TECHNOLOGY FEATURE

MICROMANAGEMENT WITH LIGHT

The optogenetics techniques that have long been used in neuroscience are now giving biologists the power to probe cellular structures with unprecedented precision.

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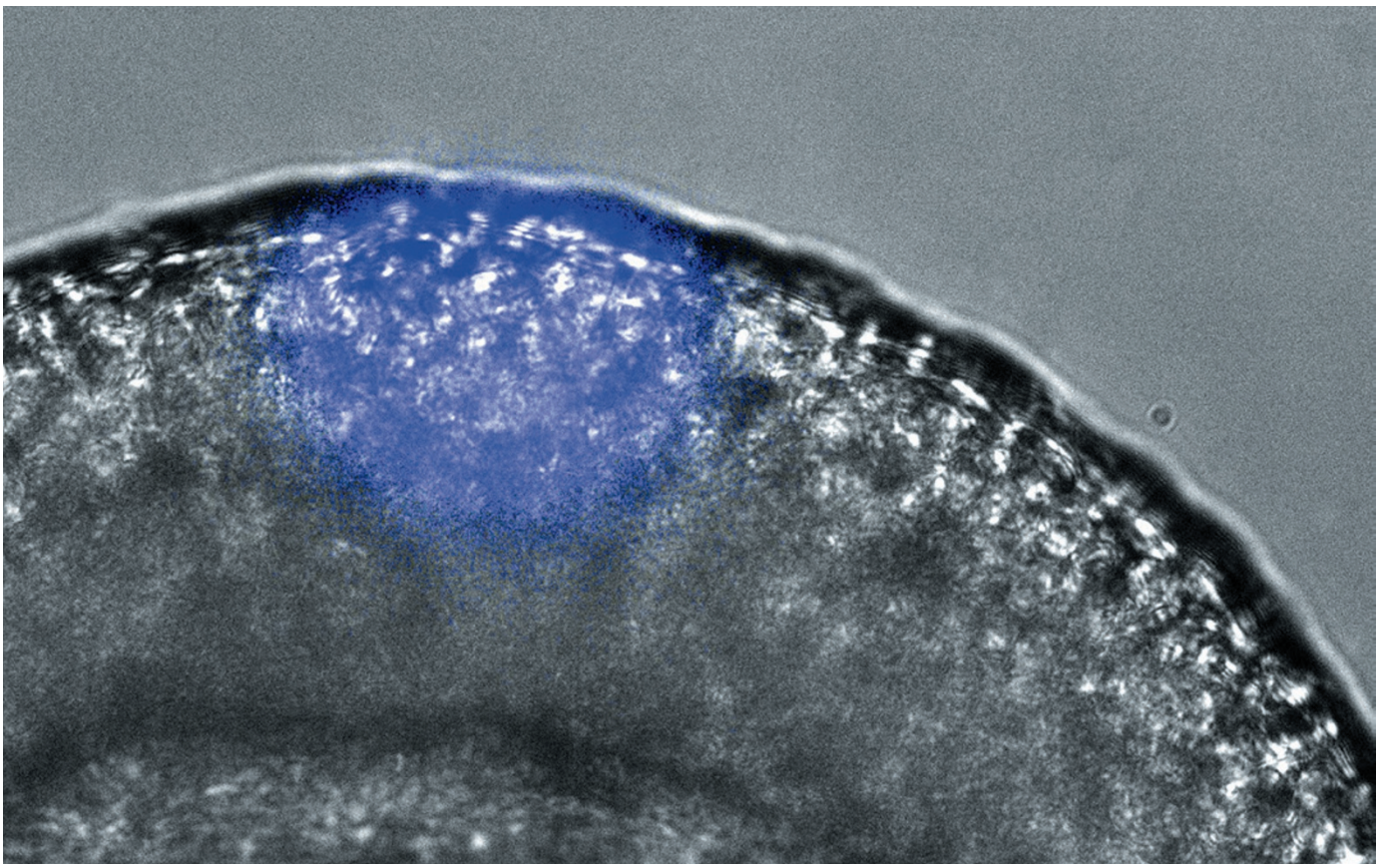


Shining blue light at just one part of an engineered zebrafish embryo enables scientists to selectively activate a light-sensitive transcription factor.

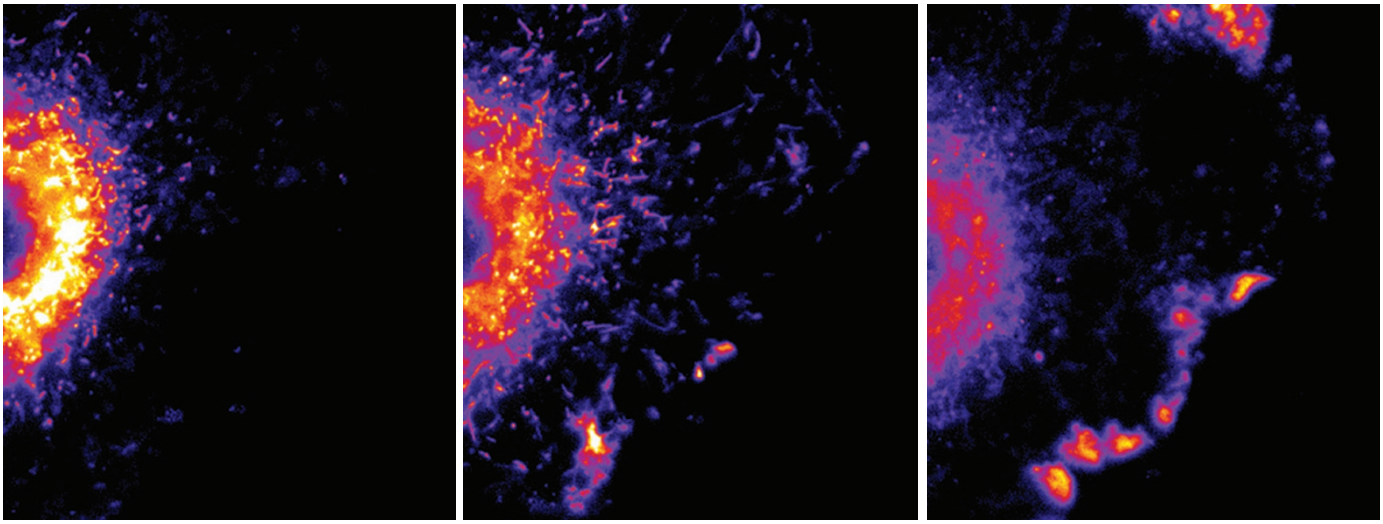
BY AMBER DANCE

Kevin Gardner opens up a mini-fridge-sized incubator and stares at the flashing blue lights inside, a scene he always finds reminiscent of a 1970s New York disco. "There are interesting things happening," he notes — but rather than the disco lights, he's talking about events at the microscopic scale.

Gardner is a structural biologist at the City University of New York's Advanced Science Research Center, where he is a leader in the use of light to control the activity of proteins, a technique known as optogenetics. Thanks to the tools that he and other protein engineers have developed, scientists can now micromanage processes such as cell signalling or movement with an LED or laser flash, rather than just observing them. They can flip proteins on and off, for example, or move organelles back and forth across a cell.

Over the past several years, protein engineers have developed nearly a dozen light-sensitive tools that they can use to accomplish such feats. Some are artificial proteins designed by scientists, but many incorporate modified versions of natural light-sensing proteins. A simple example is the light–oxygen-voltage-sensing (LOV) domain. Found in plants, fungi and some bacteria, it contains a portion that winds up into a helix. In the dark, this coil tucks in close to the rest of the protein. But under blue light, the helix lets go and lays bare the structures hidden beneath it. Plants and algae use LOV sensors to cover enzymes or DNA-binding proteins, enabling them to regulate activities such as growth towards light or rearrangements of chloroplasts. But scientists can make a custom light-activated protein by choosing what is hidden underneath the LOV coil — the active site of an enzyme, for example.

Light offers important advantages over standard methods of manipulating cellular activity. One such advantage is speed. Chemicals take minutes to enter a cell, whereas light takes ▶



LUKAS KAPITEIN, UTRECHT UNIV.

Under blue light, peroxisomes near the nucleus (left) attach to the motor protein kinesin, which drags them to the cell periphery (middle and right)[1].

fractions of a second. Thus, cell biologists can probe cellular processes such as signalling pathways or protein movement that take place on time scales of seconds to minutes, says Klaus Hahn, a cell biologist and protein engineer at the University of North Carolina School of Medicine at Chapel Hill. Likewise, a cell or organism with a knocked-out or knocked-down gene gets days, weeks or even longer to adapt to the change, and perhaps activate back-up systems. But if the protein is deactivated by a light switch on the microscope stage, there is no time to compensate — and researchers may see effects that they would not observe with conventional methods. "The cell doesn't know what hit it," Hahn says. And the effects can be reversed by simply turning off the light.

Another advantage is that optogenetics offers precise spatial control: instead of flooding every cell in a Petri dish with the same small-molecule treatment, cell biologists can use tightly focused light to flip the switch in just one cell, or even part of a single cell.

Optogenetics flourished initially in neuroscience: light-controlled channels were used to make neurons fire at will. But cell biologists have now embraced the technique enthusiastically. "You're going to see a ton of papers coming out, in every organism you can think of, using these tools, within the next 12 months," predicts Jared Toettcher, a bioengineer at Princeton University in New Jersey.

PROTEIN PARTNERS

One of the most common tricks in optogenetics is to design two proteins that will bind to each other in the presence of light, forming a 'dimer'. Scientists have been triggering dimer formation with chemicals for some time, but doing it with light is relatively new. The importance of protein–protein interactions in biology makes light-induced dimerization a game-changer, says Chandra Tucker, a biochemist at the University of Colorado School of Medicine in Denver. "If you are very creative," she says, "you can control [protein] activities in many different ways." For example, scientists can tether one of the proteins on a cellular membrane, and leave the other free-floating. When they turn on the light, the mobile partner will be captured by the membrane-bound partner, thus targeting it to that location. Or they can split a single protein into two inactive fragments and reattach them with a light switch to make the functioning version.

Lukas Kapitein, a biophysicist at Utrecht University in the Netherlands, used light-induced dimerization to move individual classes of organelles around like furniture in a house[1]. Scientists have realized lately that cells rely on a certain *feng shui*. For example, when there are plenty of nutrients around, lysosomes — metabolic organelles — hang out near the cell's edges, promoting the production of new proteins. But when cells are starved, lysosomes retreat to the cell's interior, where they encourage the cell to start digesting itself[2].

"With a flash of light, Kapitein could shuffle specific organelles inward or outward."

Organelle location can even affect a cell's shape. Neurons send out projections called axons to transmit impulses to other neurons, and axons tend to branch into two at spots where mitochondria, the cell's energy facility, have settled[3,4].

However, the effects of cellular layout can be difficult to unravel. In the past, cell biologists generally had to rely on wholesale techniques such as dissolving the cytoskeleton or changing the levels of molecular motors that deliver organelles to the right spot — relatively crude processes that tended to move all of the organelles simultaneously. Conversely, Kapitein's optogenetic method offers the ability to fine-tune the positioning of a single kind of organelle, and it is reversible. The main optogenetic tools he uses are tunable light-inducible dimerization tags (TULIPS), which are based on the LOV photosensor from oats, and an engineered protein–protein interaction domain based on the common PDZ sequence. The LOV helix hides a small peptide, which, when exposed by blue light, binds to the PDZ domain[5].

Kapitein started by attaching the LOV domain to three different kinds of organelles: mitochondria, peroxisomes (metabolic sacs) and recycling endosomes that return internalized membrane components to the plasma membrane. Then he hooked the PDZ domain to one of two different kinds of intracellular motors: kinesins, which drag their cargoes to the cell's perimeter, and dyneins, which tote cargo towards its centre. With a flash of blue light, Kapitein could shuffle specific organelles inward or outward (see 'Light switch').

The researchers applied their TULIP set-up to test how endosome location affects axon growth in neurons. They removed the endosomes from the axon tips, which stopped the axons from extending. They shoved in extra endosomes, and the axons grew faster. Thus, as with mitochondria, the position of these organelles affects the cell's shape.

The same system should work for many kinds of organelle, says Kapitein, allowing scientists to ask previously unanswerable questions about cell layout. He has received dozens of requests for his constructs from cell biologists who want to rearrange their own favourite cell structures. Looking ahead, he wants to find a way to move a single organelle (as opposed to all of the organelles of a particular class) and park it at a desired location.

SIGNAL OF INTENT

Biologists do not have to reposition entire organelles to make waves in a cell; moving a single protein will do. Many signalling pathways start with the binding of some external factor to receptors on the cell membrane, followed by a cascade of interactions that transfers the information inward from one protein to the next. The end result is some appreciable change in the interior, such as a shift in gene expression. Scientists can

often mimic these effects by identifying proteins involved in the early stages of the pathway and moving them to the plasma membrane. After they hit the membrane, the proteins act as though they have received the external signal and kick off the downstream cascade.

For example, Toettcher and his colleagues used a light-controlled system to study the effects of Ras, a signalling protein that is involved in diverse processes such as cell proliferation and determining cell fate in a developing embryo. This one signalling pathway can mediate such different processes because Ras has a different effect according to when and where in the cell it gets activated — but researchers were unable to investigate this in great detail until they had the optogenetic tools to turn Ras on and off.

Toettcher used the phytochrome B (PhyB)–PIF dimerization system, which optogenetics scientists have borrowed from the plant geneticist's favourite weed, *Arabidopsis*[6]. In the plant, visible red light causes PhyB to bind and activate the PIF transcription factor — a mechanism that *Arabidopsis* uses to turn on genes involved in processes such as germinating seeds or growing away from shade. But unlike other optogenetics systems that simply switch off in the dark, PhyB and PIF stay bound until they are hit with longer-wavelength infrared light. Toettcher hooked PhyB to the plasma membrane, and part of PIF to a Ras activator. When he turned on the red light, Ras would turn on too.

Because he could turn Ras back off with infrared rays, Toettcher could precisely control the timing of its activation over minutes or hours, and this made a difference to what happened downstream. For example, turning on Ras in one cell causes its neighbours to phosphorylate STAT3: a transcription factor that works in various processes such as cell growth and death. Two hours of continuous red light stimulated STAT3 phosphorylation. But 1 hour of red light, 15 minutes of infrared light and another hour of red light did not, Toettcher says. Although Ras activation totalled two hours in both cases, the cell could tell the difference, and responded accordingly[7]. The researchers do not know precisely what use STAT3 is being put to after extended Ras signalling, but they surmise that this kind of system would allow a cell to apply the same pathway for various purposes by varying the timing of the extracellular input.

AT THE FLIP OF A GENE

Cell-signalling systems such as Toettcher's affect the activation of genes only after a cascade of intermediate reaction steps. But optogenetic tools can also modify gene expression directly or even induce permanent changes to the genome. For example, Gardner and his colleague Laura Motta-Mena, a biochemist and cell biologist at the University of Texas Southwestern Medical Center in Dallas, have borrowed a light-activated transcription factor from bacteria to activate genes in a range of organisms[8]. At the University of Tokyo, meanwhile, chemist Moritoshi Sato and his colleagues have devised systems that use light to activate CRISPR–Cas9-based gene targeting to achieve high-precision control of gene editing or expression[9,10].

Optogenetic CRISPR tools such as these will be particularly useful for scientists who want to follow cell behaviour in entire organisms, Hahn says. For example, researchers might want to test whether a cell migrates from one organ to another. With light and CRISPR editing, they could mark the cells that they are interested in with an extra gene encoding something obvious such as green fluorescent protein. Then they could use a microscope to check where those cells go. Sato has speculated that scientists could use the optogenetics–CRISPR combination to investigate how a sequence of mutations turns a cell cancerous or how gene activation in different parts of the brain affects the organ's function.

Optogenetics techniques now allow scientists to activate individual genes or proteins with the flick of a light switch, but the next step will be to control multiple processes with a whole spectrum of light. Different proteins could be

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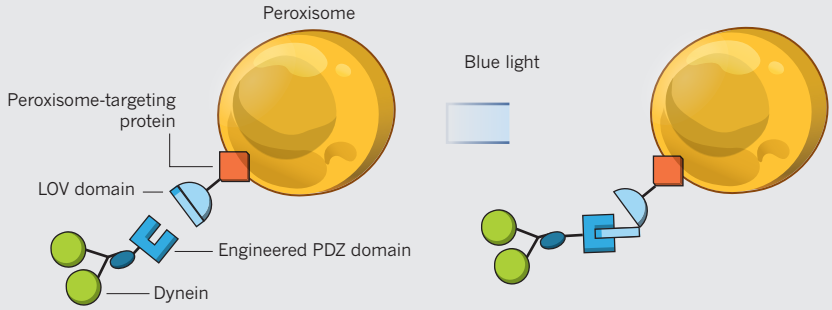
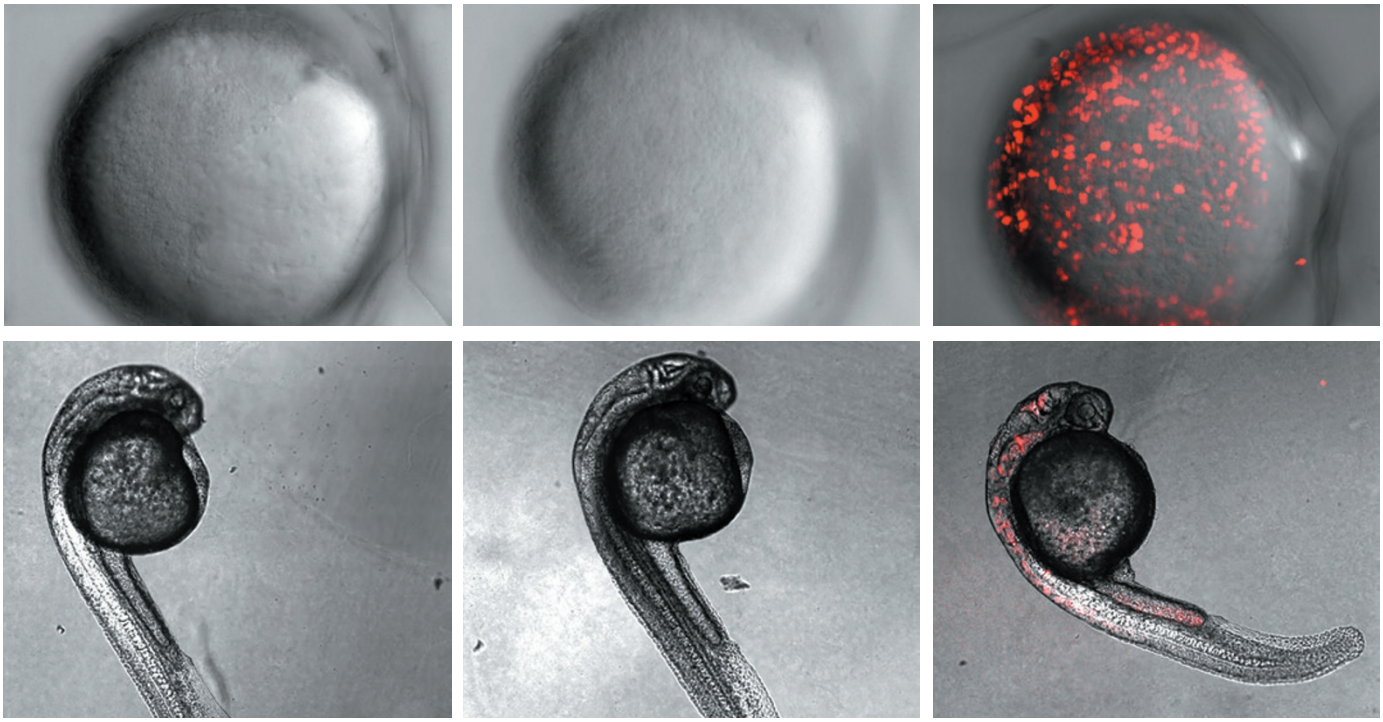
LIGHT SWITCH

To move an organelle, in this case a peroxisome, to the centre of a cell, scientists first attach a light–oxygen-voltage-sensing (LOV) domain to a protein that targets peroxisomes. They also attach an engineered PDZ domain to the motor protein dynein. In the presence of blue light, the LOV opens, the PDZ grabs it and the dynein starts dragging the whole assembly inwards.





NATURE CHEM. BIOL.

Eight-hour- (top) and one-day-old (bottom) zebrafish embryos into which a gene for red fluorescent protein has been added with a sequence that turns on the gene when it is bound by a light-induced transcriptional activator (right). In the dark (left) or when the activator is absent (middle), the gene is switched off[8].

made sensitive to different colours, so researchers could, for example, flick a blue-light switch to turn on one protein and then a green-light switch to activate another in the same cell. "I would love to see, not one red and one blue, but something like one of those big, old-fashioned organs where you have all kinds of switches and levers," says Gardner. Unfortunately, at this point, most optogenetic switches play the same note — they usually react to just blue light (although some, such as PhyB, respond to red). But researchers are working on systems that would respond to more diverse colours. Some are even exploring other parts of the electromagnetic spectrum, hoping to activate proteins with microwaves, magnetic fields or radio waves, although that work is in the early stages.

There are other disadvantages of the current optogenetics toolkit. For one, many systems are a bit 'leaky' in that they allow some activity even in the dark. And light itself can affect cellular activities such as transcription and signal transduction, points out Masa Yazawa, a stem-cell biologist at the Columbia University Medical Center in New York. This means that scientists should be careful about their negative controls, he says. Just leaving cells in the dark isn't good enough; rather, scientists should engineer a light-insensitive version of their optogenetic proteins and shine the light on those cells, too.

Another disadvantage is that some light-sensitive systems require a chemical called a chromophore, which scientists have to add if the cells they want to study do not manufacture it. This can be an inconvenience, but it also makes it easy to perform a negative control experiment — the chromophore can simply be left out.

Illumination can also be toxic in large doses. For experiments in which a quick flip of the light switch is all that is needed, this is no big deal. For Kapitein, it takes only a couple of milliseconds to activate the LOV domain and send organelles on their way, so cells have no problem. By contrast, Yazawa wants to grow cells with light-activated genes for days or weeks as they change from stem cells into brain or heart cells. Light toxicity could be a major issue, but fortunately Yazawa's switches — which are also borrowed from *Arabidopsis* — stay on after they have been stimulated, so he does not have to keep them under constant light[11]. Other scientists, such as Gardner, strobe the light on and off to limit cell exposure while keeping their optogenetic tools activated.

"The next step will be to control multiple processes with a whole spectrum of light."

A further problem is that because the tools are so new, they can still be finicky to use. "There's no plug 'n' play," says Tucker. Every cell biologist with a plan to use light will have to optimize their system, figuring out which optogenetics tools work best for them and identifying the best expression level for their light-sensitive genes. Tucker points out one rookie mistake: using the white light of the microscope to focus samples. White light contains all colours, and will activate the optogenetic sensors. It is better to use filtered light in a colour that will not stimulate the proteins.

Scientists expect that in the future, it will be easier for cell biologists to adopt optogenetics. Researchers are starting to compare different light-sensitive proteins side-by-side, and their data will help others to select the best tools for their questions. In June 2015, Gardner and Motta-Mena founded a company called Optologix, based in Dallas and New York, to offer standardized kits. Their first product will include the LOV-based gene-activation system they invented, along with an LED lamp.

On the bright side, the lighting part of the package is easy. A bench-top light may do, or the filters and lasers on many microscopes can activate proteins as desired. That accessibility could make light-based tools as standard as microscopes and pipettes in cell biology. "Ten years from now, these will be workhorse tools for everybody in developmental and cell biology," Toettcher predicts. ■

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