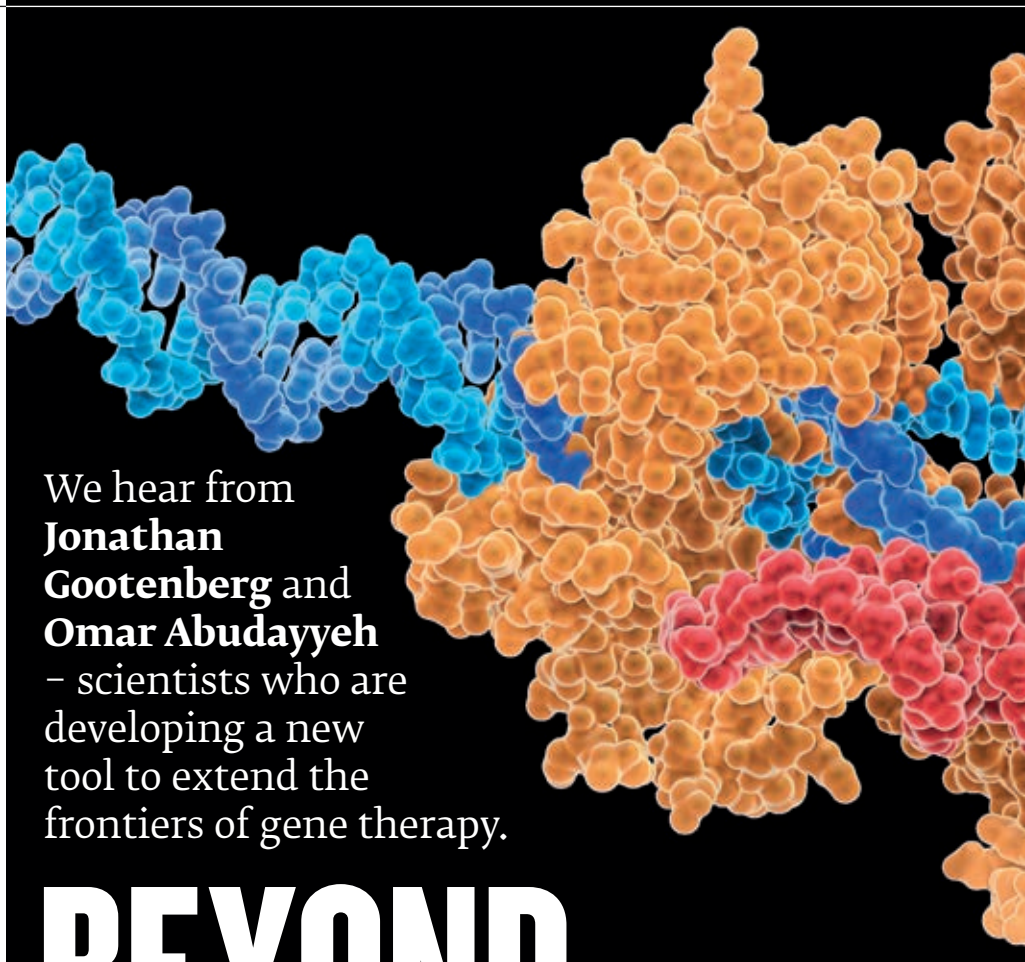


We hear from **Jonathan Gootenberg** and **Omar Abudayyeh** – scientists who are developing a new tool to extend the frontiers of gene therapy.

BEYOND CRISPR-CAS9

The development of CRISPR-Cas9 was an unprecedented advance in the field of genome editing – and the Nobel Prize in Chemistry awarded in 2020 to Jennifer Doudna and Emmanuelle Charpentier, the two scientists who designed the technology, was a recognition of this extraordinary accomplishment. With CRISPR-Cas9, it became possible for researchers all around the world to manipulate genetic material with greater precision than ever before, in ways that were all-around less complicated, time-consuming and costly. The technology opened up entirely new possibilities in the fields of gene therapy, cell therapy and immunotherapy, but also biotechnology or even agriculture.

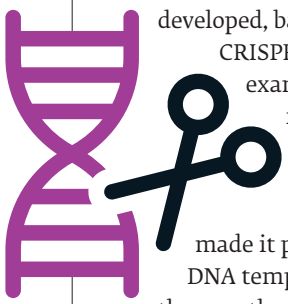
However, the technology has faced challenges, which scientists have been trying to address for a decade. Among them are Jonathan Gootenberg and Omar Abudayyeh who are building on the CRISPR gene-editing system in their lab at McGovern Institute for Brain Research at MIT. They have designed a tool to cut out and replace faulty genes with new ones.

A powerful tool

The CRISPR-Cas9 gene-editing system is made of a DNA-cutting enzyme called Cas9 and a short RNA strand that guides said enzyme to a specific area of the genome to make its cut. The cell's DNA repair processes then glue the cut back together, often deleting a small portion of the genome.

"This is a powerful tool, nevertheless this first generation of CRISPR-Cas9 just worked as a scissor. It could make a cut in the genome, leading to a deletion which can be useful for a number of genetic diseases but for many others, the system quickly reached its limits" says Omar Abudayyeh.

In recent years, in order to move beyond those limits, new tools have been developed, based on the original CRISPR-Cas9 technology. The example of "prime editing" for instance, is very interesting. By fusing specific enzymes to Cas9, this new tool made it possible to deliver a DNA template to the cells, which they are then able integrate to their genome during the repair process.



However, this process requires cells to make double-stranded breaks in their DNA, which can cause potential detrimental chromosomal deletions or genetic rearrangements.

"Furthermore, while prime editing seemed really promising at first, another limit is that for some genetic diseases, there may be many different harmful mutations involved on one gene. It's just not practical to replace each of these mutations, one at a time. We aimed to solve this problem by coming up with a system where an entire gene can be replaced – which was the original promise of gene therapy when the field started," explains Omar Abudayyeh.

Leveraging the power of natural systems

To achieve this goal, the researchers turned to a family of enzymes called "integrases". These are enzymes that

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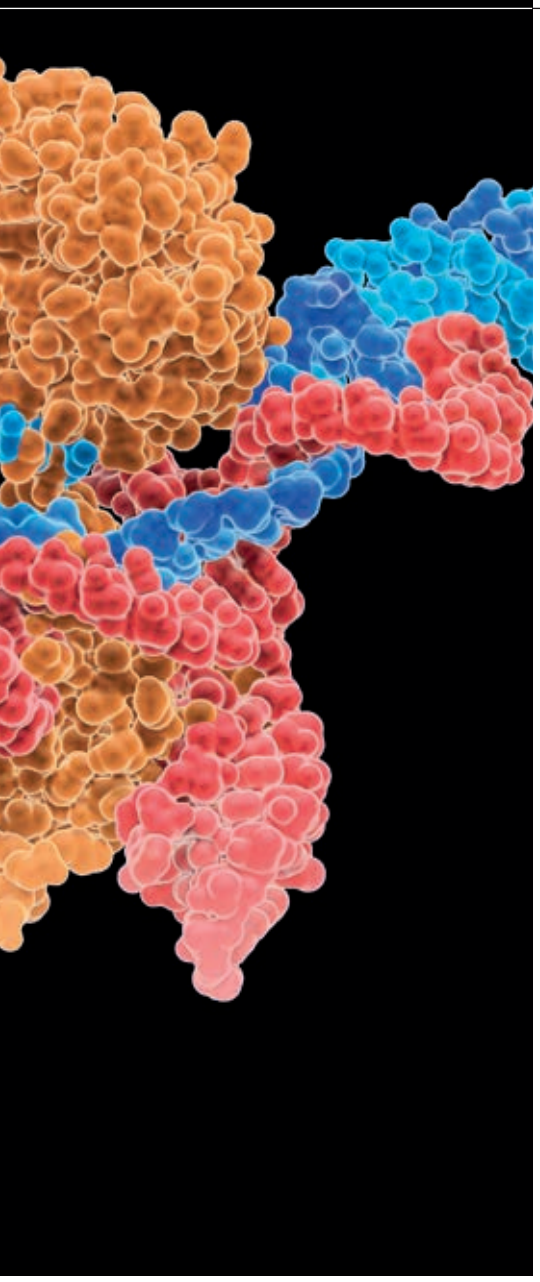

viruses known as bacteriophages use, in order to insert themselves into the genomes of bacteria.

Integrases are particularly interesting because they can insert large fragments of DNA – up to 50,000 base pairs. This makes the goal of integrating a full gene into cells much more achievable. However, the problem is that these enzymes target only specific genome sequences known as "attachment sites", and it's very tricky to reprogramme them to target other sites. So the researchers had to come up with ways to lift those limits.

"These enzymes are fascinating. They have evolved from bacterial viruses called bacteriophages that insert a hundred thousand base pairs of their genomes into bacteria. Their problem is they can't be programmed, you can't tell them where to insert the gene. In our lab, we have developed a technology to make 'programmable integrases'," Jonathan Gootenberg points out.

The scientists developed a new tool called PASTE (Programmable Addition via Site-specific Targeting Elements). To put it simply, a Cas9 enzyme first cuts at a specific genomic site, guided by a strand of RNA. An "attachment site" - which can be recognised by integrases - can then be inserted at that specific site. Once this is done, an integrase is thus able to insert a much larger DNA payload into the genome at that location.

In their new study, published in *Nature Biotechnology*, the researchers showed that they could use PASTE to insert genes into several types of human cells, including liver cells, T cells, and lymphoblasts. They also tested the tool with 13 different payload genes, including some that could be therapeutically useful, inserting them successfully into nine different locations in the genome.

"Ever since we published the paper as a preprint in 2021, scientists around the world have been contacting us and expressing an interest in PASTE.

"We have set up different academic collaborations and we are working hard to improve the tool and to make it more efficient and more accessible," says Jonathan Gootenberg.

The next step for the lab is about exploring the possibility of using PASTE as a way to replace the defective cystic fibrosis gene.

Although we are still a long way from using genome-editing technologies to cure genetic diseases, the field is getting better at translating them, at making them clinically relevant.

"Many people are starting to see programmable gene integration as the new frontier for the genome-editing field. We and other groups are trying to make this a reality, which is exciting. We want to pursue our research to see if PASTE can actually be applied to treat genetic diseases in the next decade," Jonathan Gootenberg explains.

"It is also worth noting that integrases are just the tip of the iceberg. In the coming years, we will continue to think through how we can leverage different natural systems to keep pushing the boundaries of genome editing," Omar Abudayyeh concludes. BMS

KEY NUMBERS FROM THE STUDY

- ✔ **Omar Abudayyeh** and **Jonathan Gootenberg** started their lab at MIT nearly four years ago.
- ✔ PASTE allows scientists to deliver genes as long as 36,000 DNA base pairs to several types of human and animal cells.
- ✔ The tool was successfully tested with 13 different payload genes.
- ✔ There are well over 6000 known genetic disorders – only about 600 are treatable.

"Many people are starting to see programmable gene integration as the new frontier for genome editing"