New powerful tool measures metabolites in living cells

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By engineering cells to express a modified RNA called "Spinach," researchers have imaged small-molecule metabolites in living cells and observed how their levels change over time. Metabolites are the products of individual cell metabolism. The ability to measure their rate of production could be used to recognize a cell gone metabolically awry, as in cancer, or identify the drug that can restore the cell's metabolites to normal.

Researchers at Weill Cornell Medical College say the advance, described in the March 9 issue of *Science*, has the potential to revolutionize the understanding of the metabolome, the thousands of metabolites that provide chemical fingerprints of dynamic activity within cells.

"The ability to see metabolites in action will offer us new and powerful clues into how they are altered in disease and help us find treatments that can restore their levels to normal," says Dr. Samie R. Jaffrey, an associate professor of pharmacology at Weill Cornell Medical College. Dr. Jaffrey led the study, which included three other Weill Cornell investigators.

"Metabolite levels in cells control so many aspects of their function, and because of this, they provide a powerful snapshot of what is going on inside a cell at a particular time," he says.

For example, biologists know that metabolism in cancer cells is abnormal; these cells alter their use of glucose for energy and produce unique breakdown products such as lactic acid, thus producing a distinct metabolic profile. "The ability to see these metabolic abnormalities can tell you how the cancer might develop," Dr. Jaffrey says. "But up until now, measuring metabolites has been very difficult in living cells."

In the Science study, Dr. Jaffrey and his team demonstrated that specific RNA sequences can be used to sense levels of metabolites in cells. These RNAs are based on the Spinach RNA, which emits a greenish glow in cells. Dr. Jaffrey's team modified Spinach RNAs so they are turned off until they encounter the metabolite they are specifically designed to bind to, causing the fluorescence of Spinach to be switched on. They designed RNA sequences to trace the levels of five different metabolites in cells, including ADP, the product of ATP, the cell's energy molecule, and SAM (S-Adenosyl methionine), which is involved in methylation that regulates gene activity. "Before this, no one has been able to watch how the levels of these metabolites change in real time in cells," he says.

Delivering the RNA sensors into living cells allows researchers to measure levels of a target metabolite in a single cell as it changes over time. "You could see how these levels change dynamically in response to signaling pathways or genetic changes. And you can screen drugs that normalize those genetic abnormalities," Dr. Jaffrey says. "A major goal is to identify drugs that normalize cellular metabolism."

This strategy overcomes drawbacks of the prevailing method of sensing molecules in living cells using green fluorescent protein (GFP). GFP and other proteins can be used to sense metabolites if they are fused to naturally occurring proteins that bind the metabolite. In some cases, metabolite binding can twist the proteins in a way that affects their fluorescence. However, for most metabolites, there are no proteins available that can be fused to GFP to make sensors.

By using RNAs as metabolite sensors, this problem is overcome. "The amazing thing about RNA is that you can make RNA sequences that bind to essentially any small molecule you want. They can be made in a couple of weeks," Dr. Jaffrey says. These artificial sequences are then fused to Spinach and expressed as a single strand of RNA in cells.

"This approach would potentially allow you to take any small molecule metabolite you want to study and see it inside cells," Dr. Jaffrey says. He and his colleagues have expanded the technology to detect proteins and other molecules inside living cells.

He adds that uses of the technology to understand human biology can be applied to many diseases. "We are very interested in seeing how metabolic changes within brain neurons contribute to developmental disorders such as autism," Dr. Jaffrey says. "There are a lot of opportunities, as far as this new tool is concerned."

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