EXPERIMENTAL ORGANISMS

From model to modeled: heat stressed anemones inform what to CRISPR in a coral

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Corals face an uncertain future, complicated by anthropogenic damage, pollution, and the consequences of climate change. As waters warm, researchers are keen to understand what drives thermal tolerance—or a lack thereof—in different coral species, along with the intrinsic and extrinsic factors that contribute to bleaching, the stress-induced breakdown of the symbiotic relationship between coral and the photosynthetic dinoflagellate algae that provide their hosts with food and their brilliant colors.

These are urgent questions in need of solutions, says Phillip Cleves, a molecular geneticist with a new lab at the Carnegie Institute for Science in Baltimore. “We’ve been working to expand what’s possible in coral biology by taking a biomedical approach,” he says. “As molecular geneticists, we think that understanding the basic mechanisms of what’s happening will help us understand what we can do.”

Like people, corals are big and complex creatures that don’t quite lend themselves to life in the lab—even when their health is in question. Model organisms generally make a better starting point for researchers to learn some basic biology before turning their time and efforts to the larger organism those models are intended to, well, model. As a model of coral, Cleves and his colleagues in John Pringle’s lab at Stanford have adopted a species called *Aiptasia* as their ‘lab rat.’

Aiptasia is not a coral but an anemone, a cosmopolitan cnidarian relative of the reef building species found throughout the tropics. Like corals, the anemone harbors photosynthetic residents, but it can also carry on in an aposymbiotic state without those symbionts—effectively ‘bleached.’ When in hot water, how might the presence of symbionts affect how the anemone responds to heat stress? Working with symbiotic and aposymbiotic animals, Cleves and his colleagues increased the water temperature from a balmy 27 °C to a more blistering 34 °C and used RNAseq to measure the resulting changes in gene expression.

The transcriptomic changes were drastic, resembling the early heat stress response found in other species from yeast to flies to humans, says Cleves, but there were more limited differences between symbiotic and aposymbiotic animals. This suggests the algae might not influence the anemone’s

Researchers are making use of model organisms and CRISPR tools to better understand reef-building corals, like these on the Great Barrier Reef. Credit: Francois Gohier / VWPics / Alamy Stock Photo.

ability to launch this stress response, he says. Those genes that did differ are thought to be involved with the symbiosis itself; these were downregulated in the symbiotic anemones following the heat stress but before they bleached. “We think the anemone is transcriptionally retreating from the symbiosis,” says Cleves.

Causes and consequences are still to be determined, but these results challenge one of the current models of coral bleaching: that reactive oxygen species (ROS) released by the algae cause toxicity in the host that prompts them to kick out the symbionts. In the current study, the team didn’t end up finding significant evidence that ROS genes were differentially expressed. These results make us question the current model, says Cleves. “Is photosynthetically derived ROS involved?”

As is the case with any use of a model organism, questions remain as to how relevant observations in the model are to that being modeled. Enter another tool from the biomedical field: CRISPR/Cas9. Efforts are underway to apply the genome editing scissors to the anemone model and to corals themselves. Cleves, working with Line Bay and other collaborators at the Australian Institute of Marine Science during annual trips to the Great Barrier Reef, has been working on the CRISPR puzzle in the coral *Acropora millepora*.

Acropora has been a popular study species for its reliably produced and durable eggs, but spawning events occur just once or twice a year, in the middle of

the night under a full moon in November; that means a very short window to collect zygotes for microinjection of any genetic tools. In 2018, Cleves and his colleagues reported the theoretical success of CRISPR in the coral, but the mutations didn’t prove quite potent enough to produce a meaningful phenotype. They’ve been tweaking the protocol since—improving guide designs, adding a fluorescent dye to confirm a mutation was made, and co-injecting two guides for the same gene to increase the likelihood enough cells would be mutated as the developing animal continued to divide and grow.

In a paper published in tandem with the *Aiptasia* transcriptomic work, Cleves and his colleagues in the US and Australia put their refined protocol to the test by knocking out a gene identified as over-expressed by heat-stressed anemones: *HSF1*, which encodes a protein required by a variety of different animals to tolerate heat. When the team turned up on the heat on *HSF1*-mutant *Acropora* larvae, the corals died. The results implicate HSF1 in heat tolerance in corals, a successful translation from model to ecologically relevant modeled organism, and they demonstrate CRISPR’s potential in yet another species. “It opens up an entire world of functional genetics,” says Cleves.

That won’t make *Acropora*, or any other coral species of interest for that matter, spawn more frequently or grow more quickly and reliably in the lab—there, the model will remain important. “An integrated approach is going to be very powerful,” says Cleves. In his lab, “we’re going to do year-round experiments in *Aiptasia* to try to find the important genetic players and then test those in corals during the precise times that corals spawn.”

By doing both, Cleves hopes to more quickly determine and better understand the genes and pathways that underlie coral health and their responses to their growing environmental stressors—and whether there’s anything people, the ultimate drivers of many of those stressors, can do to reverse or mitigate their damaging effects before it’s too late.

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