Understanding the evolution of cell types to explain the roots of animal diversity

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Sponges have the simplest of bodies, harboring a delicate system of canals through which water flows. So it was a surprise when researchers recently discovered that sponges have as many as 18 cell types, including one with hybrid immune and neuronal properties—even though sponges don't have any brain to speak of (1).

Researchers in Detlev Arendt's laboratory at the European Molecular Biology Laboratory in Heidelberg, Germany, used single-cell RNA sequencing technology to separate out individual sponge cells and analyze their gene expression. The team described the molecular features of a family of cells that express digestive enzymes inside the water canals, helping the sponge digest any food particles carried by the water. It turns out that these digestive cells express genes typical of the postsynaptic neuron. They also found that another family of cells had hallmarks of immune cells (i.e. the ability to eat cellular debris and microbes), and one of these also had the features of a neuron (i.e., the ability to send out chemical signals into a gap between cells).

"It's striking that the sponge cell has both neural and immune properties," says Jacob Musser, a postdoctoral fellow in Arendt's lab. Apparently, the ancestral animal cell types were multifunctional, but in the course of evolution, functions were parceled out to different cells. "This division of labor is a key way in which cell types are evolving," he says.

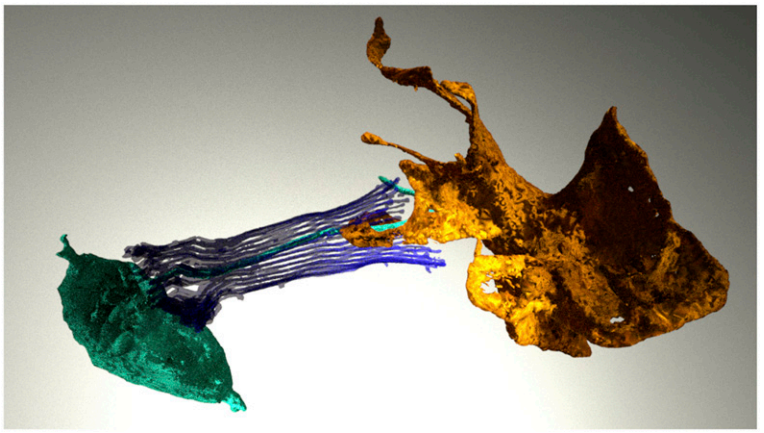
Researchers have been studying cell types developmentally and morphologically for the last 150 years. But single-cell transcriptomics recently have revealed many new, morphologically cryptic cell types not only in sponges but in other organisms as well. "By applying new tools like single-cell sequencing and single-cell proteomics, I think there's going to be a completely new era of understanding how cell types evolve," says Pawel Burkhardt, a choanoflagellate biologist at the University of Bergen in Norway.

Indeed, advances in single-cell RNA sequencing and microscopy techniques are now making it possible to study novel cell types in great detail. And these technical advances have given rise to a different way of looking at the evolution of cells and cell types: In the last decade, researchers have started to think about cells as evolutionary units in their own right. That, in turn, has led to the idea that cell types could be organized in a phylogeny that represents their relatedness, an idea that was proposed by Detlev Arendt in a 2008 article (2) and elaborated further by Arendt, Musser, Yale University biologist Günter Wagner, and colleagues in 2016 (3). The implications could be profound: Many ecologically and functionally important animal traits—such as nervous systems, extended pregnancy in placental mammals, or larval skeletons in sea urchins—all became possible when a new cell type evolved. Such types may be a significant source of evolutionary novelty that has hitherto been understudied and underappreciated.

Researchers used single-cell RNA sequencing technology to separate out individual sponge cells and analyze their gene expression. What they found surprised them: Despite the animals' simple anatomy, they have as many as 18 cell types. Image credit: Shutterstock/Allexxandar.

Origin Stories

The notion of a cellular phylogenetic framework "was a pretty radical idea," says Musser. It's not immediately clear why cell types should evolve by descent with modification because all cells in an organism share the same genome. Novel cell types are the result of cells' ability to reach new stable states of gene activity—or what researchers call stable "transcriptional regulatory states." In essence, a cell type, to be a



A sponge neuroid cell (*Right*) reaches out to a digestive cell known as a choanocyte (*Left*). Image credit: Giulia Mizzon, Jacob Musser, Constantin Pape, and Nicole Schieber (European Molecular Biology Laboratory, Heidelberg, Germany).

"type," should have a suite of stably expressed genes. Furthermore, evolution is able to modify each of these cell types independently of one another, explains Musser. The choanoflagellate's collar cell, which has an actin-filled collar and a long flagellum, is a good example. Small variations of this cell type exist in animals such as the hydra, and even *Drosophila*, Burkhardt notes. "It seems to be conserved throughout nearly all animals," says Burkhardt. "You find them in sponges, in cnidarians, in vertebrates, and so on."

Why might tracking these cell types and their origins be of interest? It turns out that the evolution of a new cell type can sometimes lead to new traits, unlocking the potential for further evolutionary diversification. One such example: Placental mammals have evolved an invasive placenta, meaning fetal tissue that invades the mother's uterus. Invasive or embedded placentation is difficult to manage: The mother's immune system needs to be modulated so that it does not reject the genetically foreign fetus and placenta. When the fetus implants and the placenta invades the mother's uterus, decidual stromal cells manage the mother's inflammatory immune response. Invasion of genetically foreign tissue is a major stressor. "The decidual stromal cells are on the front line of that stress," explains Eric Erkenbrack, a postdoctoral fellow in Wagner's lab at Yale. "The decidual stromal cells are allowing the fetus to invade."

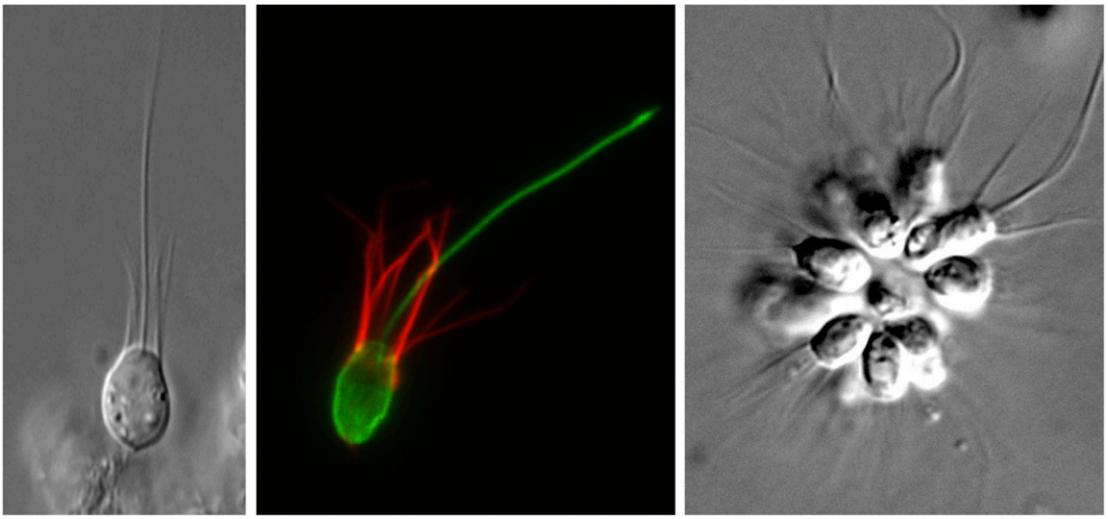
Those cells arose from a cell called the endometrial stromal fibroblast, which exists in marsupials—animals that don't have invasive placentation and lack decidual stromal cells. Erkenbrack, Wagner, and colleagues have shown that much of the genetic machinery that triggers the differentiation of endometrial stromal fibroblasts into decidual stromal cells has been co-opted from the genetic pathways involved in responding to environmental stresses (4, 5).

The researchers isolated endometrial stromal fibroblasts from humans and opossums and exposed them to pregnancy-related cues. In the opossum cells, those cues triggered a stress response (apoptosis, oxidative stress response), whereas in the human cells, those cues triggered the differentiation of the cells into decidual stromal cells. The stressor is "the seed for an alternative gene regulatory state," Wagner explains. Essentially, a new cell type co-opted the molecular machinery for responding to stress, allowing for extended pregnancy in placental mammals. It's a key life history strategy that allowed mammals to give birth to more developed offspring and may have contributed to the evolutionary success of placental mammals and their subsequent diversification.

Animal Origins

Several recent studies of cell types suggest that key animal features were already present in the cells of their ancestors—even unicellular ancestors.

Choanoflagellates, which have both single-cell and colonial life stages, offer up an example. A study by



The solitary choanoflagellate's collar cell, with its actin-filled collar, moves with a long flagellum. Small variations of this cell type exist in animals ranging from sponges to cnidarians to vertebrates. Image credit: Pawel Burkhardt (University of Bergen, Bergen, Norway).

Burkhardt and colleagues (6) on the choanoflagellate, *Salpingoeca rosetta*, showed that although all the cells in a choanoflagellate colony arise from a single cell, they can quickly differentiate into at least two different morphological types—evidence of early division of labor, Burkhardt says. "If you think about when the division of labor started, and when spatial cell differentiation happened—it could have happened even before the first animals," he says.

In another recent study, researchers in Nicole King's lab at the University of California, Berkeley described a new species of choanoflagellate, *Choanoeca flexa*, which they discovered when surveying choanoflagellates from Curaçao (7). *C. flexa* forms a sheet of cells curved in the shape of a cup. The researchers found that the curved sheet of cells could contract and change shape so that the flagella pointed outward rather than inward, allowing the unit to swim. Decreased light intensity spurred that contraction, the team found.

These findings suggest that the ability to contract in response to a stimulus (a key trait of animals, called contractility) was already latent in animals' single-cell ancestors, explains Thibaut Brunet, a postdoctoral fellow in the King lab and the first author of the article. The ability to contract in response to a stimulus is not only the foundation for muscle contraction and locomotion, but it also plays a key role in development and morphogenesis, Brunet says. He notes that ancestrally, the choanoflagellate cells are multifunctional—sensory and contractile—similar to the sponge cells that have both neuronal and immune functions, and were probably parceled out later.

Brunet suggests that some switches in the choanoflagellate cell types might have started as short-term stress responses triggered by environmental signals. Later, these responses could have come under the control of transcription factors to stabilize them, he hypothesizes.

There are still many open questions, but they are now tractable with the tools at hand. In the future, says Burkhardt, every evolutionary developmental (evo-devo) lab will work with single-cell analysis "because it opens so many avenues." At the moment, researchers are working to make a catalog of the different cell types as defined by single-cell transcriptome data. "But that is just the beginning," Burkhardt says.

Back to Natural History

In one sense, single-cell transcriptomics and the cataloging of cell types may be leading the evo-devo field back to its natural history roots. "We live in an era now where in combination with doing single-cell transcriptome sequencing and relatively cheap genomic sequencing, any lab with a reasonable budget can make a mini atlas of all the cell types and parts of their organism," says David Garfield, an evolutionary developmental biologist who recently started at Bayer in Berlin, Germany. "I don't think anyone should downplay how revolutionary that is. The question is, what can you do that goes beyond that?"

"This is the foundation of what is needed for understanding the evolution of novel traits."
—David Garfield

These technologies are essentially democratizing evo-devo by enabling researchers to study organisms other than traditional model organisms. Future studies should identify groups of related organisms in which the evolution of novel traits is linked to a novel cell type, Garfield says. Single-cell methods give researchers the ability to examine fine-scale changes as development progresses. "This is the foundation of what is needed for understanding the evolution of novel traits," he says. By comparing closely related species, it will be possible to pinpoint when a novel trait arises in the course of development—thus shedding light on the novelty's developmental and evolutionary origins with unprecedented detail.

The myriad hypotheses about how the nervous system originated, for example, can now be tested by comparing animals such as sponges, which lack nervous systems, with closely related nervous system-bearing relatives, says Rajee Rajakumar, a postdoctoral fellow at Harvard Medical School at Harvard University in Boston, MA, who will soon be launching his own lab at the University of Ottawa in Ontario, Canada. "With the cutting edge techniques that are emerging, we can really start to not only see very carefully how the nervous system develops within a single taxon; we could start comparing how the nervous system actually evolved across various taxa." New methods will allow researchers to get a "really high-resolution understanding of the transitional process of evolution," Rajakumar adds, "and at the same time, you can start to really get at questions of how novelties arise."

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