Researchers transform fluorescent proteins into a scaffold for manipulating genes

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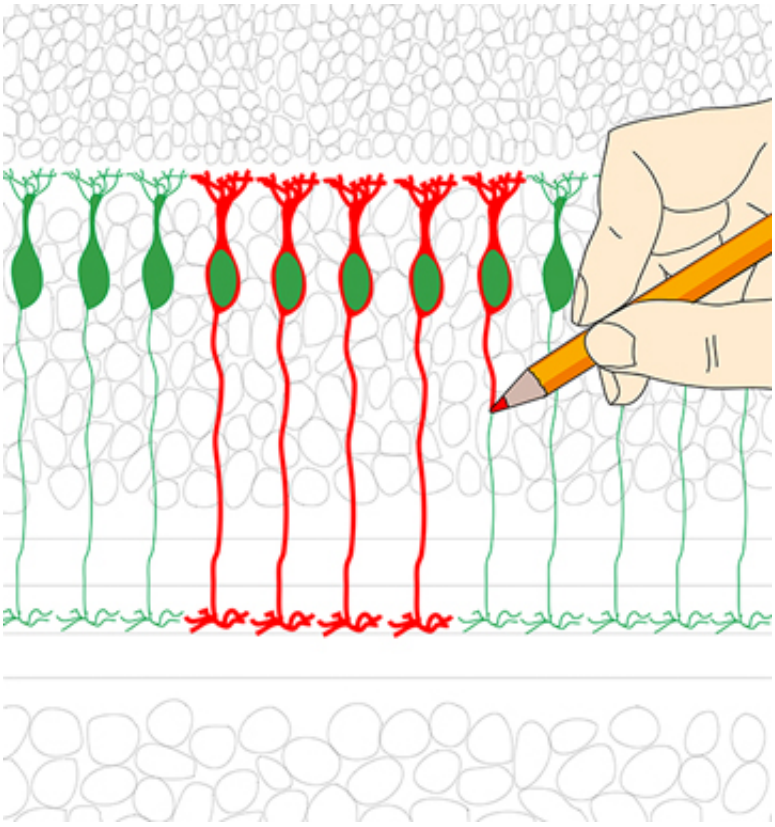

GFP (green) can be used not only as a fluorescent reporter for labeling cells, but also as a scaffold to control synthetic biological activities, such as gene expression (red). Credit: Jonathan Tang, with contributions by Tim Cherry

(Phys.org) —Jonathan Tang had a problem. A graduate student studying neural circuitry in the retina, he wanted to do more than identify fluorescent cells that send signals to the brain. He sought to understand

how these specialized cells called bipolar neurons develop and function in the eye's retina. More than "Here they are," he hoped to say, "Here's what they do."

Frustrated by the lack of available tools, he created his own. Tang, with Constance Cepko, HMS Bullard Professor of Genetics and Neuroscience, and other collaborators, transformed green fluorescent protein (GFP), a glowing biomarker borrowed from jellyfish, into a scaffold that can bring together protein fragments to enable gene manipulation, or other useful activities.

Their method, described Aug. 15 in *Cell*, enables scientists to probe how cells function not only in the retina, but also in other tissues. The tool also works with the promising technology called optogenetics, in which scientists use light to control individual cells.

To solve his problem, Tang took a step back to look more closely at GFP. The green glow produced by this jellyfish protein has become a workhorse of science, literally illuminating pathways and processes in lab dishes and living animal models since its Nobel Prize-winning discovery in 1961 and application in 1994. With it, scientists can observe molecular biology occurring in more than 1,500 transgenic GFP strains in mice and other organisms, many of which label unique populations of cell types.

An invaluable resource, GFP is nonetheless limited to tagging cells. If scientists want to control gene activity, particularly in the mouse, they turn to another workhorse molecule, Cre recombinase, an enzyme that can shuffle DNA and is primarily used in the mouse. Not nearly as many mouse strains have been made to express Cre as have GFP, so for Tang to learn not just where bipolar cells are in the retina, but also how they develop and connect to other cell types, additional transgenic mice would need to be generated and characterized.

Tang decided not to wait. After all, he had a PhD to complete.

"Jonathan is the star of the story," Cepko said. "We would sit and talk about how we had all these GFP lines we couldn't use to derive functional information. He came up with this idea that worked fantastically well, namely, to use the GFP to control biological activity specifically in cells already tagged with GFP."

To get there, Tang focused not so much on the green fluorescence in GFP as on its possible uses as a protein. By itself GFP doesn't alter cell function—an asset as a biomarker—but perhaps it could be combined with other proteins that affect gene activity. In that sense, GFP and its partners could act as both biomarker and synthetic system for controlling genes.

Searching for proteins that would bind to GFP, Tang discovered that a group of scientists in Germany had identified molecules that could bind to GFP. Derived from camel antibodies prized for their simple structure and high affinity, these proteins retained their ability to bind to GFP after they were introduced into a cell. Tang realized that this property could be exploited to create an array of tools that could enable the GFP-binding proteins to do much more. He constructed a series of chimeric, or fusion, proteins, fusing protein domains that can control transcription to these GFP-binding proteins. When two such fusion proteins that can bind simultaneously to GFP are introduced into a cell, GFP brings them together, triggering the designed co-dependent activity of the fusion proteins.

The scientists tested their tool in the retina, using it to switch on a fluorescent reporter gene in one experiment and to knock out a gene in another. As a boost to those who study neural function, they could trigger expression of channelrhodopsin, a protein that allows neural circuits to be controlled with light, thereby linking expression of GFP to

the powerful tools of optogenetics.

The tool worked in tissue culture as well as in living mice and zebrafish, another animal model favored by investigators.

"We can now use this as a way to access genetically defined cell types to see what's essential for development, behavior, neural circuit processing—or whatever one wants to study," said Tang.

"We can use these methods to probe function and development in the retina, but whatever your tissue is, you should be able to use these methods to study it," Cepko said. "You don't have to make a transgenic mouse or even have a GFP strain. You may be able to find an endogenous protein, or maybe even an RNA, that's specific to your interest and do exactly what we did to make our tool."

More information: www.cell.com/abstract/S0092-8674%2813%2900892-1

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