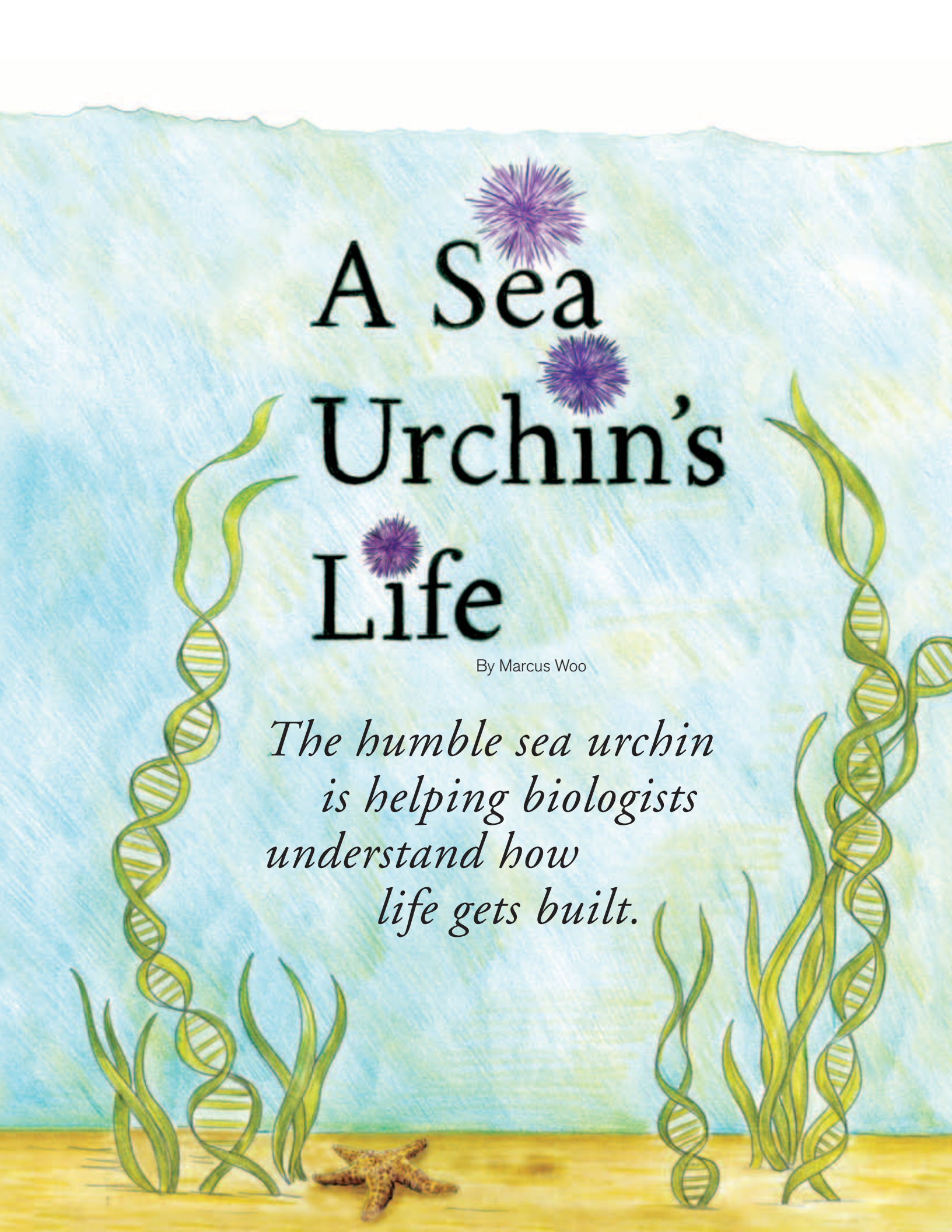


A Sea Urchin's Life

By Marcus Woo

The humble sea urchin is helping biologists understand how life gets built.

It's spawning season for the purple sea urchin. A female has released a milky cloud of millions of tiny eggs; her male counterpart has released millions of sperm, which swim toward the eggs.

When egg and sperm meet, it will mark the beginning of an intricate and complex process of development that will not only result in a long-lived, seafloor-dwelling adult sea urchin, but will also provide a window into the ways in which all organisms—humans included—go from egg to embryo to adult.

Indeed, for over a century, sea urchins have been helping scientists piece together the whys and wherefores of embryology. In developmental biologist Eric Davidson's lab at Caltech, biologists have been studying the development of sea-urchin embryos for 40 years, thereby helping to unfold one of the most important origin stories of all—how a fertilized egg transforms into a complex organism.

Much of what biologists know today about fertilization, embryology, and development they know thanks to the sea urchin. For example, sea-urchin research in the early part of the 20th century helped scientists discover that heredity is rooted in chromosomes, the folded structures of DNA and proteins that carry an organism's genetic information.

Over the past few decades, Davidson and his colleagues have worked out the principles by which the sea urchin's genes control its development. In 2006, researchers at the Human Genome Sequencing Center at the Baylor College of Medicine sequenced the genome of the purple sea urchin, and a team of 240 researchers from 70 institutions—led by Davidson, Caltech biologist Andrew Cameron, and others—analyzed and annotated it. This achievement has been crucial in helping scientists pinpoint the exact mechanisms behind the organism's development. And with recent advances that allow biologists to analyze hundreds of genes simultaneously, Davidson and his colleagues—together with biologists around the world—are unraveling the fundamental basis of development with a level of detail that was never possible before.

GROWING A SEA URCHIN

Under a microscope, the cells of a developing sea urchin look almost translucent, each containing a visible nucleus and hairlike cilium, which provides locomotion to the cell. But, at the molecular level, there's a lot more going on than meets the eye. During the first few hours after fertilization, the cells of a sea-urchin embryo divide synchronously, forming a hollow sphere of cells called a blastula within about 12 hours. By then, the cells will have started to separate into the precursors of the specialized types that will form the completed embryo: some will form the muscles, some the skeleton, some the nervous system, some the immune cells, others the gut. By the time it reaches the 24-hour mark, the embryo will have become a lot more complicated. It is now composed of about 500 cells, with the interior cells already expressing the genes needed to form the skeleton. At about 30 hours postfertilization, an indentation forms on the outer surface of the sphere, producing a cavity that later elongates into a tube, becoming the gut; the tube's ends become the sea urchin's mouth and anus. This process marks the transformation from blastula into what's called a gastrula. In just about three days, what began as a single cell about 70 microns in diameter (a human egg is about 100 microns) has become a triangular larva called a pluteus.

When Davidson arrived at Caltech in 1971, he already knew that to understand each and every one of these developmental milestones in the most fundamental way possible, he would have to explain how they're encoded in the "letters" of the double-helical DNA molecules of which the genome is composed.

It is the genome that controls any organism's form and function. It is the genome—specifically, the interplay between genes in that genome—that determines that we humans will have two legs and two opposable thumbs, that elephants will have trunks, that turtles will have shells. It is the genome that dictates which cells of the embryo will become the gut, which will turn into muscle, and which will form the skeleton.

But the genome is more than just a blueprint—the genes responsible for development are more like a computer program that tells the organism how to build *itself*. As Davidson explains, it's like a building that starts off with a single brick that can multiply into many bricks, which then can assemble themselves into walls, form a roof, and grow all the necessary wires, pipes, and fixtures.

A PROGRAM FOR LIFE

Davidson's basement office is lined with shelves of books; his desk is covered with papers. Intricate line drawings of sea urchins adorn the wall. On his computer screen, he pulls up an image of about 900 transparent, identical-looking sea-urchin plutei. For most people, it might be just a pretty picture. But for a biologist like Davidson, it encapsulates one of the fundamental facts of life: that the genome defines an organism.

"That's a field of sea-urchin embryos," he says. "Every one is exactly like every other one. Once you look at that, you know development is a hardwired, programmed process." In other words, since every embryo looks like the one right next to it, they all must be operating under the same set of rules—the program for embryonic development that's resident in their genomes.

Over the last decade, Davidson's group has made tremendous progress in figuring out how the sea urchin's genes control its development. As it turns out, you can engineer a sea urchin to take on certain genetic traits simply by injecting those genes into its egg. And, perhaps most importantly, while reproduction in the wild is a game of luck, it's pretty easy to make large numbers of normally developing purple sea urchin embryos in the laboratory.

To do this, you first stick a needle into the underside of an adult sea urchin, through a soft spot just next to its mouth, and inject a solution of potassium chloride. The solution triggers the muscles in the sea urchin's reproductive organs to squirt out eggs or sperm, depending on the animal's gender. Then you just introduce the sperm to an egg and let nature do the rest.

Using a number of different techniques, beginning with these simple procedures, Davidson and his colleagues recently showed definitively that gene regulatory networks—interconnected webs of genes that control other genes—dictate development. Regulatory genes are segments of DNA that encode proteins that recognize certain DNA sequences and thereby tell other genes to turn on or off.

When a gene is on, the information encoded in its sequence of letters is used to produce a protein with a specific biological function—a process called gene expression. Sometimes, specific classes of genes have the job of regulating other genes. Ultimately, these genetic switches form a network that controls the expression of every gene that encodes the proteins responsible for the organism's structure and function. This allows a cell to "know" that it's meant to form a mouth, essentially because its mouth-forming genes are turned on.

"The essence of how you get from an egg to a complicated organism lies in those genes going on and off in different cells at different times," says Cameron, who is also the director of the Center for Computational Regulatory Genomics at Caltech. In fact, he says, it's the regulatory interactions between genes—rather than the genes themselves—that lie at the heart of what makes you a sea urchin

Left: Eric Davidson's computer screen features an image of adult sea urchins.

Below: A sea-urchin larva, called a pluteus, is about half a millimeter long. After drifting in the ocean for two to three months, it settles on the seafloor and transforms into its spiky adult form.

instead of a sea star, or a human instead of an elephant. "Different regulatory genes go on in different places at different times," Cameron explains, "and you get a nose instead of a trunk."

MAPPING IT ALL

In order to fully understand how gene expression is regulated, you need the entire genomic code of the creature you're studying—in the case of the sea urchin, that's 23,000 genes, plus their control sequences. And that's precisely what biologists at the Human Genome Sequencing Center at Baylor pinned down in 2006. Davidson and Cameron helped coordinate the program and organized the interpretation of the sequence. Armed with the sequenced genome, researchers can now analyze every line in the genetic program. "This is clearly the most fundamental way to look at the developmental process," Cameron says. "You can't get any more basic than that, can you? You're right there in the molecules and the DNA."

Of those 23,000 genes, a few hundred are regulatory genes—the roles of which need to be uncovered, one by one, if you want to completely understand how a fertilized egg becomes a purple sea urchin.

There are a number of ways to go about this sort of genetic treasure hunt. If you're trying to figure out if one gene holds sway over another, you can use a specific molecule that will turn that gene off and see how other genes are affected. Researchers in Davidson's lab are using this so-called perturbation method to analyze the effects of each regulatory gene on all other regulatory genes operating in each tissue of the developing embryo, and thus obtain information about all the gene interactions in the network.

These sorts of high-throughput methods enable researchers to analyze the flood of information encoded in a genome thousands of times faster and much more cheaply than they ever could before, says Davidson. "The era of working on just this gene or that gene is over," he adds.

THE URCHIN-WIDE WEB

It was these fast-tracked methods of gene analysis that allowed biologists, led by Davidson and his Caltech colleagues, to map out almost all the switches and connections that control a sea-urchin embryo's gene regulatory network during the first 30 hours of its life, until it begins its gastrula stage. "We've been able to understand the gene regulatory network of the sea-urchin embryo more completely than other embryonic networks," Davidson says.

As part of building that map, senior research fellow Qiang Tu and former staff scientist Paola Oliveri (now at University College London), together with Davidson, were able to piece together the details of the part of the regulatory network responsible for generating the cells that build the biomineral rods that ultimately form the sea-urchin embryo's skeleton. By mapping this skeleton-forming network, Davidson notes, the researchers became the first to explain completely—in terms of the underlying DNA instructions—how genes interact to govern the development of a particular type of cell in a developing animal embryo.

In an accompanying commentary to the paper, biologist and former Caltech professor Leroy Hood (BS '60, PhD '68) called the work a "tour de force."

"This paper will be the model for many more that will undoubtedly follow, transforming the landscape of developmental biology and ultimately elucidating the molecular systems that drive development," Hood wrote.

He was indeed prescient. Just last year, Davidson and senior research fellow Isabelle Peter published an analysis

of the 14-gene network responsible for the development of another type of embryonic cell—endoderm cells, which are the ones that eventually form the sea-urchin larva's gut. Because of this work, Davidson says, biologists now know more about how endoderm cells develop in the sea urchin than any other kind of cell.

What Peter and Davidson found was that gut-regulating genes begin expressing themselves in specific cells just a few hours after the egg is fertilized—well before the gut begins to take shape. In fact, it is becoming clear that an egg is created with some regulatory features that foreshadow its cellular destiny. Even though the egg is a single cell, its composition is not the same throughout; certain gene-regulatory proteins are more prevalent at one pole of the egg than another, for instance. As a result, when the fertilized egg divides, only some cells will contain those proteins. And if those proteins are the ones that, for instance, are needed to turn on the gut-forming genes, then only those cells will know to begin expressing the regulatory genes that will direct the program of gene expression that ultimately results in the formation of a gut tube in the embryo.

To get an even deeper understanding of their findings, Peter, Davidson, and postdoc Emmanuel Faure are working on a Boolean computer model to generate in the computer the outputs of the gene regulatory networks of endoderm and mesoderm cells, which respectively form muscles and parts of the immune system. The model is a series of if-then statements that compute the consequences of turning each gene on and off. With this computational tool in hand, the scientists can compare the model to a real embryo, hour by hour, seeing which genes in the network are being expressed, and which are being shut down. So far, Davidson says, the model is remarkably good at reproducing what their experiments have found in real sea urchins. The researchers can even do virtual experiments by seeing what happens when they tweak the model network. The model is so good that the results of those virtual experiments match the real ones, further demonstrating that the model represents a complete understanding of the network and, more significantly, that the network itself encapsulates almost the entire control system that determines what genes will be expressed, when, and where for this part of embryonic development.

Within a couple of years, Davidson says, biologists will have analyzed most of the sea-urchin embryo's genetic networks and will thoroughly understand the control system underlying its development. In working toward that goal, Stefan Materna (PhD '12), Caltech staff biologist Andy Ransick, senior research fellow Smadar Ben-Tabou de-Leon, and postdocs Enhu Li and Julius Barsi are probing the networks responsible for the rest of the embryo, which includes the cells that form the outer wall and its various structures—the mouth and parts of the neuronal system, for instance—and the cells that differentiate into the diverse kinds of mesoderm cells, including pigment and immune cells. Isabelle Peter and two graduate students, Miao Cui and Jon Valencia, are analyzing the later processes of gut development, in which the stomach, intestine, and foregut are formed. Senior research fellow Qiang Tu and Cameron have analyzed all the RNA that is produced every few hours during gene expression while the sea-urchin embryo develops. Meanwhile, members of the Davidson laboratory are constantly improving the technology for studying gene regulatory networks. For example, scientific research associate Jongmin Nam recently invented a way to speed up analysis of gene regulation more than 100-fold.

EVOLUTION AND MODULES

On paper, a sea urchin's regulatory network looks like an overly complicated subway map, with colorful lines and arrows that skitter across the page. And yet, Davidson says, these seemingly dense and intimidating sets of data are not as complex as they appear.

For one thing, the overarching networks are composed of smaller subcircuits—mini-networks, you might call them—with each involving just a few interacting genes. And they all consist of one of just a few dozen basic designs, each with a specific type of function. One such design, for example, might involve three genes in a positive feedback loop that stabilizes other genes downstream. Another subcircuit might result in boosting the expression of a particular gene in a specific type of

Below: Just a few of the myriad interactions among the regulatory genes that control a sea urchin's development are illustrated here. Scientists are discovering that these gene-regulatory networks have an inherent modular structure, which means the networks may actually be simpler than they appear.

cell while repressing it everywhere else.

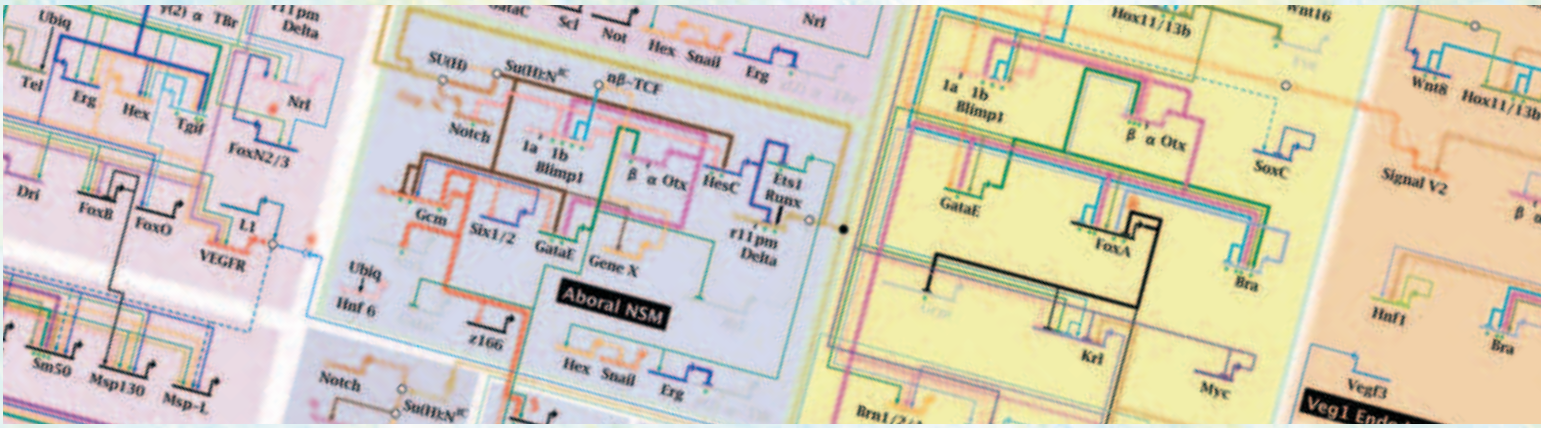
In other words, these modules are like building blocks for a regulatory network, offering a powerful and simple way to make sense of hundreds of interacting genes. According to Davidson, there's evidence that similar module-based building blocks also exist in flies, worms, zebrafish, and frogs. This suggests that understanding the design of the subcircuits in sea urchins will help biologists understand other species—even if their embryonic development looks nothing like that of the sea urchin. "Understanding sea-urchin embryos blazes the trail for understanding other embryos," Davidson says.

They're also gaining some insight into the way in which one creature evolves into another—a process that would, by definition, rely on changes in the gene regulatory networks. In fact, says Davidson, evolution can be thought of as the assembling and reassembling of these network subcircuits. Over the course of millions of years, as mutations cause changes in regulatory genes, they may not only affect what an organism looks like, but what species it is. And so, by tracking how regulatory networks change from species to species, biologists can follow the precise mechanism of evolution—an achievement Charles Darwin could never have anticipated.

These changes can be directly observed by comparing different species. For example, scientific research associate Feng Gao is comparing a regulatory system found in both sea stars and sea urchins. Similarly, Eric Erkenbrack—a graduate student—is comparing the genomes and regulatory networks of the purple sea urchin with those of *Eucidaris tribuloides*, a cousin whose ancestors diverged from the purple sea urchin's about 275 million years ago. Although both species are sea urchins, much of their regulatory networks are wired differently, resulting in slightly contrasting developmental processes. For example, the two species form their larval skeletons from cells that have different embryological origins and behave in different ways.

To better understand such disparities, Erkenbrack is now trying to insert the set of regulatory genes responsible for the purple sea urchin's skeleton into *E. tribuloides*. The goal is to reprogram *E. tribuloides* to grow a skeleton just the way its purple relative does. The researchers call this synthetic experimental evolution. And if it works, it will also confirm that the genes the researchers have identified as the purple urchin's skeleton builders really *are* behind the differences in the way these two species build their skeletons.

"With all these advances, it's now feasible to think about understanding the whole genome," Cameron says. "It was great to be able to describe how one gene interacts with another gene. But the whole thing? Think about that. That's pretty cool." E&S



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