Sugar metabolism tracked in living plant tissues, in real time

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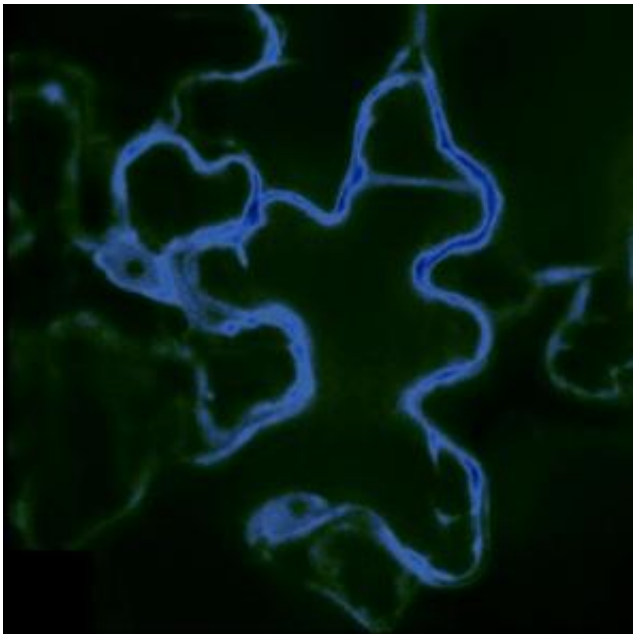

This false-color image shows a cell from the epidermis of an Arabidopsis thaliana plant; the cell has been marked with fluorescent imaging sensors designed to detect the sugar glucose. In this image, only the densely packed interior of the cell in which most metabolic functions occur -- called the cytosol -- is targeted by the glucose sensors. The dark area sits inside the vacuole -- a large storage organelle that can occupy up to 90 percent of the cell's volume. Credit: Image courtesy Sylvie Lalonde and Wolf Frommer

Scientists at Carnegie's Department of Plant Biology have made the first real-time observations of sugars in the cells of intact and living plant

tissues. With the help of groundbreaking imaging techniques, the group has determined that plants maintain extremely low levels of sugar in their roots--as much as 100,000 times lower than previous estimates. The new technology will enable new studies of sugar metabolism in plants, which will inform the effort to engineer higher crop yields for food and biofuel production.

Led by Carnegie staff member Wolf Frommer, the researchers designed genetically-encoded fluorescent tags to monitor glucose, an important sugar, in leaf and root tissues of the model plant Arabidopsis thaliana. The technique has allowed the researchers to track glucose over time and space at unprecedented detail, in living and undisturbed plant tissues. The work appears in the September issue of the journal Plant Cell. The group has also developed a FRET sensor for sucrose, a major transport sugar in plants. This work will appear in the September issue of the *Journal of Biological Chemistry*.

"Until now, we have had few clues regarding how much sugar is in an individual cell in a multicellular plant," Frommer said. "We normally grind up a leaf or a root and average the information for all cells, but if sugar levels rise in one cell and drop in another, we would see no change in this average." Also, because the cell can distribute sugar among subcellular organelles, it is nearly impossible to know how much sugar is in any cell compartment at a given time.

"Time resolution is another problem," Frommer added. "We can sample tissue at intervals, but if the sugar changes in waves, we might miss the right time point. Our new technology addresses all of these problems by measuring sugar flux in real time in individual cells, with subcellular resolution."

Frommer and his colleagues have used similar imaging tags, called fluorescent resonance energy transfer (FRET) sensors, to track sugars

and neurotransmitters in animal cells. Most recently, the group used FRET sensors to study glutamate, an important mammalian neurotransmitter. Frommer has tracked glucose in cultured mammalian cells, but until now, plant tissues had proven problematic because of interference from the plants' virus defense mechanisms, as well as high background fluorescence in some plants.

To surmount these issues, Frommer's team dramatically improved the sensors, while inserting them in mutant Arabidopsis plants with disabled defense genes. The fluorescent tags worked well where they had failed before.

"It may not be ideal to use defense-mutant plants--the ideal would be for the sensors to work in any wild-type genetic background," Frommer explained. "But proving that the sensors can work in plants is an important first step. Now we can begin addressing important questions about the way plants manage sugar distribution while we continue to improve the sensors."

In preliminary experiments, Frommer's group compared fluctuations in glucose levels in root tissue and leaf epidermis--the topmost layer that absorbs sunlight--and found that the plant maintained glucose at higher levels in leaf tissue than in roots. In fact, the researchers found that root cells contain sugar at concentrations at least 100,000 times lower than previous estimates.

FRET sensors are encoded by genes that, in theory, can be engineered into any cell line or organism. They are made of two fluorescent proteins that produce different colors of light--one cyan and one yellow--connected by a third protein that resembles a hinged clam shell. The two fluorescent proteins are derived from jellyfish, and the third from a bacterium; the shape of the clam shell protein determines which sugar or other molecule the sensor can detect. When a target molecule

such as glucose or sucrose binds to the third protein, the hinge opens, changing the distance and orientation of the fluorescent proteins. This physical change affects the energy transfer between the cyan and yellow markers.

When the researchers hit the tags with light of a specific wavelength, the cyan tag starts to fluoresce. If the yellow tag is close enough, the cyan tag will transfer its energy to the yellow tag, causing it to resonate and fluoresce as well. This energy transfer affects how much cyan and yellow fluorescence can be seen, and by calculating this ratio, researchers can accurately track molecules such as glucose and sucrose in both time and space.

"The strength of this technology lies in its elegant simplicity; with the power of computational design, we can potentially design FRET tags to detect virtually any small molecule in living cells," Frommer said. "Imaging techniques like this are the next frontier in the study of metabolism, and will help to answer some of the most pressing questions on plant biologists' minds, such as the role of individual genes in the distribution of sugars. This in turn can help us engineer plants to produce more biomass."

Source: Carnegie Institution