to make is encoded on a strand of RNA. The longer that strand gets, the more likely it is to be damaged by enzymes in the cell.

"Different flavours of genome-editing platforms are still going to be needed for different types of edits," says Sontheimer.

But prime editing seems to be more precise and versatile than other CRISPR alternatives. Those include modified versions of CRISPR–Cas9 that enable researchers to swap out one DNA letter for another, and older tools such as zinc-finger nucleases, which are difficult to tailor to each desired edit.

Freedom through control

CRISPR–Cas9 and prime editing both work by cutting DNA at a specific point in the genome. CRISPR–Cas9 breaks both strands of DNA at once and then relies on the cell's own repair system to patch the cuts and make the edits. But that repair system is unreliable and can insert or delete DNA letters at the points where the genome was cut. This can lead to an uncontrollable mixture of edits that vary between cells.

Even when researchers include a template to guide the edits, the DNA repair system in most cells is much more likely to make those small, random insertions or deletions than to add a specific sequence to the genome. That makes it difficult for researchers to use CRISPR–Cas9 to overwrite a piece of DNA with a sequence of their choosing.

Prime editing bypasses these problems (see 'Precision editor'). It, too, uses Cas9 to recognize specific DNA sequences, but the prime editor's Cas9 enzyme is modified to nick only one DNA strand. Then, a second enzyme called reverse transcriptase, guided by a strand of RNA, makes the edits at the site of the cut.

The prime-editing enzymes don't have to break both DNA strands at the same time to make changes, freeing researchers from relying on the cell's genome repair system — which they can't control — to make the edits that they want. This means that prime editing could enable the development of treatments for genetic diseases caused by mutations that aren't easily addressed by existing gene-editing tools.

Previously, researchers, including Liu, thought that they would need to develop gene-editing tools specific to each category of change they wanted to make in a genome: insertions, deletions or DNA letter substitutions. And the options were limited when it came to making precise substitutions.

An older technique, called base editing, which is comparable in precision to prime editing, chemically converts one DNA letter directly into another — changing a T to an A or a G to a C — without breaking both DNA strands. That's something CRISPR–Cas9 can't do. Developed by Liu, base editing could be useful for correcting genetic diseases caused by single-letter mutations, including the most common form of sickle-cell anaemia.

But base editing can't help with genetic disorders caused by multi-letter mutations such as Tay–Sachs disease, a usually fatal illness typically caused by the insertion of four DNA letters into the *HEXA* gene. So Liu and his colleagues set out to create a precise gene-editing tool that gave researchers the flexibility and control to make multiple types of edits without having to create bespoke systems.

"It's fantastic," says Sontheimer. "The breadth of the mutations that can be introduced is one of the biggest advances. That's huge."

Liu's team, and others, will now need to carefully evaluate how well the system works in a variety of cells and organisms. "This first study is just the beginning — rather than the end — of a long-standing aspiration in the life sciences to be able to make any DNA change at any position in an organism," says Liu.

RUSSIAN SCIENTIST EDITS HUMAN EGGS IN EFFORT TO ALTER DEAFNESS GENE

Denis Rebrikov says he does not plan to implant gene-edited embryos until he gets regulatory approval.

By David Cyranoski

Russian biologist Denis Rebrikov has started editing genes in human eggs with the goal of repairing a mutation that can cause deafness. The news, detailed in an e-mail he sent to *Nature* on 17 October, is the latest chapter in a saga that kicked off in June, when Rebrikov revealed his controversial intention to create gene-edited babies resistant to HIV using the popular CRISPR tool. So far, only one person has claimed to have created a baby from a gene-edited embryo — the Chinese scientist He Jiankui, in November 2018.

Rebrikov's e-mail (see Q&A on page 466) follows a September report in the Russian magazine *N+1,* in which he said a couple who both have a genetic mutation that impairs their hearing had started procedures to collect eggs that would be used in an attempt to create a gene-edited baby. The eggs that Rebrikov has edited so far are from women without the genetic mutation. He says the goal of those experiments is to learn how to allow couples with the mutation to have a child with unaffected hearing.

He also wants to better understand potentially harmful 'off-target' mutations, which are a known challenge of using the CRISPR–Cas9 system to edit embryos.

Rebrikov says he does not plan to use the tool to create such a baby yet — and that his previously reported plan to apply this month for permission to implant gene-edited embryos in women has been pushed back.

Instead, he says, he will soon publish the results of his egg experiments, which also involved testing CRISPR's ability to repair the gene linked to deafness, called *GJB2*, in body cells taken from people with the mutation. People with two mutated copies of *GJB2* cannot hear well without interventions such as hearing aids or cochlear implants. Rebrikov says that these results will lay the groundwork for implanting an edited embryo.

Rebrikov adds that he has permission from a local review board to do his research, but that this does not allow transfer of gene-edited eggs into the womb and subsequent pregnancy.

Apart from the couple who agreed to start undergoing egg collection, he is in discussion with four other couples in which both would-be parents have two mutated *GJB2* genes, he says.

Rebrikov also provided further information about the couple who agreed to the procedures. In September, *N+1* reported that the couple hadn't signed a consent form and had backed away from the idea of creating a gene-edited child, citing personal reasons.

"I will definitely not transfer an edited embryo without the permission of the regulator."

But Rebrikov now says that this is only a temporary hurdle. He notes that the woman who donated the eggs has taken a one-month pause while she gets a cochlear implant.

Rebrikov also emphasized that he will not move forwards without approval from the Ministry of Health of the Russian Federation. "I will definitely not transfer an edited embryo without the permission of the regulator."

That might not come any time soon. Earlier this month, the ministry released a statement saying that production of gene-edited babies is

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Denis Rebrikov plans to publish his experiments to repair genes in human eggs soon.

ANDREY RUDAKOV/BLOOMBERG/GETTY

premature. Rebrikov says "it is hard to predict" when he will get permission, but that it will be after all the necessary safety checks.

Rebrikov shot to fame in June when he told *Nature* of his plans to make HIV-resistant babies. The news shocked international researchers, who feared that he was following in the footsteps of He Jiankui.

Those plans involve using CRISPR to disrupt the same gene that He did — *CCR5*. The protein made by the *CCR5* gene allows HIV to enter cells, and people with a mutated copy of this gene

"The project is clearly unethical and damages the credibility of a technology intended to help, not harm."

are much less likely to get the virus. But many scientists say that the benefits — possible resistance to HIV — are not worth the unknown risks of gene editing, because there are other ways to prevent HIV passing from parent to child.

Rebrikov says he started looking for women with HIV who wanted to have a baby and who have responded poorly to HIV drugs. He argues that such people might be good candidates for the procedure because they have an elevated risk of passing the virus to their children, although many scientists think that any attempt to use gene editing in embryos to modify *CCR5* is misguided. In his latest e-mail, Rebrikov told *Nature* that he is still looking for suitable women. "But there are very few of them," he says.

In the meantime, Rebrikov has taken on the project to repair the *GJB2* gene in human embryos. Some scientists also question the benefits of this procedure because hearing loss is not a fatal condition. "The project is recklessly opportunistic, clearly unethical and damages the credibility of a technology that is intended to help, not harm," says Jennifer Doudna, a pioneer of the CRISPR tool at the University of California, Berkeley.

In the wake of He's explosive revelation, the World Health Organization (WHO) tasked a committee with developing an international framework to govern the clinical use of gene editing. In August, the WHO committee also launched an international registry of clinical research using gene editing in humans to oversee this practice. An international commission created by the US National Academy of Sciences, the US National Academy of Medicine and the United Kingdom's Royal Society is also preparing a framework to guide clinical research in germline gene editing. This is expected to be released by mid-2020. The commission will hold a public meeting on 14–15 November to gather ideas.

Rebrikov said last month that he wants to follow regulations that have been internationally agreed on when moving gene editing to the clinic, according to the Bloomberg news agency. But he also expressed frustration that none exists yet.

Robin Lovell-Badge, a developmental biologist at the Francis Crick Institute in London and a member of the WHO committee, says that Rebrikov should wait until such a framework has been agreed, which will take time. "This is not a simple matter, and it is ridiculous to think that we can come up with global solutions to regulation in a very complex scientific and potentially clinical area in a few months."

Q&A

Denis Rebrikov

Below are edited versions of the questions that *Nature* sent to Rebrikov, and his answers.

Some scientists and bioethicists say that, because deafness is not a life-threatening condition, it should not be the target of a risky treatment like this.
Any new drug carries certain risks. The deafness model is the most appropriate for applying genomic editing at the zygote [newly fertilized egg] stage.

In particular, scientists worry about off-target mutations — which are potentially dangerous and could be introduced away from the intended edit.
Of course we worried about those. We have a long and reasonable algorithm for checking off-target activity. I'd like to discuss the algorithm for checking the efficiency and safety of the technology, rather than the method's prematureness.

Some also warn that because the CRISPR repair mechanism is inefficient, there is a high likelihood of producing children with mosaicism — a mix of edited and unedited cells. Are you worried about this?
Yes. Unfortunately, due to the impossibility of a complete analysis of the embryo — we only look at a biopsy of five to seven cells — we will never be completely sure of the absence of mosaicism in transferred embryos. But statistically (in experiments), it is possible to show either the permissible percentage of mosaicism or its absence.

The Russian health ministry said earlier this month that it follows the position of the World Health Organization committee: it is too early to do such experiments. Will you apply anyway?
What does it mean, too soon? Lenin said, "yesterday was too early, tomorrow it will be too late."

Those working on international frameworks to guide the clinical application of human-embryo editing have suggested that, until they are done, clinical research should slow down.
Are you serious? Where did you see the researcher willing to slow down?

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