

## **Plant toughness: Key to cracking biofuels?**

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Along with photosynthesis, the plant cell wall is one of the features that most set plants apart from animals. A structural molecule called cellulose is necessary for the manufacture of these walls. Cellulose is synthesized in a semi-crystalline state that is essential for its function in the cell wall function, but the mechanisms controlling its crystallinity are poorly understood. New research from a team including current and former Carnegie scientists David Ehrhardt (Carnegie), Ryan Gutierrez (Carnegie), Chris Somerville (U.C. Berkeley), Seth Debolt (U. Kentucky), Dario Bonetta (U. Ontario) and Jose Estevez (U. de Buenos Aires) reveals key information about this process, as well as a means to reduce cellulose crystallinity, which is a key stumbling block in biofuels development. Their work is published online by *Proceedings of the National Academy of Sciences* for the week of February 20-24.

A plant's <u>cell wall</u> serves several essential functions including mechanical support: Allowing the plant to withstand the onslaughts of wind and weather, and permitting it to grow to great heights-- hundreds of feet for trees like the giant Redwood--and providing an essential barrier against invading pathogens. The cell wall is also the source of materials that have long been utilized by humans, including wood and cotton, in addition to serving as a potential source of biofuel energy.

Cellulose is the primary constituent of the cell wall and as such is the most abundant <u>biopolymer</u> on the planet. It is also the key molecule providing the cell wall its essential mechanical properties.

To address the question of its manufacture in plant cells, the research



team, led by Seth DeBolt of the University of Kentucky, focused on different aspects of cellulose-synthesizing complexes.

Working in conjunction with Chris Somerville, Ehrhardt developed a method for observing this complex by tagging it with a fluorescent marker derived from jellyfish and imaging the tagged protein using a technique called spinning disk confocal microscopy. This technique allows individual biosynthetic complexes to be seen and studied in living cells, producing an unusually high level of resolution.

Dario Bonetta of the University of Ontario Institute of Technology, Debolt, Somerville and Ehrhardt all participated in screening a large number of small molecules to determine which ones interfere with cell wall building. Those that interfered were then examined at the cellular level—using the fluorescent marker—in order to see how they affect the cellulose-synthetic complexes.

Once interesting candidates were identified, a search was undertaken to look for mutant plants that showed reduced responses to these molecules. It was assumed that, because these plants were either unaffected or differently affected by these molecules, then they would have <u>plant cell</u> walls that are compromised or in some way unusual.

Using this process of elimination, two mutations, called CESA1 and CESA3, were found in the genes that encode certain cellulose synthase proteins and these mutated genes were further studied. Both of these mutations are predicted to be found in the part of these proteins that cross the plant cell's membrane, which forms just inside the cell wall.

Other members of the team analyzed the cellulose manufactured by plant cells that had these mutations and found defects in the structure of cellulose that these altered proteins produced.



Normally, the individual sugar chains that make up cellulose bond to each other to make a semi-crystalline fiber. This crystalline structure gives cellulose its essential <u>mechanical properties</u>, such as rigidity and tensile strength. This structure is also is responsible for cellulose's resistance to digestion, which provides a key barrier to utilizing cellulose as a source to produce liquid fuel.

The mutant CESAs, 1 and 3, produced cellulose with lower <u>crystallinity</u>. This cellulose was also more easily digested, a process that is needed to liberate sugars from cellulose so they can be converted to useful fuels.

"The team made a connection between the structure of the proteins that produce cellulose, and the structure of their product," Ehrhardt said. "This is a first step in understanding how this important property of <u>cellulose</u> may be regulated, opening possibilities for development of useful biomaterials and for cellulosic biofuel crops."

Provided by Carnegie Institution

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