

SEX4, starch and phosphorylation

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Some of the new molecular mechanisms and regulatory components in starch metabolism have been identified by Dr. Samuel Zeeman and his colleagues. Dr. Zeeman, of the Institute of Plant Sciences, ETH Zurich, in Switzerland, who is the 2007 recipient of the Charles Albert Shull Award, will be presenting this work at the opening Awards Symposium of the annual meeting of the American Society of Plant Biologists in Mérida, Mexico (June 27, 2:30 PM). Mutational and structural analyses by Dr. Zeeman and his colleagues have revealed that starch degradation in *Arabidopsis* leaves at night differs significantly from the versions traditionally described in textbooks. Specifically, mutations at the Starch Excess 4 (SEX4), Maltose Excess 1 (MEX1) and other loci produce plants unable to metabolize starch to a usable form.

When we use starch in the lab or cook with it, we tend to think of it as an amorphous mass, but it is really a complex, ordered substance. Starch consists of two polysaccharides (polymers of the simple sugar glucose)--amylopectin and amylose. Both are long chains of connected glucose molecules, but amylopectin is also highly branched and forms a tree-like structure. The branches are then packed so that double helices can form between the chains, which are arranged into concentric layers forming semi-crystalline starch granules. This exquisite structure is extremely stable to enzyme activity and can thus be stored by the plant for later use. However, when needed, starch must be broken down to its component sugars for export to the rest of the plant. It appears that a number of proteins are major players —debranching enzymes, glucanotransferases and amylases, among others-- and that in leaves, their actions are finely tuned to the diurnal changes in photosynthesis

and the circadian rhythms of the plant.

Some of the new proteins that have been identified by Dr. Zeeman and other researchers in the field act as glucan kinases and phosphatases, that is, they place and remove phosphate groups on the starch molecules. Among these proteins are glucan water dikinase (GWD), phosphoglucan water dikinase (PWD) and SEX4. It is thought that GWD and PWD act in concert to place phosphate groups on starch molecules. The highly charged phosphate group may act as a wedge, disrupting the semi-crystalline packing in the starch and allowing degradative enzymes access to the glucose chains and branches. SEX4 then removes the phosphate groups. Although the exact mechanisms of how these proteins coordinate starch metabolism are still unknown, the importance of phosphate groups in the process is now well established. Mutants of all of these proteins result in plants with an excess of undigested starch.

Sequencing analyses have shown that GWD is conserved over many plant taxa, and proteins similar to SEX4 have been found in other plant species, including rice, maize, and tomato. The amylopectins of leaf starches in different plant species have also been found to be decorated with phosphate groups. Studies of Arabidopsis and potato leaves suggest a common mechanism for starch breakdown, although different pathways may operate in other plants. Further research is needed to establish conservation of the process as well as the proteins in the plant kingdom.

Elements of the newly-discovered mechanism of starch breakdown may also be conserved across kingdoms. Amylopectin has similarities to glycogen, the soluble storage carbohydrate accumulated in animals, fungi, and bacteria. The SEX4 phosphatase is related to laforin, a protein involved in animal glycogen metabolism. When laforin is missing, insoluble starch-like polyglucosans (Lafora bodies) accumulate, which results in neuronal dysfunction, severe epilepsy, and death. The

similarities between the animal and plant processes suggest common regulatory mechanisms, which may be the result of evolutionary convergence or conservation.

Understanding the molecular mechanisms of starch metabolism has direct implications for genetic engineering of plants for biofuels such as ethanol. It could also be important in adjusting the balance of protein and carbohydrate in plants needed to feed a growing global population.

Source: American Society of Plant Biologists

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