Special News Feature

TO EDIT OR NOT: THE NgAgo STORY

New genome-editing approaches always receive widespread attention. But in the case of a novel Argonaute-based technique published last spring, attention has been particularly intense.

Credit: Ernesto del Aguila III, National Human Genome Research Institute, National Institutes of Health.

With an ever-growing array of applications being reported in the scientific literature, CRISPR/Cas9 remains the hot molecular biology technology of the moment. Yet there continues to be significant interest in refining and expanding current genome-editing tools. So when a team of researchers reported this spring that they had developed a new genome-editing technique that could overcome some of the downsides of CRISPR, there was tremendous excitement. But just as soon as scientists set off on a whirlwind of experiments, questions started to emerge.

A New Way to Edit Genes

It all started in May 2016 when Hebei University researchers Chunyu Han and his colleagues reported in the journal *Nature Biotechnology* that *Natronobacterium gregoryi* Argonaute protein (NgAgo) could function as a DNA-guided endonuclease capable of genome editing in human cells. The results were powerful since, unlike Cas9, NgAgo did not seem to require a protospacer-adjacent motif (PAM), making it easier to use for targeted genome editing. In addition, Han's data showed that NgAgo resulted in low guide-target mismatch and high efficiency when editing difficult templates, such as GC-rich genomic targets. These features made the new system particularly attractive to researchers, many of whom were eager to test the method for themselves. In fact, according to a guest blog post on the Addgene (a plasmid repository) website by MRC Centre for Regenerative Medicine postdoctoral fellow Pooran Dewari, more than 400 requests for the NgAgo plasmids have been made since publication.

The NgAgo system is not without its own shortcomings though—in vitro assembly of NgAgo/single-stranded DNA (ssDNA) complexes for targeting requires incubation at 55°C, a high temperature indeed for enzymes. Plus, the system involves co-transfection of the NgAgo plasmid with the targeting ssDNA

guide, another cumbersome step. Still, with the possibility of little off-target editing, researchers could see potential for the tool.

The scientific community weren't the only ones who had taken note of the publication; NgAgo gene editing quickly made its way into the general media. One Chinese news outlet quoted Han as saying that in the future, "With this technique, middle-aged men with bald heads can probably gain their hair through genetic repair." The South China Morning Post detailed Han's work in a story on underfunded researchers making significant contributions to science. And on the *Nature Biotechnology* website, Han's NgAgo original article was getting more downloads and views than any other. Expectations and interest in NgAgo were sky-high right out of the gate.

Wrinkles Appear

The internet has become a haven for researchers of all disciplines looking for suggestions and tips to improve their experiments. Open sharing of ideas and strategies has become increasingly commonplace online, so it is no surprise that there is an active genome-engineering Google Group focused on methods and tools for genome modification.

The first entry in that Google Group mentioning NgAgo appeared on June 23, 2016. It was from a researcher who tried a transfection with a NgAgo plasmid acquired from Addgene and a 5'P oligonucleotide targeting green fluorescent protein (GFP) for modification. This should have been a simple experiment where gene editing decreases GFP fluorescence. The problem was, there was no noticeable change in fluorescence after transfection, according to the post. The researcher wondered if anyone else had experienced similar issues with the NgAgo system. A day later, another poster reported they too had tried targeting genes with the same NgAgo plasmid and had also failed to see the expected editing result.

It's not unusual for researchers to have hiccups with new techniques in their first outing—there might be specific steps in a protocol that need to be exactly adhered to in order to achieve the intended result. But following those posts on June 23rd and 24th, more scientists posted their data. In time, trouble arose as there seemed to be two camps: some believed that the system might be working, while others agreed with the first two posters after they, too, did not see the expected editing results. Confusion started to weave its way through various posts in the Google group.

It's worth taking a step back here and noting that life science research is in the middle of a reproducibility crisis. Several recent high-profile reports that eventually had to be retracted (two *Nature* articles on STAP cells, for example) have led many to question new findings to a greater extent than in the past. As of early July 2016, vetting of the NgAgo technique became increasingly intense as more researchers posted their results and the details of how they did their experiments, often then asking for assistance in getting the technique to work.

In an effort to gain additional insight on the NgAgo controversy and where the technique stood, Dewari sent out a survey to users to assess their NgAgo success. By early August, the results were in. When asked if indels (insertions and deletions) had been seen using the NgAgo system, only 5.1% said yes, while 51.5% said no. Using NgAgo for other genome editing applications yielded very similar numbers. Based on the percentages, the NgAgo technique was in trouble, but the question that remained was whether the problems lay in the method itself or with the researchers trying to implement it in their own labs.

Consensus is a Complicated Road

Han has been quiet for the most part. According to reports, the researcher rarely travels outside of China. And while he has provided an additional protocol and detailed conditions for using NgAgo, as well as noting that researchers in other labs have been able to replicate his technique, the criticism has not been silenced. In fact, the additional information he provided actually raised more concerns amongst critics due to its specific time requirements and conditions.

Australian National University researcher Gaetan Burgio has been reporting the results of his NgAgo experiments online. Such open science efforts have led to many questions about the technique. Credit: Multimedia, JCSMR, ANU.

Perhaps the best summation of this curvy road for NgAgo up to this point came from Gaetan Burgio, a group leader at the Australian National University. On July 29, 2016, Burgio wrote a comprehensive blog entry on his experiences trying to get NgAgo to work for gene editing.

Initially, Burgio said his PCR results indicated that NgAgo had in fact edited the beta-spectrin gene he targeted using a NgAgo plasmid, vindication for NgAgo and Han. Burgio posted this result on Twitter and mentioned it to colleagues at a conference he was attending at the time. But shortly after Burgio's first PCR results came in and the initial excitement died down, things changed.

In a follow-up experiment, Burgio started seeing small holes in his NgAgo gene-editing validation data. Other enzyme tests he tried indicated that NgAgo had not edited the beta-spectrin gene as that first PCR gel suggested. Sequencing the PCR products again showed what Burgio suspected could be edited sequences. However, after isolating individual PCR bands and sequencing them, Burgio finally concluded that those sequences were in fact random and that no NgAgo editing ever occurred in his experiments. The last blog entry from Burgio was posted in late August where he stated that he could see no evidence of editing in any of his NgAgo experiments.

Burgio's results have only bolstered the contention for many that the NgAgo

system simply does not work. Some continue to argue there are specific cell lines and conditions that are needed for the gene-editing activity of NgAgo to be effective. Burgio himself noted his experiments were based on using NgAgo and were not attempts to directly replicate Han's experiments and results from the *Nature Biotechnology* article. Still, critics counter that even if this is true, it simply means the technique is not robust and therefore is not a viable substitute for CRISPR/Cas9.

At the moment, *Nature Biotechnology* is investigating the NgAgo paper after being contacted by several researchers who were unable to replicate the findings. And it has been reported that Han's university has also launched an inquiry.

While the future of NgAgo is still up in the air, according to Dewari's survey the majority of scientists (64.5%) are now holding off on NgAgo experiments, waiting to see what happens. Indications are it will take time before we learn if NgAgo, in any form, ends up in the genome-engineering toolbox.

Reference

Gao et al. 2016. DNA-guided genome editing using *Natronobacterium gregoryi* Argonaute. *Nat. Biotechnol.* 34, 768-773.

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