Vibrio goes with the flow

The human body may host thousands of different species of bacteria, which interact with their host and each other to influence numerous aspects of health and disease. The Hawaiian bobtail squid contains just one: *Vibrio fischeri*, luminescent marine bacteria that inhabit light organs, or photophores, in the squid. The vibrio are thought to help camouflage the nocturnal squid against moonlight above the water while the squids provide shelter and nutrients. Biologists like Margaret McFall-Ngai at the University of Hawaii have been using this simple symbiotic relationship as a model to investigate the development of the microbiome. For over twenty five years, she and others working with the squid-vibrio model system have been teasing out the biochemical signals and changes in gene expression that influence how the two organisms communicate and establish their relationship.

How the vibrio get to the right spot in the first place, however, remained somewhat of a mystery. Most assumed by brute force as newly hatched squid inhale and exhale surrounding seawater. But when McFall-Ngai first introduced the problem to Janna Nawroth, a PhD student at Caltech with an interest in biophysics and fluid dynamics, and Eva Kanso, an applied mathematician and engineering professor at the University of Southern California, they both agreed that the physics didn't quite add up. So they jumped into the wet lab, bringing fresh eyes and mathematical modeling with them (*Proc. Natl. Acad. Sci. USA* 114, 9510–9516; 2017).

The photophore is surrounded by ciliated appendages that disappear shortly after the animals hatch, Kanso explains, “so there were indications that the cilia could be playing a role.” But when Nawroth started digging into the literature, she found plenty of static confocal images of cilia, but no sign of any video. “No one, it seemed, had ever taken any live recordings of the cilia or really looked at what they look like when they beat,” she says. McFall-Ngai showed her how to dissect tiny hatchlings—just a few millimeters long—to expose the light organ, and Nawroth began staining and recording cilia in motion and tracking where vibrio cultures as well as different sized particulates ultimately ended up.

(a) Location of the ciliated light organ (white square) in the mantle cavity of a juvenile *E. scolopes*. (b) SEM of a light organ showing the position of the two pairs of ciliated appendages. Adapted with permission, *Proc. Natl. Acad. Sci. USA* 114, 9510–9516; 2017.

She found some novel observations about cilia. Rather than uniform structures, Nawroth saw short (10 μm) and long (25 μm) variations. The long cilia beat synchronously, as expected, but the short ones beat randomly. The literature says that asynchronous cilia are a sign of something pathological, Kanso explains, but Nawroth recorded it in healthy young squid.

She also observed mechanical sorting—vibrio and vibrio-sized particles would make it into the photophore while larger ones would be diverted away. The flow produced was also faster than what vibrio could be expected to swim through, eliminating another idea of how the bacteria get to the light organ. “I found a very interesting flow field and very distinctive structural features, and so it seemed like the obvious question was, how do these relate to each other?” Nawroth says.

With Nawroth's physical observations, Kanso created a physics-based computational model to decipher the relationship. The model suggests that the long, evenly beating cilia create two distinct flow zones that sort large particles from small (an important consideration for an animal that lives in eutrophic coastal waters, Nawroth says—without that sorting, the photophore could easily become clogged) and then isolates small particles in a sheltered zone on the photophore, where the short cilia enhance molecular mixing. The team thinks that mixing creates the right microenvironment for the vibrio and the squid to communicate without their chemical signals quickly washing away.

According to McFall-Ngai, “it was very interesting to see that the cilia would size

select particles about the size of the bacterial cell.” In most ciliated marine organisms, the cilia, though of similar size to those in the squid, harvest larger suspended food particles from the water column, she explains. “These data suggest that there has been selection pressure to modify a ciliated field on an invertebrate surface so that it will capture a specific size, i.e., the size of its symbiont. That said, many non-specific bacteria are the size of the symbiont, so the activity of the short cilia in mixing the biomolecules that favor the symbiont may be the final selection step.”

A squid may seem pretty far removed from a human, but ciliated surfaces aren't unique to marine organisms. The human trachea and lungs contain cilia, and Kanso and Nawroth wonder if the mechanical selection they observed in the squid could one day help inform how the respiratory system establishes beneficial bacteria and becomes susceptible to harmful ones.

For now, Kanso and Nawroth are excited to continue working with the squid model. “One thing we are trying to decipher is, what is the relative role of the mechanical environment versus chemical signaling,” Kanso says. “The mechanics, the flow, the chemicals, it's all coupled together, so we are trying to develop microfluidic platforms outside the animal to isolate the role of every ingredient in the full story.” While Kanso pursues microfluidics, Nawroth is looking to tissue engineering, with plans to change the structure of the cilia in order to test different modeling predictions.

To answer questions about the microbiome, it sometimes helps to just go with the flow.

Ellen P. Neff