Building better base editors

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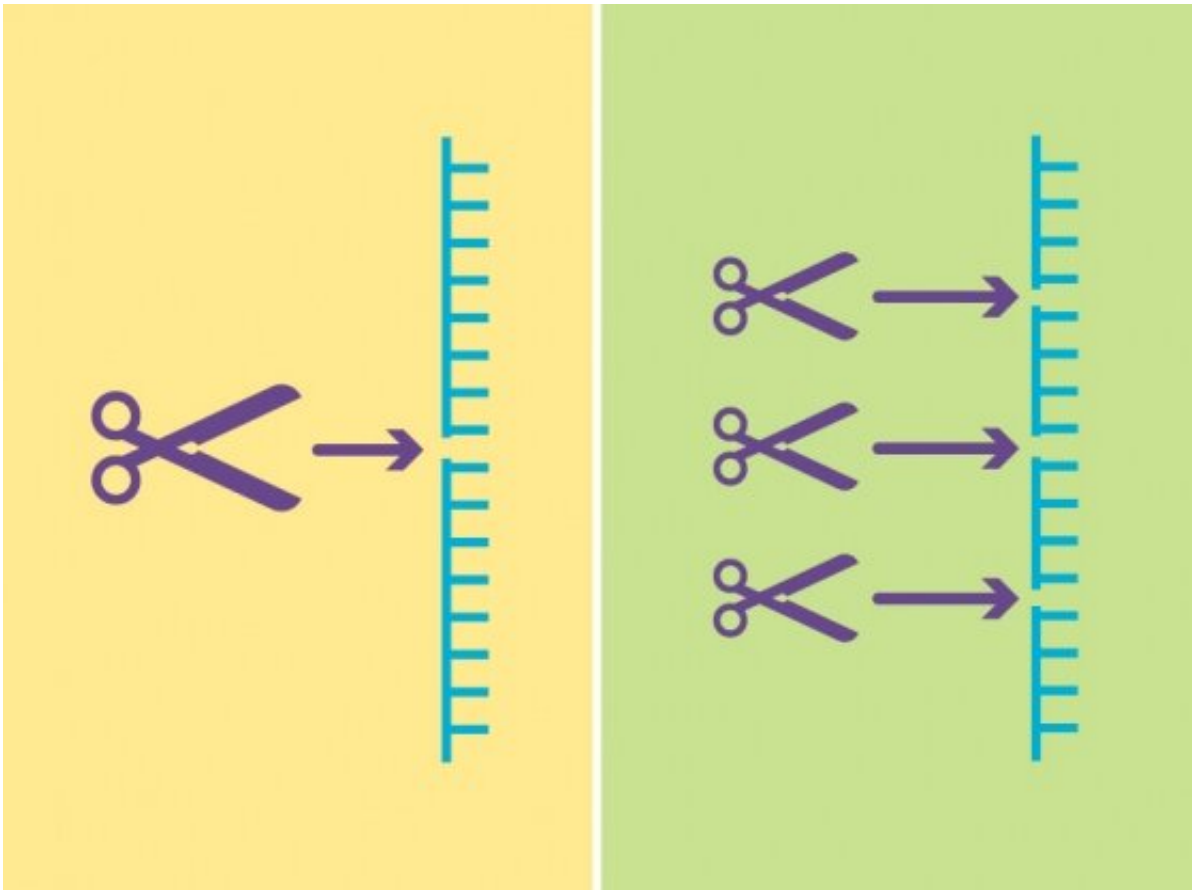

Credit : Susanna Hamilton, Broad Communications

CRISPR-based gene editing has potential therapeutic benefits but also some technical shortcomings. One set of these gene editing tools, base editors, can rewrite the four individual DNA letters, or bases— A, C, T or G—which represent key chemical building blocks of DNA, adenine, cytosine, thymine, and guanine.

Now, in two papers published in *Nature Biotechnology*, researchers the Broad Institute of MIT and Harvard, Harvard University, and the Howard Hughes Medical Institute have invented new CRISPR tools that address some of the challenges of base editors by improving their precision and genome-targeting ability.

Base editors work by homing in on specific areas of DNA and swapping out certain bases for others. After that swap, base editors—like the cytosine base editor that converts C•G to T•A—sometimes perform unwanted, off-target edits. And until now, even the best CRISPR tool—SpCas9—can only bind to about one in 16 locations along DNA, leaving many genetic mutations out of reach.

In the first paper, the research team designed new cytosine base editors that reduced an elusive type of off-target editing by 10- to 100-fold, making these new variants especially promising for treating human disease. In the second study, the team evolved a new generation of CRISPR-Cas9 proteins capable of targeting a much larger fraction of pathogenic mutations, including one responsible for sickle cell anemia. This mutation was, until now, prohibitively difficult to access with previous CRISPR methods.

"Since the era of human genome editing is in its fragile beginnings, it's important that we do everything we can to minimize the risk of any adverse effects when we start to introduce these gene editors into people," said David Liu, senior author of the two papers. "Minimizing this kind of elusive off-target editing, called Cas9-independent editing, is an important step toward achieving that goal. This type of off-target editing can occur at random locations in the genome. When you run the experiment 10 times, you get 10 different answers. That makes it so challenging to study."

Liu is the Richard Merkin Professor and director of the Merkin Institute

of Transformative Technologies in Healthcare at the Broad Institute, a professor of chemistry and chemical biology at Harvard University, and a Howard Hughes Medical Institute investigator.

In search of off-targets

One way to detect Cas9-independent edits is to sequence the entire genome many times. But such experiments are time-consuming and expensive—tens of thousands of dollars. Instead, Liu and his team designed five new tests that detect these edits quickly and at low cost, without the need for whole-genome sequencing. In one test, they send in a different CRISPR protein to hold the strands of the DNA double helix open at six different locations in the human genome. Base editors strongly prefer to edit single-stranded DNA, so the open strands attract any misbehaving base editors. "By holding the strands open," Liu said, "this assay invites a cytosine base editor to come in and edit in the opened DNA if it's prone to do so."

Then, Liu and his team searched for base edits in the six open DNA strands. They sequenced the whole genome to verify their assays matched the results of the slower and more expensive, but proven, method—and they did.

Next, Liu's team tested every major type of cytosine base editor—14 in all—to determine which produced fewer off-target edits. A variant called YE1 won. "Even if we held a bunch of DNA loops open enticingly for it to edit, it wouldn't bite," Liu said. Since YE1 had a smaller editing reach than other variants—when parked on DNA, it could only edit the three closest bases—he and his team engineered the tool to reach farther—across five bases. The result is a more precise, selective and versatile suite of base editors.

More parking spots

CRISPR has another hurdle to overcome: its limited ability to access the entire human genome. "You can't actually park Cas9 anywhere in the genome," Liu said. "You can only park it in places that have a small constant sequence of DNA—called a PAM."

Until now, the most common PAM was NGG, where "N" is any base. But two consecutive Gs only occur in about one in 16 places in the genome—just 6.25 percent of the genome. "One out of 16 is bad odds," Liu said.

To increase those odds, Liu and his lab evolved variants of Cas9 that could recognize some DNA sequences with just one G, expanding Cas9's reach to one in four locations in the genome. "But among the untrodden territories of SpCas9 are PAM 'deserts' that don't have any Gs," said Liu.

Using a previous invention from the Liu lab—phage-assisted continuous evolution (PACE)—the researchers forced SpCas9 to evolve quickly, creating many new generations of the protein in about a week. Without PACE, this process would take months or years. Their goal was to produce new SpCas9 proteins that had all the talents of the parent protein but greater versatility. Only proteins capable of recognizing PAM sequences without a G survived the Darwinian selection.

"And out came these three families of SpCas9 variants," Liu said. Collectively, they can direct both cytosine and adenine base editors and park at almost any NR, where R is either an A or a G, giving them access to roughly half of all DNA sites. Because base editors can reach across a five-base window to perform edits, the likelihood of a five-base window without an A or G is just 5 percent. "Ninety-five percent of pathogenic point mutations that we know of have an NR PAM in the right place to support base editing," Liu said. This means base editors can now reach

and correct up to 95 percent of point mutations that cause disease.

The researchers also showed that their new SpCas9 variants could base edit the mutation that causes most cases of sickle cell anemia, which has been difficult to access. "By bad luck, it doesn't have an NGG in the right place for previous SpCas9 base editors to work optimally on the sickle cell mutation," Liu said. "As a result, it's challenging to use published base editors to go after that site."

"And sure enough," Liu added, "using one of the evolved SpCas9 variants, we can now base edit this mutation quite efficiently."

More information: Jordan L. Doman et al. Evaluation and minimization of Cas9-independent off-target DNA editing by cytosine base editors, *Nature Biotechnology* (2020). DOI: 10.1038/s41587-020-0414-6

Shannon M. Miller et al. Continuous evolution of SpCas9 variants compatible with non-G PAMs, *Nature Biotechnology* (2020). DOI: 10.1038/s41587-020-0412-8

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