EXPERIMENTAL ORGANISMS

CRISPR for choanoflagellates expands the genetic toolbox for our sister group

Booth, D.S. and King, N. *eLife* **9**, e56193 (2020). https://doi.org/10.7554/eLife.56193

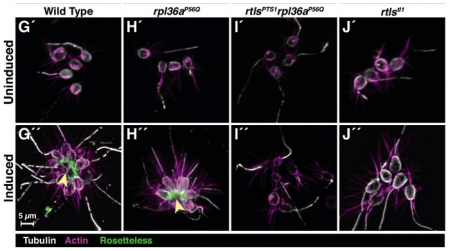
Throughout the waters of world, from the tropics to the poles and from salty seas to freshwater ponds, you'll find curious eukaryotes called choanoflagellates. Some live freely as single cells while others will form multicellular colonies in various shapes and configurations. They can be mobile, propelled through their watery habitats in a sperm-like manner by flagella, or found attached to different substrates in their environment.

These predatory organisms aren't quite animals, but they aren't that far off: they form the sister group to metazoans, making choanoflagellates the closet living non-animal relatives to the 'true' animals, which include everything from sponges, worms, and flies to fish, mice, and humans.

That relationship, plus their relative morphological, physiological, and behavioral simplicity, make choanoflagellates a unique organism in which to explore the origins of animals. "It's pretty clear from genomes, it wasn't just one key innovation that led to animals," says University of California San Francisco biochemist and choanoflagellate researcher David Booth. "Instead, it's really a lot of interconnected genes that help tune cellular architecture and gene regulation."

Although some time has passed since the organisms split from their last common ancestor with metazoans—some 600 million years, give or take—genome sequencing of several choanoflagellate species has revealed a number of homologous genes and gene families that the simple organisms share with their multicellular relatives. These include genes involved in development, cell signaling, adhesion, and differentiation, and even some such as *p53* and others more commonly found in the biomedical literature as tumor suppressors and proto-oncogenes. "That is beckoning for high-throughput genetic screens to discover new connections between genes that we couldn't get just from genomic surveys," says Booth.

But to make the most of any model organism, researchers need a well-supplied genetic toolbox. For choanoflagellates, that's been in the works. A few years ago, Nicole King's lab at the University of California Berkeley established forward genetic screening methods, but the mutations made in such screens are essentially random. A new paper published in *eLife* and accompanying protocol on protocols.io from Booth's time as a post-doctoral fellow with King now establishes genome editing in *Salpingoeca rosetta* using CRISPR-Cas9.



Salpingoeca rosetta choanoflagellates can live as single cells (top panels) but will form multicellular rosette colonies when induced by environmental cues (wild type, bottom left); CRISPR-Cas9 editing the *rosetteless* gene disrupts this formation. Reprinted from Booth and King (2020), eLife.

The process is not unlike editing mammalian cell cultures—a guide RNA that matches a genetic sequence in the organisms' DNA complexes with the Cas9 enzyme, which then makes a cut at that particular location in a cell; this can lead to mutations in a particular gene or be used to introduce new genetic sequences. But at the time Booth and King were getting started on their genome editing project, plasmids to deliver the necessary CRISPR components weren't yet readily available in choanoflagellates; instead, they had to figure out how to transfect the cells with the editing complex. There, says Booth, the salty sea water *S. rosetta* prefers made fine-tuning the required buffers a bit meticulous and painstaking.

Nevertheless, choanoflagellates have proved amenable to CRISPR-Cas9 editing. Booth and King established their CRISPR protocol by engineering cycloheximide resistance as a selectable marker into *S. rosetta*, which provided a relatively simple assay to determine how well a given set of parameters was at facilitating editing.

With baseline conditions determined, Booth and King then targeted *rosetteless*, a gene discovered in forward screens that is required for the development of multicellular rosette colonies and hypothesized to be similar to those that gave rise to multicellularity in early animals. A reverse screen with genome-edited choanoflagellates revealed a similar, rosette-less phenotype in the resulting mutant strains.

There is much still to improve, says Booth, including the ability to engineer larger fragments into the choanoflagellate genome, such as green fluorescent protein tags. But with full genomes and transcriptomes and a means now to edit the organism, Booth is forging ahead in search of the genes that underlie the formation of different cellular identities in choanoflagellates and that mediate cell fate in response to different environmental conditions. "I'd like to be guided by their biology," he says.

The genome editing lessons learned aren't just limited to *S. rosetta*. "We thought that a lot of the things we would do for choanoflagellates would be unique to them and wouldn't give us any general principles for other organisms, but that turned out not to be the case," says Booth. The work is providing editing insight to others working with various choanoflagellate species and different marine microorganism as well, such as the protists *Aboemaforma whisleri* and several *Micromonas* species.

With other choanoflagellates being characterized in their native environments and in the lab—where they are relatively easy and cheap to work with, even if there is a subtle art to culturing them—Booth suggests animal researchers might think more deeply about the evolution of the molecular mechanisms they are exploring and look back in time at simpler organisms, such as choanoflagellates, to help reconstruct that history. "With new tools, that will become easier to do," he says, "We should start thinking beyond the organisms that we've grown accustomed to and be able to make whole menageries in our labs to really dissect molecular mechanisms."

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Published online: 13 July 2020
https://doi.org/10.1038/s41684-020-0613-2