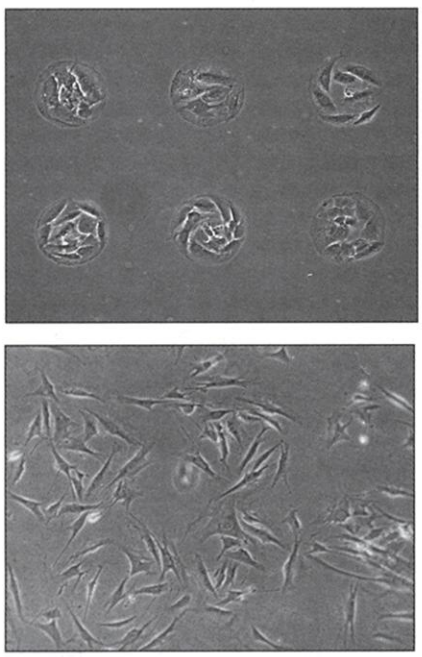
BIOCHEMISTRY

Ligand Chip Helps Manipulate Cells

Scientists at the University of Chicago have found an electrochemical way to control interactions between cells and a chip. It's an improvement over existing methods of influencing cell behavior, none of which allows control over cell-surface activities with chip-bound molecules. Researchers say the achievement could be a key step toward a wide variety of applications—everything from new drug assays to prosthetic aids that replace damaged neurons.

The behavior of cells is controlled by any of a large number of different receptors on their surfaces that respond to hormones, growth factors, and various other types of regulators. Chicago's Milan Mrksich and his colleagues devised a way to first attach certain peptide regulators, or ligands, to their chip, and then to release them, through electrical means. Because cells bind to the ligands, this allowed the researchers to turn the chip on for cell attachment or turn it off, thus releasing the attached cells. "The technique would be useful for studying subjects in cell biology like cell migration that depend critically on the cell's surface composition," says synthetic chemist George Whitesides of Harvard University, a former mentor of Mrksich. "In the longer term, it could serve as a basis for new types of assays for drug screening."



Moving pictures. Cells attached only to regions on the substrate coated with fibronectin (*top*) are able to spread and fill the entire surface (*above*) after electrochemical oxidation.

CREDITS: (LEFT TO RIGHT) MILAN MRKSICH/UNIVERSITY OF CHICAGO; NASA

Researchers have previously designed ligand-coated substrates for attaching cells, but these have been limited by the static composition of their surfaces. Mrksich wanted to develop a dynamic substrate—a chip that could actively modulate the behavior of attached cells. To achieve that, he and his colleagues coated a gold film with an alkanethiolate, a type of organic molecule that carries hydroquinone groups. Applying a tiny electric voltage to the film triggers an electrochemical reaction, converting the hydroquinone groups to quinones. When molecules containing a ligand bound to another organic group called cyclopentadiene are added to the chip, they will then react with the quinones. This reaction installs the ligand on the surface of the chip, thereby turning it on for cell attachment. Reapplying the electric voltage to the film converts the quinone groups back to hydroquinones, thus causing tethered ligands to break away and release the cells from the substrate—in effect, turning off the chip.

Mrksich and his colleagues have shown that they can induce cells to migrate on the chip by taking advantage of its switching-on aspect (see images). They first coated small circular regions on the gold substrate with a protein called fibronectin that causes connective tissue cells called fibroblasts to adhere to the spots, leaving the remaining areas covered with alkanethiolates. Then the researchers turned on the chip in the presence of a solution containing a conjugate of cyclopentadiene and a small peptide known to mediate cell adhesion. This caused the conjugate to bind to the chip and, as a result, the cells moved out from the circles and lodged themselves uniformly across its surface.

Such a system can be used, Mrksich says, to screen either for drugs that promote cell migration, which might be helpful in wound healing, or for drugs that inhibit it, which might have antimetastatic effects. For example, the team has tested two compounds, known for their antimigratory properties, and found that both blocked cell migration on the chip. "One goal of the work is to improve the design of cell-based sensors for drug screening," says Mrksich, whose results are in press at *Angewandte Chemie*.

Strategies for precise and selective cell attachments, such as the one used by Mrksich's group, should allow scientists to engineer small surfaces with multiple cell types. That could help in the miniaturization of cell-based sensors and thereby speed up drug screening. "The technology would enable us to screen drug candidates at a much more specific and fine level than what is possible with traditional assays," says Tom Schall, founder of ChemoCentryx Inc., a drug-discovery company based

ScienceScope

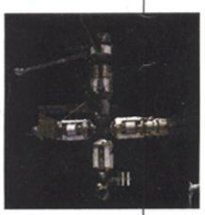
Frosted Biomedical researchers may be nipped by an early freeze this winter thanks to Congress's inability to pass a spending bill for the National Institutes of Health (NIH). Institute chiefs learned last week that the delay will probably force them to suspend cost-of-living increases for continuing grants due to go out early next month, and they plan to make cuts in new and competing grants as well.

Congress is considering a bill that would give NIH a 15% increase, but election year politics has stalled work until at least next month. Mary Hendrix, president of the Federation of American Societies for Experimental Biology, says the uncertainty "is a very serious threat to biomedical research" and could be "devastating" as faculty plan for research and staffing in the next year.

Sacked Indian scientists are in an uproar over the sudden removal last week of the head of the Indian Council of Agricultural Research (ICAR) for alleged financial mismanagement. Agricultural minister Nitish Kumar said that Rajendra Singh Paroda, a prominent scientist who has headed the $300 million ICAR for the past 6 years, was sacked to allow for an "independent investigation" of allegations that he mishandled the purchase of computers as part of a loan from the World Bank. But Narendra Gupta, executive secretary of the National Academy of Agricultural Sciences, says that he doubts the allegations will hold up. The unprecedented removal of a well-regarded technocrat, he says, makes scientists "like sacrificial lambs in the hands of politicians."

ICAR, with 5000 scientists, is India's premier agriculture research agency and has played a crucial role in ushering in the Green Revolution. An ICAR spokesperson said that "there was a deathly silence after the news of the removal broke."

MirCorpse? After numerous resurrections, Russia's Mir may finally be headed for a fiery death. President Vladimir Putin's cabinet agreed last week to deorbit the space station, launched in 1986, in February 2001. The decision is bad news for Amsterdam-based MirCorp, which wanted to lease the station for science and tourism. But company officials still hold out hope, with one noting that Russian officials have "killed the station at least four times" before.



Contributors: Eliot Marshall, Pallava Bagla, Andrew Lawler

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.

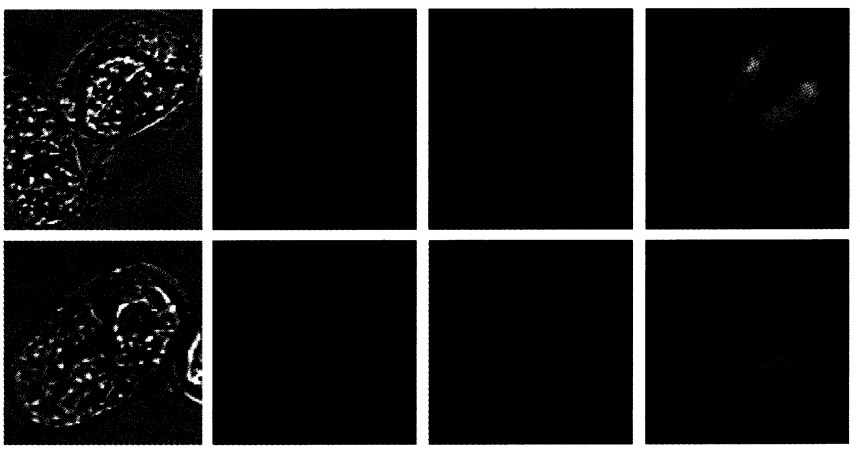
The researchers did not realize at first, however, that there is more to drFP583 than just a new red-colored tag. In fact, recent work described in the 24 October issue of the *Proceedings of the National Academy of Sciences* by Roger Tsien's group at the University of California, San Diego, and also 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