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REDOX-REGULATED BAM1 AND ITS ROLE IN DIURNAL STARCH DEGRADATION

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Possibly due to their sessile nature, plants show a huge metabolic plasticity, essential both for their normal growth and in response to environmental change. Post-translation modifications take part in plant metabolic plasticity, regulating the activity of metabolic enzymes. Protein phosphorylation and thiol/disulfide redox modulation are common modifications of plant enzymes and as such they are involved in the control of plant metabolism.

Starch is the major storage biopolymer synthesized by plants, and leaf starch is transiently accumulated in a daily cycle. Soluble carbohydrates resulting from starch degradation are mainly exported to sink tissues. Alternatively, starch-derived soluble sugars can also participate to osmotic adjustment under water stress or take part in the osmotic regulation of specialized cells, notably guard cells.

Here we report the characterization of BAM1 (At3g23920), a plastid-targeted beta-amylase of *Arabidopsis thaliana* specifically activated by reducing conditions. Under oxidizing conditions, the catalytic activity of BAM1 is close to zero. Among all major plastid thioredoxin isoforms, BAM1 was preferably activated by thioredoxin f1, followed by thioredoxins m1, m2, y1, y2, and m4. Alternative activation of BAM1 was also achieved by plastid-localized NADPH-thioredoxin reductase (NTRC), which allowed the recovery of about half of the BAM1 maximal activity.

In contrast with the timing of starch metabolism in mesophyll cells, redox regulation of BAM1 activity suggests that this enzyme would be mainly active in the light rather than in darkness. To elucidate this inconsistent behaviour, knockout (KO) mutants and promoter activity of BAM1 were analyzed.

Differently from KO mutants for BAM3 (At4g17090; the major chloroplast beta-amylase, insensitive to thiol/disulfide redox modulation) which show a reduced phenotype characterized by starch accumulation at night (sex phenotype), a T-DNA insertion line in which BAM1 expression was nil displays normal growth but reduced stomata opening and increased starch content in illuminated guard cells.

To study the promoter activity of BAM1, the reporter genes GUS and YFP were placed under the control of BAM1 promoter, and Arabidopsis transgenic plants were analyzed. In non-flowering plants, both YFP and GUS plants showed expression of BAM1 in leaves and roots, but expression in leaves was mainly restricted to guard cells, in agreement with the regulatory properties of the enzyme. Interestingly, BAM1 expression appeared in mesophyll cells of young plants in response to

osmotic stress. Total β-amylase activity also increased, together with its redox-sensitive fraction, in osmotically stressed wild type plants but not in KO mutants. Taken together these data suggest that thioredoxin-regulated BAM1 activates a starch degradation pathway in illuminated mesophyll cells upon osmotic stress, similar to the diurnal pathway of starch degradation in guard cells that is also dependent on thioredoxin-regulated BAM1.