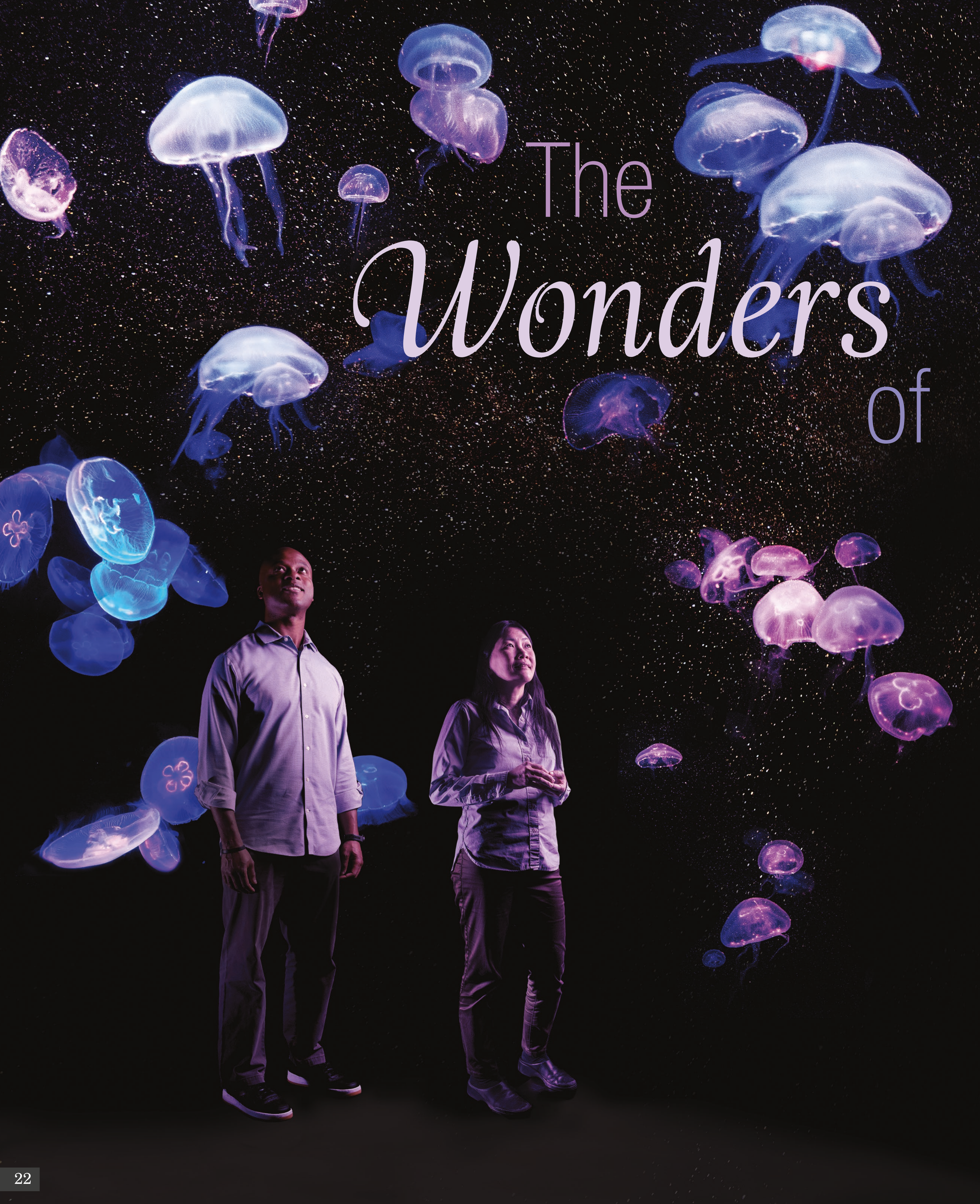
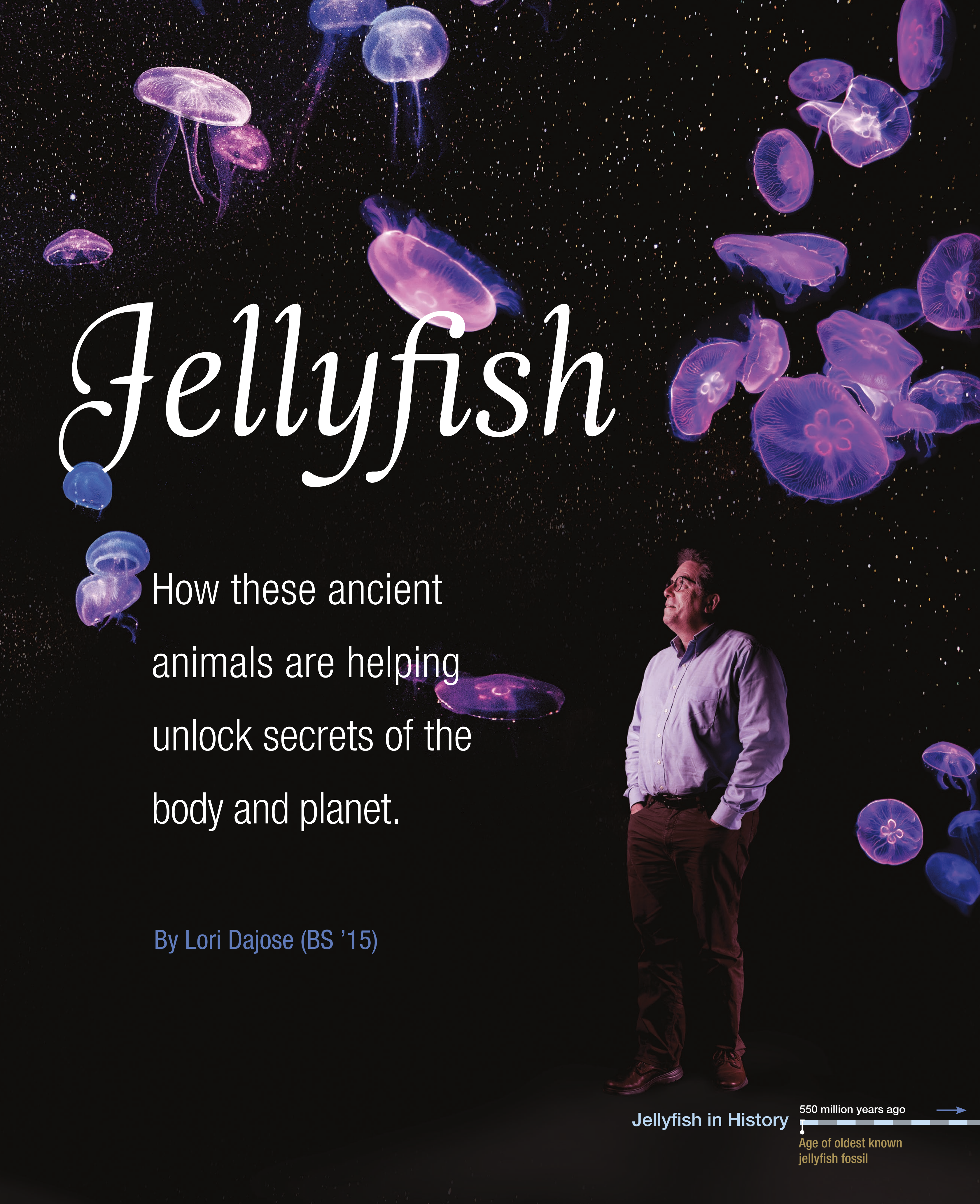
The *Wonders* of





Jellyfish

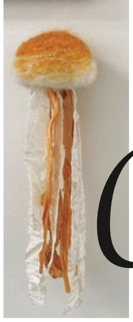
How these ancient animals are helping unlock secrets of the body and planet.

By Lori Dajose (BS '15)

Jellyfish in History

550 million years ago

Age of oldest known jellyfish fossil



Caltech's Chen Neuroscience Research Building is a maze of pristine white hallways and white tile floors, long lab benches, and whirring machinery around every corner. Deep within these corridors, if you can find it, is a wooden door marked not with a sign but rather a stuffed jellyfish made of felt and ribbons. Sliding back this door reveals a darkened room lined with identical vats of gently bubbling water, each illuminated by a row of dim lights. Look closely, or you might miss them: ghostly translucent circles, smaller than a pinkie nail, disappearing and reappearing as they drift through the liquid.

Older than the dinosaurs, older even than the rings encircling Saturn, jellyfish have been swimming Earth's oceans for 550 million years, surviving drastic environmental changes relatively unscathed. Over the past decade, Caltech researchers David Anderson, John Dabiri (MS '03, PhD '05), and Lea Goentoro have begun to tap into the powerful abilities of these primitive animals to answer questions about our changing planet and the common properties underlying life itself. In the process, the translucent creatures have opened new windows into our understanding of neuroscience, the deep ocean, and biological regeneration.

"There aren't a lot of labs in the country that have jellyfish," notes Dabiri, an aeronautical and mechanical engineer. "Which is why it's exciting that we now have three labs at Caltech working on them."

Reading minds

The felt jellyfish marks the entrance to the laboratory of Anderson, a neuroscientist and the director of Caltech's Tianqiao and Chrissy Chen Institute for Neuroscience. Over the past four years, he and postdoctoral scholar Brady Weissbourd have worked to develop a toolbox of genetic methods they use to tinker with the brain cells of the microscopic jellyfish *Clytia hemisphaerica* in order to study the neuroscience of behavior.

Jellyfish are perhaps a counterintuitive animal with which to study neuroscience, because the animals do not actually have a brain as we know it. Rather than being centralized in one part of its body, the jellyfish brain, which is composed of approximately 10,000 neurons, is diffused across the animal's entire body like a net. The Anderson lab, which focuses on the neurobiology of emotion, decided to develop a jellyfish model for studying how behavior is coordinated in the absence of a centralized brain.

Brady Weissbourd, postdoctoral scholar in the Anderson laboratory, examines batches of genetically engineered *Clytia hemisphaerica.*

(Years are approximate)

"These types of extreme biology are useful to understanding life as we know it," Weissbourd says.

Over 580 million years ago, while Earth was thawing from extensive glaciation, primitive nervous systems began to appear in animals. It was then that a sort of schism of evolution happened: on one side, the decentralized nervous systems of cnidarians like sea anemones, coral, and jellyfish, and on the other, essentially everything else, with a few exceptions.

The lack of a complex centralized brain, however, has not seemed to hinder jellyfish evolutionarily. Their unusual brains have myriad different abilities than ours; for example, their decentralized control of behavior enables a surgically removed jellyfish mouth to carry on "eating" autonomously without the rest of its body.

"By studying these weird creatures that branched off evolutionarily a long time ago, we might be able to learn about what the first neurons might have looked like or the first neural networks," Weissbourd says.

To gain these insights, Anderson's team genetically modified *C. hemisphaerica* so their neurons would light up with a fluorescent protein when firing. The idea behind this was to enable the researchers to observe jellyfish neural activity under a microscope in real time as the animals swim around and behave normally. Though this kind of modification has been done in other laboratory animals such as mice and fruit flies, developing a new so-called transgenic animal was not easy. The main challenge for the team was determining how to insert new genetic material that encodes for the fluorescent protein into the jellyfish genome and link it with the processes that make neurons fire.

"There was a lot of uncertainty at the beginning," Weissbourd says. "We were trying a lot of different methods. David [Anderson] and I would talk about, 'How long are we going to try to get a transgenic jellyfish before we stop trying?'"

After nearly four years, it worked. Using this new tool, the laboratory first sought to crack the neural code underlying how *Clytia* eat. When a jelly snags a brine shrimp in one of its tentacles, it folds its body to bring the tentacle to the mouth at its center. By examining the chain reactions of neural activity as the animals ate, the team identified certain neurons responsible for that inward folding of the body.

Clytia seen from above. One centimeter across when mature, the jellyfish has a central mouth and symmetric tentacles along its circular body.

Additionally, the researchers found a remarkable degree of organization in the network of jellyfish neurons, which originally seemed diffuse and unstructured, and only became visible with their fluorescent system; as it turns out, *Clytia*'s neurons are arranged, surprisingly, in radial wedges, like slices of a pizza.

"Studying how neural circuits control jellyfish behavior should give us insights into which aspects of human brain function can be traced back to the earliest stages of neural evolution and which aspects are more recent inventions," Anderson says. "In addition, understanding how their behavior and reproduction are controlled will help us learn to live with, control, and conserve jellyfish."

Undersea explorers

While Anderson continues to uncover the secrets of the jellyfish mind, Dabiri and his colleagues are hoping to harness the animals' abilities in their natural habitat to help researchers explore the deep ocean and improve our understanding of climate change.

The ocean comprises 99 percent of Earth's habitable volume and is home to at least 2 million marine species. Because the deep ocean is opaque to most of the electromagnetic spectrum, it is, in a way, invisible. But the processes in the deep ocean are critical to human survival on this planet; for example, the ocean acts as a reservoir to sequester carbon and prevent it from being released into the atmosphere and warming the planet. Yet, human understanding of these processes has been limited by our ability to access the ocean's depths. Most of the data available are thus biased to a thin strip of its surface.

"We know more about the surface of Mars and Venus than we do about the deep ocean," says Dabiri. "If I was given the choice between suiting up and going to Mars versus going to the bottom of the ocean, of course I would pick the ocean."

However, Dabiri is hoping that humans will not have to develop complex deep-ocean suits to perform this feat. Instead, he plans to use jellyfish, which are natural explorers in these environments.

The jellyfish species *Aurelia aurita* has been discovered as deep as the Marianas Trench, nearly 7 miles under the surface. The Dabiri lab is currently developing tiny, lightweight devices that can be attached to these jellyfish, like a harness onto a horse, and that can steer the animals around the ocean while simultaneously making measurements of oceanic parameters such as oxygen

400 million years ago

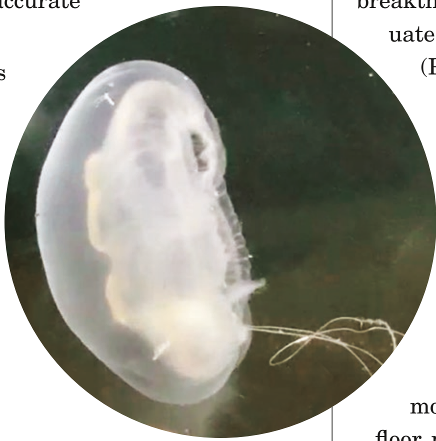
First land plants evolve on Earth

levels and temperature. Being able to measure these parameters would be important to creating more accurate models and predictions of climate change.

The team has already shown that the devices can be attached to the animals and induce them to swim faster and more efficiently with no damage or stress to the jellies. The next step is to program the devices with artificial intelligence algorithms that will enable the small-scale apparatuses to autonomously steer the jellyfish through strong ocean currents to desired locations. Ultimately, Dabiri envisions being able to send millions of these tech-enhanced jellies out into the ocean.

"I think it's poetic that jellyfish can help us to understand climate change, since our success in the effort will ultimately help to protect their natural habitats," Dabiri says.

The depths *Aurelia* will explore are echoed by the setting of Dabiri's subterranean lab, which sits two floors below the surface of Caltech's campus. There, gently drifting *Aurelia* live in an aquarium with a rounded bottom, which prevents them from getting stuck in a corner. The lab also houses a huge, 4,300-gallon tank with which researchers can measure the performance of the bionic jellyfish; behind the tank, a mural of the futuristic jellies is lensed through the distortion of the water. For even larger-scale research, a two-story tank, closer to 5,000 gallons, is just a few doors away down the underground corridors.



An electrical current is used to regulate the *Aurelia's* pulse in order to increase its speed and efficiency in the water.

Sleep with the jellyfishes

Goentoro, a biologist, first met Dabiri when she joined the Caltech faculty in 2011. Originally, her research focused on how cells send messages to one another through proteins and other molecules. But those projects were taking months to set up and, in the meantime, over chance conversations at Caltech's Red Door Café, Goentoro had become intrigued by Dabiri's jellyfish studies. So she devised a side project for one of her graduate students to study the so-called moon jellyfish, *Aurelia aurita*.

"Lea has so many creative ideas, and she is an innately creative person," Dabiri says. "For a long time, I had preached the gospel that there's so much about jellyfish we don't know, largely from an engineering perspective. And now, she's come across these really interesting biological discoveries."

Goentoro had no idea that, within five years, this side project would reshape the entire focus of her research, which seeks to understand how biological systems are built and how they function. A major jellyfish-related breakthrough came in 2017: in a study led by then graduate students Ravi Nath (PhD '18), Claire Bedbrook (PhD '18), and Michael Abrams (PhD '18), the team made headlines for discovering the seemingly simple fact that the jellyfish *Cassiopea* sleeps, just like humans. Goentoro's lab partnered on the research with fellow Caltech researchers Paul Sternberg, the Bren Professor of Biology, and Viviana Gradinaru (BS '05), professor of neuroscience and biological engineering, in whose labs Nath and Bedbrook worked.

Cassiopea is a genus of jellyfish that spends most of its life sitting upside down on the ocean floor, pulsating every few seconds. The team showed *Cassiopea* exhibits reduced pulsating at night, and it takes longer to arouse in this period of decreased activity. The team also prevented the animals from entering this quiescent state by pulsating water at the jellies throughout the night and saw that they were more likely to fall into the quiescent state during the day. These discoveries provided the evidence needed to prove the animals do indeed sleep.

"It may not be surprising that jellyfish sleep; after all, simple organisms like worms and fruit flies have also been shown to sleep," Goentoro says. "But our discovery that *Cassiopea* also enters into a regular sleeping state, just like us, now makes jellyfish the most evolutionarily ancient animal known to sleep. Finding out things that are

Graduate student **Nina Mohebbi** swaps out an old seawater battery for a new one on a test platform in the Dabiri lab. These batteries operate in the ocean by using ambient seawater as the battery's electrolyte.

(Years are approximate)

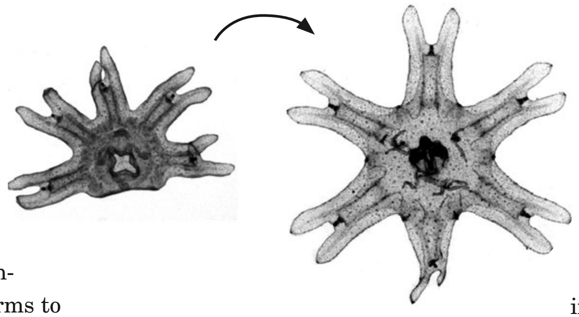
common to all life makes you see the world, and yourself, in a profoundly different way. I look around me: the birds, the beetles, the trees, we are all deeply connected. The collaboration between the three laboratories really made this possible."

Coaxing regeneration

The sleep study was a major turning point for Goentoro's research, but the definitive pivot occurred with a study of *Aurelia* ephyra, the infant stage of the jellies studied by Dabiri's lab.

Although the mature *Aurelia* can grow to the size of a dinner plate, its ephyra are just 5 millimeters across. Their eight tiny symmetrical arms pulsate in rapid synchronization like a blinking eye or beating heart as they swim through water. When they are not actively swimming, you could easily mistake them for pieces of debris or algae.

In 2015, Abrams, the graduate student who worked on the sleep paper, was studying how ephyra repair themselves after losing one or more of their eight arms, in the same way human skin repairs itself after a cut. The team discovered that within the first two days after injury, an *Aurelia* ephyra would reorganize its existing arms to be symmetrical and evenly spaced around the animal's disc-like body. This so-called symmetrization occurred regardless of whether the animal had as few as two limbs remaining or as many as seven; ultimately, the process was observed in three additional species of jellyfish ephyra.



Under certain conditions, *Aurelia* ephyra will regrow a missing arm.

Abrams and co-author Ty Basinger also observed another phenomenon while studying symmetrization: in some rare cases, the jellyfish would begin to regrow a missing arm. While some animals, including certain species of jellyfish, are known to have the ability to regenerate damaged body parts, this had never been shown in *Aurelia*. This discovery gave the team an opportunity to look for ways to enhance these unexplored regeneration abilities. Over the next five years, the team examined various molecules and conditions that might induce arm regeneration in *Aurelia*. They found that all it took was the hormone insulin and the amino acid leucine.

"These are simple molecules that are promoting regeneration," Goentoro says. "This was surprising because, if regeneration could be induced at all, we expected to find signaling molecules and transcription factors that pattern tissues. Insulin and amino acids are typically associated with metabolism, how our body processes food into energy. Our work suggests that modulating metabolism may be what it takes to unlock regeneration."

The researchers decided to see if these same simple conditions could induce regeneration in two other laboratory animals not known to regenerate: specifically, the fruit fly *Drosophila melanogaster* and the laboratory mouse. Though *Drosophila* have never been shown to regrow limbs, the team found that increased levels of insulin and leucine in the flies' food led to some degree of regrowth in 49 percent of flies. In mice, 10 percent were able to regrow at least part of an amputated toe when their diets were supplemented with leucine and sugar.

The work suggests that there is a latent, untapped ability to regenerate after injury that is shared by animals as primitive as jellyfish and as complex as mice.

"Some people have thought for some time that all animals have a latent ability to regenerate," Goentoro explains. "The biggest surprises are how simple the key is that can unlock this capacity, and that the same key works across animals. Just a common amino acid and sugar can coax these complex structures to regrow. The simplicity brings closer the hope that someday we can coax body parts to regrow in humans."

Regeneration, Goentoro says, will be the focus of her lab going forward, as she and her team seek to learn more about the molecular pathways that are triggered during regeneration and the simple dietary conditions that might trigger them.

"I had no idea things would go this route," she notes. "That's what I love about Caltech. I get to be inspired by the outside-of-the-box research my colleagues are doing."

David Anderson is the Seymour Benzer Professor of Biology; the Tianqiao and Chrissy Chen Institute for Neuroscience Leadership Chair; director of the Tianqiao and Chrissy Chen Institute for Neuroscience; and a Howard Hughes Medical Institute investigator. His work is funded by the Howard Hughes Medical Institute, Caltech, National Institutes of Health, and the Caltech Center for Evolutionary Science.

John Dabiri (MS '03, PhD '05) is the Centennial Professor of Aeronautics and Mechanical Engineering. His work is funded by a MacArthur Foundation fellowship, the National Science Foundation, and the Office of Naval Research.

Lea Goentoro is a professor of biology. Her work is funded by the Natonal Science Foundation, National Institutes of Health, and Caltech's Center for Environmental Microbial Interactions.

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