAS9 in all of its cells[7]. Use of this mouse eliminates having to introduce CAS9 into the cells of interest, allowing researchers to add only the guide RNAs needed.

One big advantage of CRISPR for creating mouse models is that it allows researchers to closely mimic the autism-linked mutations seen in people. Traditional methods crudely knock out both copies of a relevant gene, a distant simulation of mutations that might affect only one copy of a gene, for example. What's more, a CRISPR mouse model can be up and running — literally — in three months as opposed to the typical six months or more.

Another significant edge is that the method makes it possible to easily insert multiple mutations into a single animal, unlike the traditional method of painstakingly creating sets of mice for each animal and then interbreeding them over years.

Autism is thought to stem from the additive effect of multiple mutations, so this advantage is a particularly powerful one for research on the condition.

Flips and swaps:

Michael Talkowski and his team at Harvard University are exploring how mutations in CHD8, a leading autism candidate, influence gene expression.

Some people with autism carry one mutated copy of CHD8. Last year, in a bid to study **how CHD8 might lead to autism**, Talkowski's team used the traditional method of RNA interference, in which a string of RNA binds and blocks expression of a gene — in this case, CHD8 — and tracked the effects. This method is not ideal because researchers are guessing at the level of expression when one copy of the gene is mutated. CRISPR, by contrast, allows researchers to reproduce those effects directly.

"CRISPR has enabled a way to very accurately, repeatedly and robustly model genome mutation

in ways that mimic what we see in actual [people]," says Talkowski, assistant professor of neurology at Harvard University.

His team has now switched to using CRISPR. Another team has used CRISPR to generate stem cells that lack one copy of CHD8[8]. The mutation is stable over generations, providing a ready and renewable source for future experiments.

CRISPR can also recreate deletions or duplications of long stretches of DNA that occur in people with autism. In October, Talkowski's team reported at a conference that they have been able to engineer **duplications or deletions of the 16p11.2** chromosomal region, both of which are associated with autism. They have since also recreated duplications and deletions of 15q13.3, another candidate region.

"Microduplications, especially, is something we're able to do now that I never could have imagined being able to do before," says Talkowski. He says he plans to reconstruct **even more complex rearrangements in DNA**, such as DNA inversions that flank duplications or deletions. "Everybody's working on different things to do here, and I think it's really exciting," he says.

Autism zoo:

To make these intricate manipulations, Talkowski is banking on the continued sharpening of the tool, in ways that enable even greater precision.

In January, Zhang and his colleagues reported that CRISPR can not only block but **boost the expression of target genes**. This tweak opens up therapeutic avenues — by, say, compensating for the mutant copy of a gene by enhancing expression from the unaffected copy. In another study published this month, Zhang tinkered with CAS9 to minimize its binding to unintended target regions[9].

Even before these tweaks, CRISPR held the promise of opening up autism research to more sophisticated animal models than mice. "One of the reasons why we have very little progress [in autism research] is probably that we don't have adequate animal models for it," says Feng.

The rat genome is more complex than the mouse, and not as responsive to traditional genetic engineering methods. Using CRISPR will make engineering rats easier, says **Rodney Samaco**, assistant professor of molecular and human genetics at Baylor College of Medicine in Houston. **Comparing the rat and mouse model** of the same mutation may enhance our understanding of the mutation's effects better than studying it in one type of animal, he says.

Primates are even more difficult to manipulate than rats, says Feng, who has tried many approaches. To create mouse models, researchers typically engineer mutations in embryonic stem cells so they can easily select for those that carry the mutation. They then inject these mutant cells

into mouse embryos, transforming some small proportion of germ, or reproductive, cells. (Once in a germ cell, a mutation is retained across generations.) This implantation technique has never worked in primates.

CRISPR instead allows researchers to tweak DNA directly in a single-cell embryo, so that it is present in every resulting cell in the body of primates, including humans. Feng and his colleagues are using CRISPR to generate a breeding colony of marmoset monkeys with autism mutations. Their **first planned model** is a monkey lacking SHANK3.

Tricky treatments:

Because CRISPR makes it easy to create changes that can remain stable over generations, it offers promise as a gene therapy. Its application in people still seems far off, however.

In April, Chinese researchers used the method to modify the gene involved in a sometimes-fatal blood disorder, called beta thalassemia, in fertilized embryos[10]. To circumvent ethical concerns, the researchers **used embryos from a local fertility clinic** that were not viable. They reported that the method replaced the gene with low efficiency and may have unintended consequences.

The study reignited a long-held debate about how far researchers should go to make genetic fixes in people. Early this month, a group of nearly 500 scientists and ethicists concluded that the scientific community needs to **address potential misuses** of the technology before allowing any more experiments on human embryos.

Still, the idea that CRISPR may have a therapeutic use is exciting, says Feng.

“This is really opening the doors for many things we could potentially do,” he says. “[CRISPR] changed our ways of thinking and approaching autism research. Instead of focusing on research, maybe we should also think about therapeutic approaches.”

REFERENCES:

1. Cong L. *et al. Science* **339**, 819-823 (2013) **PubMed**
2. Mali P. *et al. Science* **339**, 823-826 (2013) **PubMed**
3. An M.C. *et al. PLoS Curr*. **6**, ecurrents.hd.0242d2e7ad72225efa72f6964589369a (2014) **PubMed**
4. Firth A.L. *et al. Cell Rep.* **12**, 1385-1390 (2015) **PubMed**
5. Mandal P.K. *et al. Cell Stem Cell* **15**, 643-652 (2014) **PubMed**
6. Tsuchiya Y. *et al. Genes Cells* Epub ahead of print (2015) **PubMed**
7. Platt R.J. *et al. Cell* **159**, 440-455 (2014) **PubMed**

8. Wang P. *et al. Mol. Autism* **6**, 55 (2015) **PubMed**
9. Slaymaker I.M. *et al. Science* Epub ahead of print (2015) **PubMed**
10. Liang P. *et al. Protein Cell* **6**, 363-372 (2015) **PubMed**