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NEW TOOL HIGHLIGHTS ACTIVITY OF KEY CELLULAR SIGNAL

Scientists at Johns Hopkins and the University of Texas Medical Branch have created a new tool that easily reveals when and where a key cellular signal is active. The development, described in the early edition of the *Proceedings of the National Academy of Sciences*, should speed identification of the signal's triggers and effects in normal processes and in conditions such as asthma, allergy, inflammation, lung disease and heart disease.

The tool -- a special fluorescent protein -- probes the activity of cyclic AMP in living cells and represents biology's growing application of a fluorescent phenomenon to study the molecular changes that reveal cells' inner workings.

Much like a child might pass along a visitor's request to a grown-up, cyclic AMP carries messages from hormones or other molecules "knocking" at the cell's door to proteins inside the cell. But because cyclic AMP uses just a handful of proteins to pass on many messages, scientists have had a hard time figuring out how it can trigger the right cellular response to each one.

"Scientists suspected that timing and location of cyclic AMP activity was important, but there was no easy way to study cyclic AMP inside cells in real time and in real space," says Jin Zhang, Ph.D., senior author of the study and an assistant professor of pharmacology and molecular sciences and of neuroscience in Johns Hopkins' Institute for Basic Biomedical Sciences. "This new fluorescent protein can be directed to the nucleus or to other parts of the cell, so we can now follow cyclic AMP activity in real time and space."

The new fluorescent protein takes advantage of the fact that fluorescent molecules can "talk" to one another when they are close together, affecting the color of light emitted -- a phenomenon called fluorescent resonance energy transfer (FRET). If the distance between the fluorescent molecules changes, the color of light emitted may change as well.

In the early 1990s, biologists began harnessing this phenomenon to study molecular changes in cells. A team led by Roger Tsien, Ph.D., at the University of California San Diego created the first FRET probe of cyclic AMP activity by attaching small fluorescent molecules to the ends of a protein called PKA. Once activated by cyclic AMP, PKA breaks in two, separating its two fluorescent "caps."

The first all-protein fluorescent version of PKA was developed a few years later.

But the PKA-based probes, which Zhang used as a postdoctoral fellow at the University of California San Diego, were difficult to use because PKA is made of four parts. For a simpler and easier to use probe, Lisa DiPilato, now a second-year graduate student in pharmacology, added fluorescent caps -- one cyan, the other yellow -- to a one-piece protein called Epac that is also activated by cyclic AMP.

By using a special microscope to measure how the fluorescence of the probe changed in response to cyclic AMP, DiPilato proved the probe's ability. Addition of a genetic "address label" then allowed her to direct the fluorescent probe to go to particular places in cells -- keeping it at the cell membrane, sending it to the nucleus, or directing it to the cell's power plant, the mitochondria.

"The probe has already provided new information," says Zhang, who arrived at Hopkins in September 2003. "The probe itself shows that Epac changes shape when it's activated, which had not been directly observed. And while some scientists had suggested cyclic AMP could get into the mitochondria, our studies are the first to show that it's there."

Stimulating cyclic AMP production in a human embryonic kidney cell line containing the targeted fluorescent Epac proteins caused an immediate build-up of cyclic AMP and activation of Epac at the cell membrane, where cyclic AMP is produced, the researchers report. Within seconds, build-up of cyclic AMP also began in the cytoplasm of the cell, the nucleus and the mitochondria.

Because cyclic AMP does so many things, understanding how it works has broad implications, says Zhang. For example, the main enzyme that degrades cyclic AMP is being targeted by drug companies for potential application in inflammation, allergy, asthma or chronic lung disease, conditions that may be helped by increasing cyclic AMP. More recently, altered regulation of cyclic AMP has been tied to the risk of stroke occurrence and to heart conditions including dilated cardiomyopathy.

Zhang says her lab will use the new and other fluorescent probes to study the role of cyclic AMP in smooth muscle cells, such as those in blood vessels, in inflammation, and in fat cells, where it may be involved in diabetes-related processes.

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