# Metabolite damage and repair associated with bisphosphatase reactions of the Calvin-Benson cycle

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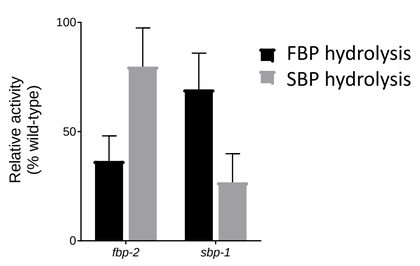
## Background

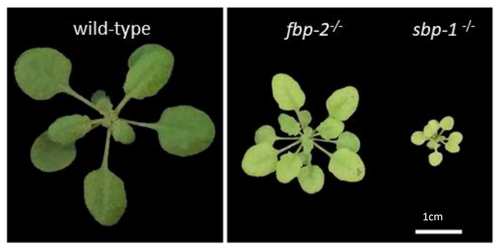
Fructose-1,6-bisphosphatase (FBPase) and Sedoheptulose-1,7-bisphosphatase (SBPase) are two enzymes involved in the regeneration of the $CO_2$ acceptor molecule in the Calvin-Benson cycle.




FBPase catalyzes the dephosphorylation of Fructose-1,6-bisphosphate (FBP) to Fructose-6-phosphate (F6P). SBPase catalyzes the dephosphorylation of Sedoheptulose-1,7-bisphosphate (SBP) to Sedoheptulose-7-phosphate (S7P).

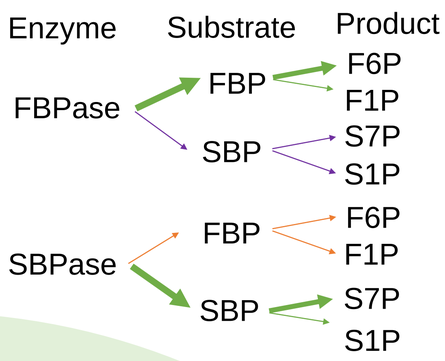
Knockout mutants of both FBPase and SBPase, respectively, are viable, but show reduced photosynthetic activity, leaf chlorosis and severe retardation in growth[1].

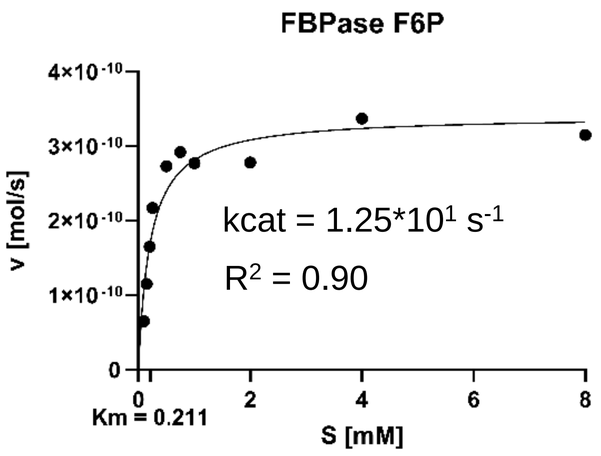
The FBPase mutant still has residual FBPase activity when spiked with FBP and recombinant SBPase was shown to have FBPase activity. The same was found for the SBPase mutant and recombinant FBPase. However use of the non-canonical substrate leads to production of the non-canonical metabolites Fructose-1-phosphate (F1P) and Sedoheptulose-1-phosphate (S1P). Both metabolites are also found at elevated levels in the respective mutants[1].

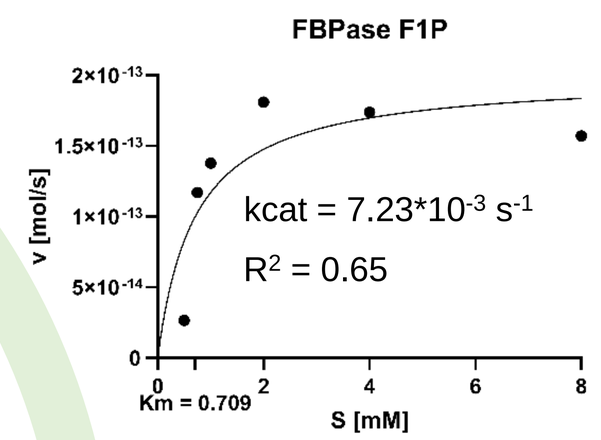



## Metabolite damage

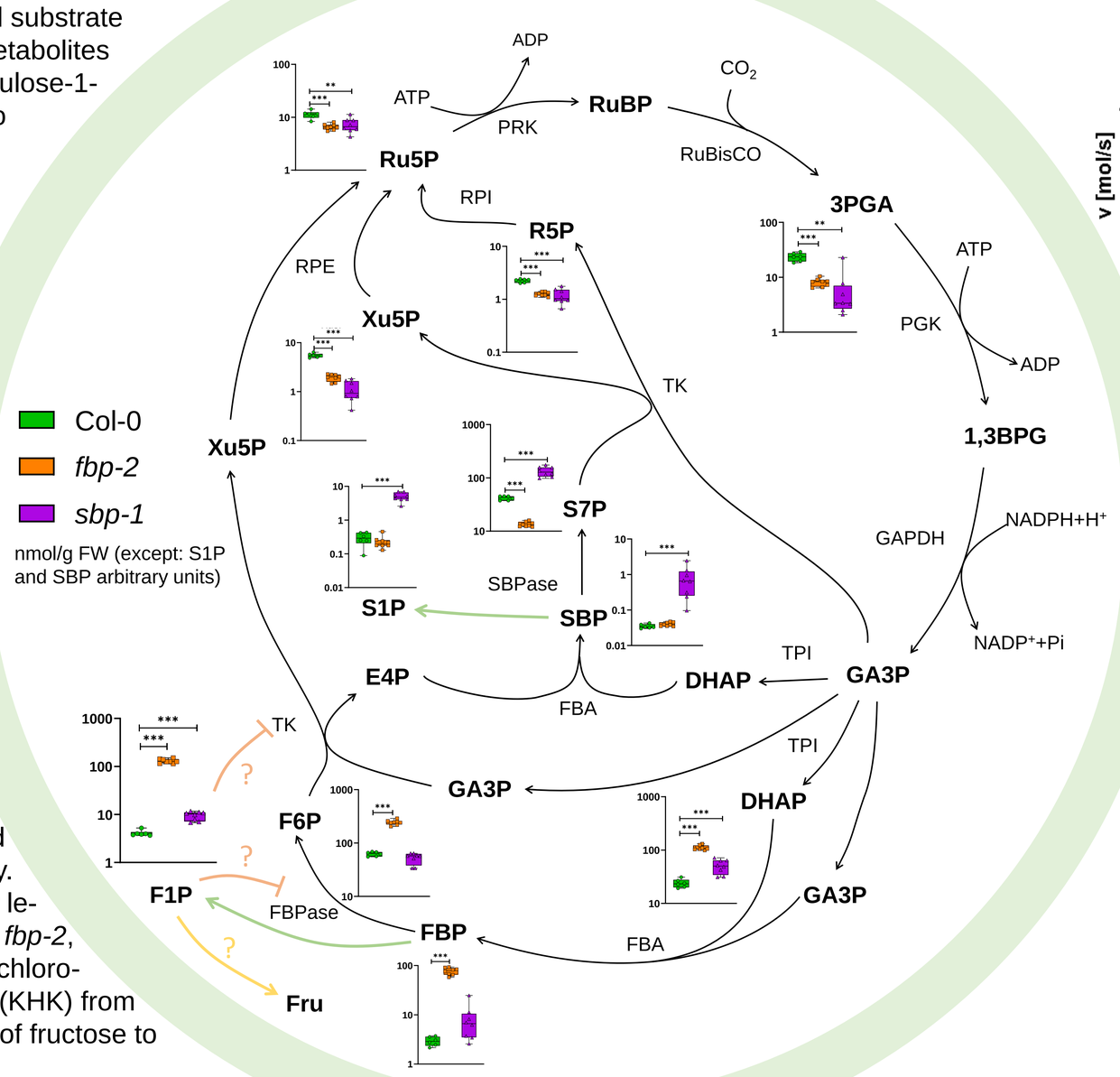
Assessement of the kinetic parameters of recombinant FBPase and SBPase on their canonical as well as non-canonical substrates will give us insight into the viability of the knockout mutants and the accumulation of the non-canonical products F1P and S1P.



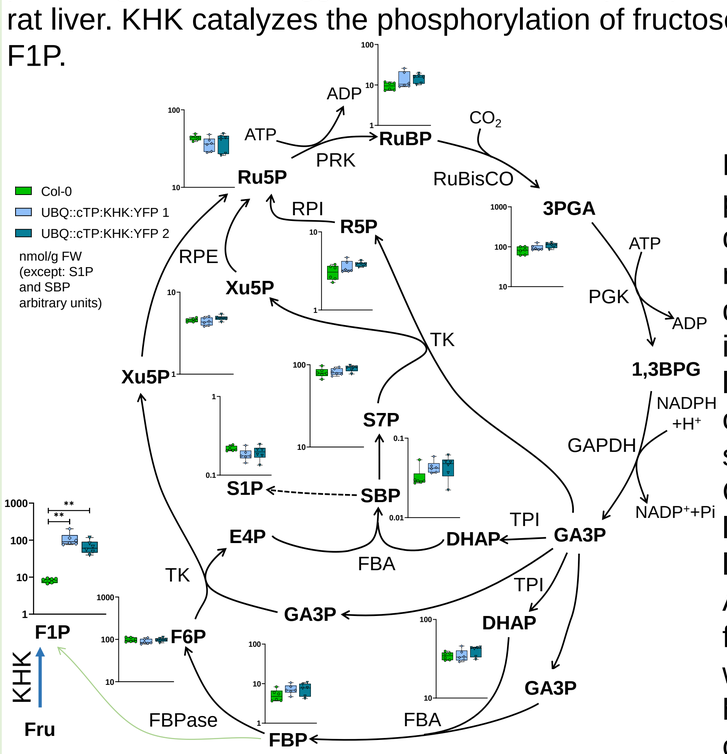




Recombinant FBPase preferentially catalyzes the production of F6P, but also generates F1P at low rates.



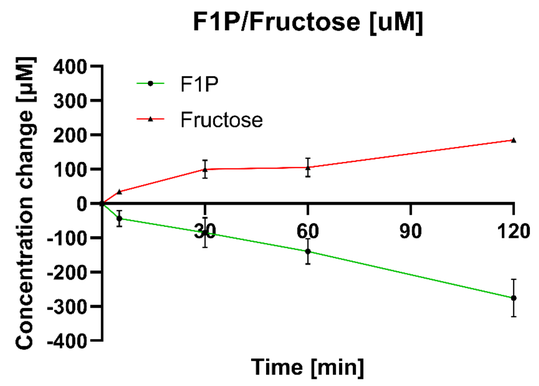

## Effect of F1P on metabolism

The metabolite profile of the *fbp-2* (high F1P, low pentose phosphates) and slightly reduced Transketolase (TK) activity of total soluble protein extracts spiked with F1P lead to the hypothesis that F1P inhibits TK activity. To create a plant with high chloroplastic F1P levels but without the metabolic impairment of *fbp-2*, wild type *A.thaliana* was transformed with a chloroplast targeted, YFP tagged Ketohexokinase (KHK) from rat liver. KHK catalyzes the phosphorylation of fructose to F1P.



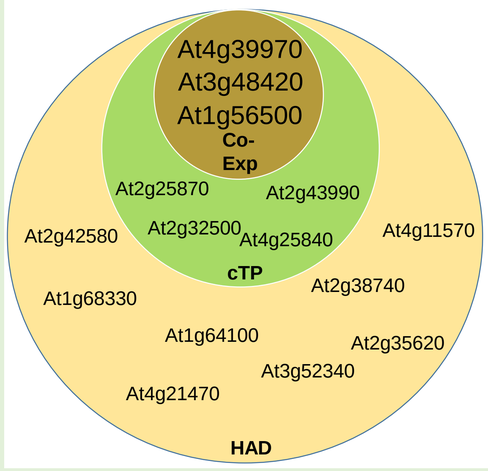

Expression of KHK in potato plants reportedly leads to strong developmental defects and a reduction of photosynthesis despite no detectable increase in F1P[3]. Although 10-fold higher levels of F1P were detected in our KHK transformed Arabidopsis plants, no changes in the Calvin-Benson cycle metabolome were measured in these lines compared to wild type. Also no apparent growth defects were observed. Therefore, we conclude that F1P does not have a strong negative effect on chloroplast metabolism.

## Metabolite damage repair




F1P → ? → Fructose

Stable-isotope labelled $CO_2$ incorporation measurements and measurements of F1P throughout the diel cycle in wild type suggest active turnover of F1P. Assaying wild type total soluble leaf protein extracts by LC-MS/MS and HPAEC-PAD upon addition of 1mM F1P indicate fructose as the breakdown product of F1P metabolism.




Three haloacid dehalogenase-like phosphatases (HADs) from *E.coli* have been reported to show high specificity for F1P as a substrate[2]. Using high similarity to the F1P specific *E.coli* HADs, localisation to the chloroplast and Co-Expression with FBPase as selection criteria three Arabidopsis proteins were identified as candidates for F1P metabolising enzymes. Assays using F1P as substrate in plant protein extracts of the respective knockout mutants will us allow to identify the F1P metabolsiing enzyme.

1. Zanella, Martina. (2018) Regulatory aspects and alternative carbon fluxes in the Calvin-Benson cycle of Arabidopsis. *Doctoral Thesis.* ETH Zürich
2. Kuznetsova, Ekaterina, et al. (2006) Genome-wide Analysis of Substrate Specificities of the *Escherichia coli* Haloacid Dehalogenase-like Phosphatase Family. *Journal of Biological Chemistry* 281(47)
3. Geigenberger, Peter, et al. (2004) Heterologous expression of a ketohexokinase in potato plants leads to inhibited rates of photosynthesis, severe growth retardation and abnormal leaf development. *Planta* 218(4)