

## UNDERSTANDING STARCH METABOLISM IN PLANTS AND THE POTENTIAL TO IMPROVE STARCH CROPS

ZEEMAN S.C.

Department of Biology, ETH Zurich (Switzerland)

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Starch is our primary source of nutrition and a key renewable resource used by industry (e.g. as a feedstock for bioethanol production). It is composed of branched and linear glucans with an architecture that allows the formation of insoluble, semi-crystalline granules. Understanding the metabolism of starch in plants gives us options for starch crop improvement by altering starch structure and properties, and by increasing yields. Much progress has been made by studying starch metabolism in model species such as *Arabidopsis thaliana*. In *Arabidopsis*, as in most plants, starch is a primary product of photosynthesis in leaves, where it is temporarily stored in chloroplasts for use during the night. Functional genomic studies have advanced our understanding of both starch synthesis and breakdown.

Recent data are consistent with the idea that starch synthesis requires both synthetic enzymes (starch synthases and starch branching enzymes) and degradative enzymes (debranching enzymes). Together, these enzymes create the correct glucan structure to allow crystallization. A wealth of data from different systems shows that starch structure can be manipulated in a rational way by altering the complement of these enzymes. As a result, glucans with altered properties can be obtained (e.g. in the degree of crystallinity or solubility, the ease of hydrolysis to fermentable sugars), some of which may be better suited to industrial applications than wild-type starches.

Advances have also been made in understanding starch degradation. For example, glucan phosphorylation, mediated by glucan water dikinases (GWD and PWD), is required for normal degradation to occur. Phosphorylation disrupts the starch granule surface, rendering the glucans accessible for degrading enzymes. However, dephosphorylation, mediated by the chloroplastic phosphatase SEX4 (Starch EXcess4), is also required for starch degradation. Phosphorylated intermediates of starch breakdown accumulate in *sex4* mutants. This is because the phosphate groups, while necessary to disrupt the granule surface, can also obstruct enzymes of starch degradation such as beta-amylases. Experiments with starch granules *in vitro* show that the rate of degradation is increased by simultaneous phosphorylation and dephosphorylation, corroborating the hypothesis that reversible glucan phosphorylation and glucan hydrolysis are synergistic processes. Plants have two homologues of SEX4, LSF1 and LSF2 (Like Sex Four). With collaborating labs, we recently demonstrated that the loss of LSF1 also causes a starch-excess phenotype. However, the roles of LSF1 and SEX4 differ; phospho-oligosaccharides do not accumulate in *lsf1* and recombinant LSF1 protein has no phosphatase activity. These findings indicate additional complexity in the process of transient phosphorylation of the granule during starch degradation, which is the subject of ongoing research in our lab.