in San Carlos, California.

In addition, Mrksich and his colleagues say the technique should work with any ligand, making it useful for studying a wide range of cellular behaviors. These would include cell differentiation during embryonic development and programmed cell death, which culls damaged or excess cells, as well as cell migration. Indeed, says Nicole Sampson, a chemist at the State University of New York, Stony Brook, the chips should provide a handy tool to "help determine the mechanisms of cellular signaling."

–YUDHIJIT BHATTACHARJEE

Yudhijit Bhattacharjee writes from Columbus, Ohio.

CELL BIOLOGY

## Color-Changing Protein Times Gene Activity

For years, researchers trying to capture the bustling activity of genes in living cells have mostly had to make do with snapshots, which clearly fell short of the mark. But now their frustration may be over. On page 1585, a team led by Alexey Terskikh and Irving Weissman of Stanford University and Paul Siebert at Clonetech Laboratories in Palo Alto, California, describes a new fluorescent protein that turns bright green when it is first made, then changes to red over several hours—providing the ability to witness how genes alter their activities over time.

This new fluorescent timer should be widely applicable, enabling developmental biologists, for instance, to monitor how the activities of genes change as cells migrate in the developing embryo. "If a picture is worth 1000 words, then a movie should be worth a novel," says developmental biologist Randall Moon of the University of Washington, Seattle.

The Stanford-Clonetech group's work builds on the discovery last year, by Mikhail Matz and colleagues at the Russian Academy of Sciences in Moscow, of a fascinating protein, dubbed drFP583, found in coral. The researchers identified the protein based on the similarity of its sequence to that of "green fluorescent protein," which is widely used to track proteins and cells in living organisms. But drFP583 glowed a bright red. Thus, it could add a new color to cell biologists' palette of protein markers, enabling them to more easily study two separately labeled proteins at once and track their interactions.

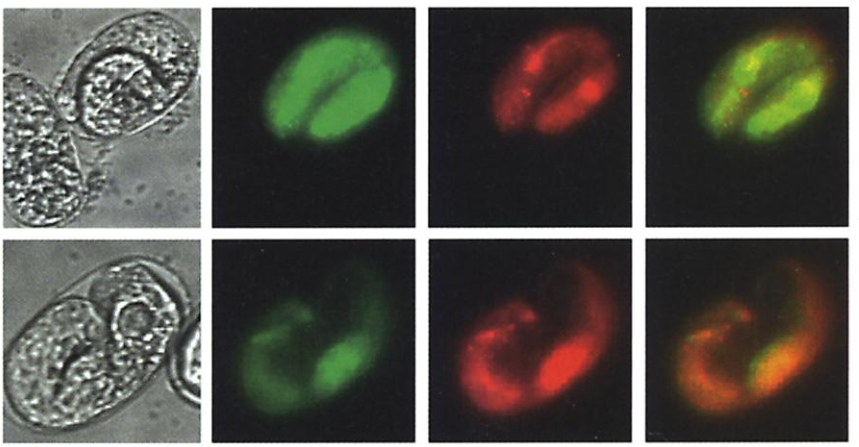
The researchers did not realize at first, however, that there is more to drFP583 than just a new red-colored tag. In fact, recent work described in the 24 October issue of the *Proceedings of the National Academy of Sciences* by Roger Tsien's group at the University of California, San Diego, and also by Watt Webb at Cornell University in Ithaca, New York, suggested that the protein had some significant drawbacks. Among other things, drFP583 is a very dim green right after synthesis and takes hours to days to develop a red color intense enough to be of practical use. Biologists tracking fast-paced changes in gene expression usually can't afford to wait so long.

Shortly after drFP583's discovery, Terskikh generated a collection of mutants, hoping to find proteins that were brighter and developed their color faster. He soon spotted several mutants worthy of attention—and one apparent inconsistency in his record keeping. He noticed that E5, a mutant he had labeled bright green one day, turned a deep fluorescent red the next day. Assuming he had made a mistake in recording the color the first time, Terskikh repeated the experiment and regrew the stock. But once again, the mutant appeared green at first and turned red the next day.

The change occurred no faster than that of the unaltered drFP583. But E5's initial green fluorescence is much brighter than that of its parent protein, making it easily detectable. To their delight, Terskikh and his colleagues realized they now had a protein that could be used as a fluorescent timer of gene activity. They could simply attach the E5 coding sequence to regulatory sequences of the gene they wanted to track, and the color of the resulting protein would provide an estimate of when the gene had become active. In this context, the protein's delayed color shift, originally considered a detriment, became an asset, because its timing function would have been lost if it turned red immediately after synthesis. "These people, you might say, have figured out how to make lemonade out of a lemon," says Tsien.

Terskikh and his colleagues have since gone on to test their fluorescent timer in living organisms. In one set of experiments, they placed the E5 gene under the control of a regulatory sequence from a gene for a so-called heat shock protein; this sequence causes the genes containing them to be turned on by a boost in temperature. The researchers then introduced the hybrid gene into the roundworm *Caenorhabditis elegans* and monitored its behavior. When the worms were kept at room temperature, the gene was silent and the worms remained colorless. But when placed at 33°C for an hour, the worms turned green, indicating that the gene had become active. As E5 aged, the embryos acquired a yellowish-orange hue, and 50 hours after the heat shock, they were mostly red.

To see whether they could track changes in gene expression during embryonic development, the Stanford workers also linked E5 to a regulatory sequence from *Otx-2,* a gene needed for normal formation of the nervous system. They injected the gene into embryos of the frog *Xenopus laevis* and then examined the brains of the resulting tadpoles. As predicted by previous studies of *Otx-2* expression, they could see that the gene was expressed in some areas of the brain before others. Those where the gene came on earlier and then turned off appeared orange, while others were greenish, indicating the gene's recent expression.



**Color shift.** At 11.5 hours after E5 expression in *C. elegans* was activated by heat shock (*bottom panels*), the protein's green color has decreased compared with that at 7 hours (*top panels*), and its red color has increased.

CREDIT: ALEXEY TERSKIKH *ET AL.*

Although enthusiastic about the results, Tsien cautions that the E5 mutation likely hasn't solved all of drFP583's problems. Like drFP583, E5 probably aggregates to form tetramers. That could cause problems if researchers try using E5 to track proteins by creating hybrids, because they could clump as well. In addition, E5 requires oxygen to change colors, and it's currently unclear how variations in oxygen concentrations in different biological systems will affect the rate of color change. But Tsien and others suspect it will be possible to engineer new proteins that don't aggregate and, if

necessary, are less dependent on oxygen.

Even before that happens, researchers are eager to test the new timer protein. Leonard Zon of Children's Hospital and Harvard Medical School in Boston is using it to follow the development of blood cells in zebrafish. And many others are lining up after him. "The people [who] would like to use it are more than I can actually handle," says Terskikh, who is already involved in several collaborations. **–MARINA CHICUREL**

Marina Chicurel is a freelance writer in Santa Cruz, California.

NEUROSCIENCE

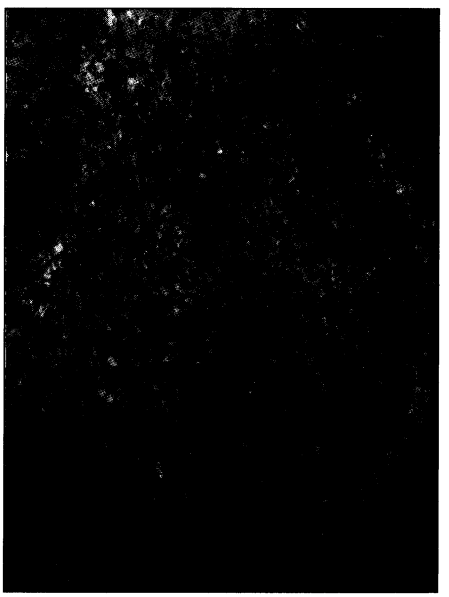
## Stem Cells Hear Call of Injured Tissue

**NEW ORLEANS**—Like a superhero who can hear cries for help from miles away, ever versatile stem cells somehow sense danger in the brain and spinal cord and rush to the rescue. In animal models, at least, injected stem cells travel to tissue injured by stroke, Alzheimer's-like plaques, contusions, or spinal cord bruises, sometimes traversing long distances. Several teams reported these surprising results this month at the Society for Neuroscience annual meeting. No one knows exactly how stem cells detect these different kinds of damage, but researchers hope that the cells' migratory powers can be harnessed to either replace dead tissue or deliver therapeutics right where they're needed.

Researchers have known for years that stem cells migrate widely if added to young brains but are fairly dormant in healthy adult brains. Now studies are showing that injuries somehow prompt stem cell movement even in adult brains. "In the abnormal brain, there are new rules. The whole terrain is changed," says neuroscientist Evan Snyder of Harvard Medical School in Boston. And this newfound motility is being put to use: "A number of us find that we can pull cells out from the nervous system, grow them in a dish, and put them back," where they migrate to damaged tissue and sometimes repair it, Snyder says.

The research teams are using a variety of sources for their stem cells. Some come from established cell lines that originated either from human or mouse cells. Other researchers pluck neural precursor cells from where they occur naturally in the primate brain, in a layer of tissue that surrounds the ventricles. Others implant stem cells from embryonic tissue. Regardless of their origin, the cells share one trait: They still have developmental decisions to make. And under the right circumstances, they can be coaxed to turn into neurons or other brain cells called glial cells.

In many disorders that strike the motor system, replacing glial cells might be a better strategy than building new neurons, says neuroscientist Jeffery Kocsis of Yale University. Most spinal cord injuries, for instance, don't completely cut the axons that run through the cord. But bruising or crushing the cord kills the tissue, called myelin, that insulates the axons, leaving axons exposed and unable to conduct signals. To see whether stem cells could act as glial cells and build up myelin around bare axons, Kocsis and his team made small, demyelinating lesions in the spinal cords of monkeys. Using punch biopsy, they then plucked neural precursor cells from the rim of the injured monkeys' ventricles, multiplied them, and injected the cells back near the injury. The cells appeared to seek out damaged axons and rewrap them with myelin. Kocsis's team is now testing whether the newly insulated neurons conduct nerve impulses better than untreated ones. "The feasibility is there" for remyelinating exposed axons, Kocsis says, a strategy that could also be useful in treating multiple sclerosis.



**To the rescue.** Stem cells (red) infiltrate a brain tumor (green).

In another model of motor system damage, Jeffrey Rothstein of Johns Hopkins University and colleagues found that stem cells can migrate along the entire length of the spinal cord, at least in mice and rats. The team infected the animals with a virus that causes the same sort of neuronal damage as amyotrophic lateral sclerosis does in humans. The virus kills neurons at the base of the spinal cord, paralyzing the animal. When the Hopkins team injected mouse neural stem cells into the cerebrospinal fluid, the cells migrated from the top of the spinal cord to the base and clung to injured areas. Untreated control animals remained fully paralyzed. But 8 weeks after receiving the stem cells, half of the treated rats could move their limbs somewhat. Human-derived stem cells didn't work reliably, Rothstein says; they're not sure why.

Stem cells can also find their way to damaged spots in the brain. To model Alzheimer's disease, researchers led by Barbara Tate of Children's Hospital in Boston injected amyloid, a protein that accumulates into plaques characteristic of Alzheimer's, into one side of rats' brains. In control rats, they injected benign proteins. The researchers then injected stem cells into a ventricle on the opposite side of the brain. The cells crossed to the opposite hemisphere and found their way to the amyloid deposits but ignored the control protein.

Stem cells don't always make the cross-hemisphere trek successfully. In a model of traumatic brain injury in mice, stem cells injected near the injury migrated to it, as well as to diffuse white-matter damage throughout the injured hemisphere, reported Tracy McIntosh of the University of Pennsylvania in Philadelphia. There, they apparently repaired at least some of the damage: 12 weeks later, treated mice were walking relatively gracefully across a moving cylinder while the others stumbled about. But when the team injected stem cells into the opposite hemisphere, the cells did not find the injury or help recovery.

Looking at a different axis, Snyder's team has found that stem cells do move reliably from the back of the brain to the front. They injected stem cells into the rear brains of adult rats with induced strokes in the forebrains. The cells found their target, coating the rim of the stroke lesion and restoring some movement. But many questions remain, says Snyder. They don't know whether the stem cells replace dead cells near the lesion, improve connections among remaining cells, or perform some other function.

Stem cells might also be enlisted to deliver therapeutics to the right spot, according to a report in the 7 November *Proceedings of the National Academy of Sciences*. Brain tumors send tentacles throughout large areas of tissue, making tumors tough to eradicate. Karen Aboody of Children's Hospital in Boston, working with Snyder and colleagues, inserted into stem cells a gene for a molecule that shrinks tumors. They then injected the cells into several sites in rat brains. The stem cells surrounded the tumors and "chased down" the cancer cells the tumor spins off, they report, thereby shrinking the animal's tumor burden.

These studies, especially Aboody's, "show in a clinical context that we might be able to use this ability" of stem cells to migrate, says Ron McKay of the National

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