To Edit or Not: The NgAgo Story

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

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 for more efficient use. On the heels of the expansion of CRISPR, researchers introduced an argonaute-based genome editing approach that seemed to overcome some of CRISPR’s downsides.

A New Way to Edit Genes

In May 2016, Hebei University researchers Chunyu Han and his colleagues reported in the journal Nature Biotechnology that *Natronobacterium gregoryi* Argonaute (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, and many were eager to test the method for themselves. In fact, according to a blog on the Addgene website (a plasmid repository) posted 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 the NgAgo/single-stranded DNA (ssDNA) complex 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 though 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 out of the gate.

Wrinkles Appear

The internet has become a haven for researchers of all disciplines looking to find 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 the Google genome-engineering group associated with NgAgo appeared June 23, 2016. It was from a researcher who tried to transfect with an NgAgo plasmid acquired from Addgene and a 5´P oligonucleotide targeting GFP for modification. This should have been a simple experiment where the editing results in a decrease in GFP fluorescence. The problem was that there was no 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 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 for achieving the intended result. But following those posts on June 23 and 24, 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 posts after they also did not see the expected results. Confusion wove its way through various group posts.

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 (regarding 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, and requested assistance in getting the technique to work.

In an effort to gain some insight on the NgAgo controversy and where the technique stood, Dewari sent out a survey to users to assess their NgAgo research 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 similar numbers. Based on the percentages, NgAgo was in trouble, but the question remained whether the problem lay in the technique itself or the researchers trying to implement it in other 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 the technique, the criticism has not been silenced. In fact, the additional protocol actually raised more concerns amongst critics with its specific time requirements and conditions.

Perhaps the best summation of the curvy road for NgAgo up to this point came from Gaetan Burgio, a group leader at 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 edited the beta-spectrin gene he targeted using an NgAgo plasmid in a way that was similar to CRISPR/Cas9, vindication for NgAgo and Chunyu. Burgio posted this result on Twitter and mentioned it to colleagues at a conference he attended at the time. But shortly after Burgio’s first PCR results came in and the initial excitement died down, things started to change.

In a follow-up experiment, Burgio began to see small holes to the 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.

Burgio’s results and conclusions have only bolstered the contention for many that the NgAgo system simply does not work. Others argue that there are specific cell lines and conditions that are needed for the gene-editing activity of NgAgo to be effective, but critics counter that even if true, this simply means the technique is not robust and therefore a not a viable substitute for CRISPR/Cas9.

Nature Biotechnology is currently investigating the NgAgo paper after being contacted by several researchers who were unable to replicate the findings. And Han’s university has also launched an inquiry.

While the future of NgAgo is still up in the air as scientists work to determine whether or not the enzyme is in fact capable of gene editing, Dewari’s survey indicates most (64.5%) are now holding off on experiments, waiting to see what happens. Time will tell if NgAgo ends up in the genome-engineering toolbox.

Reference


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