Metabolic Phenotyping & Plant Performance

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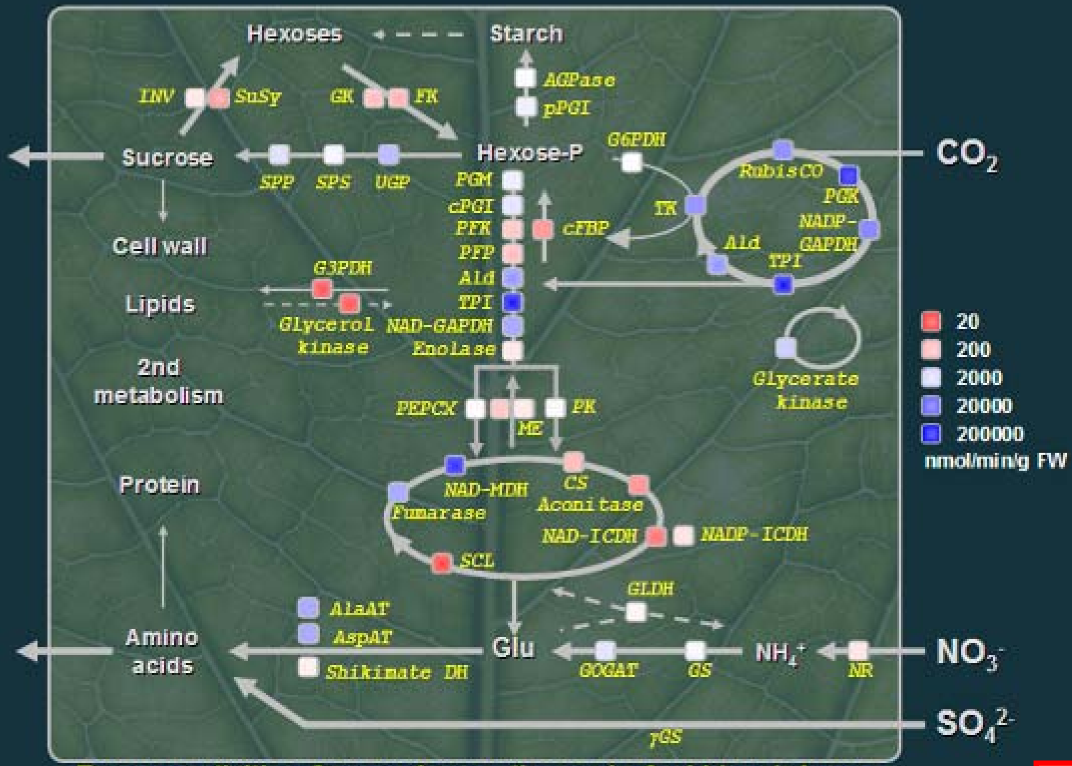
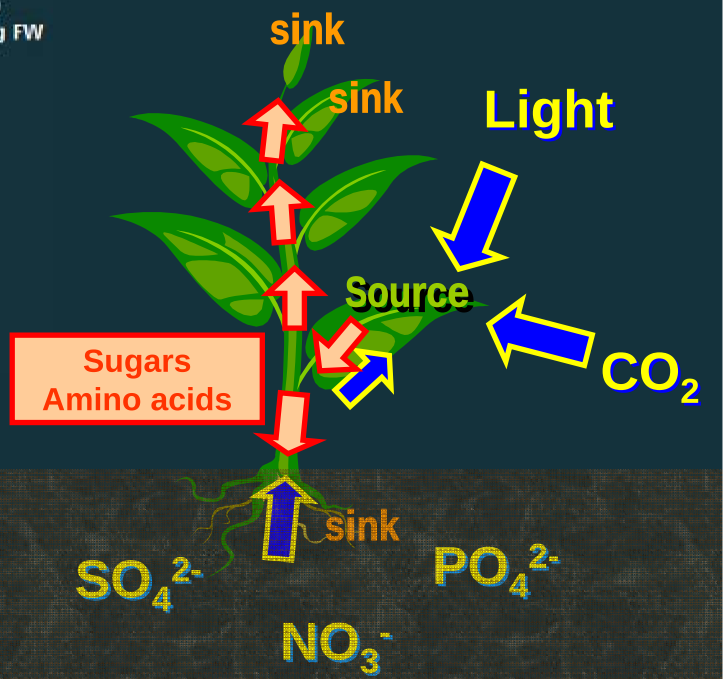
Objectives

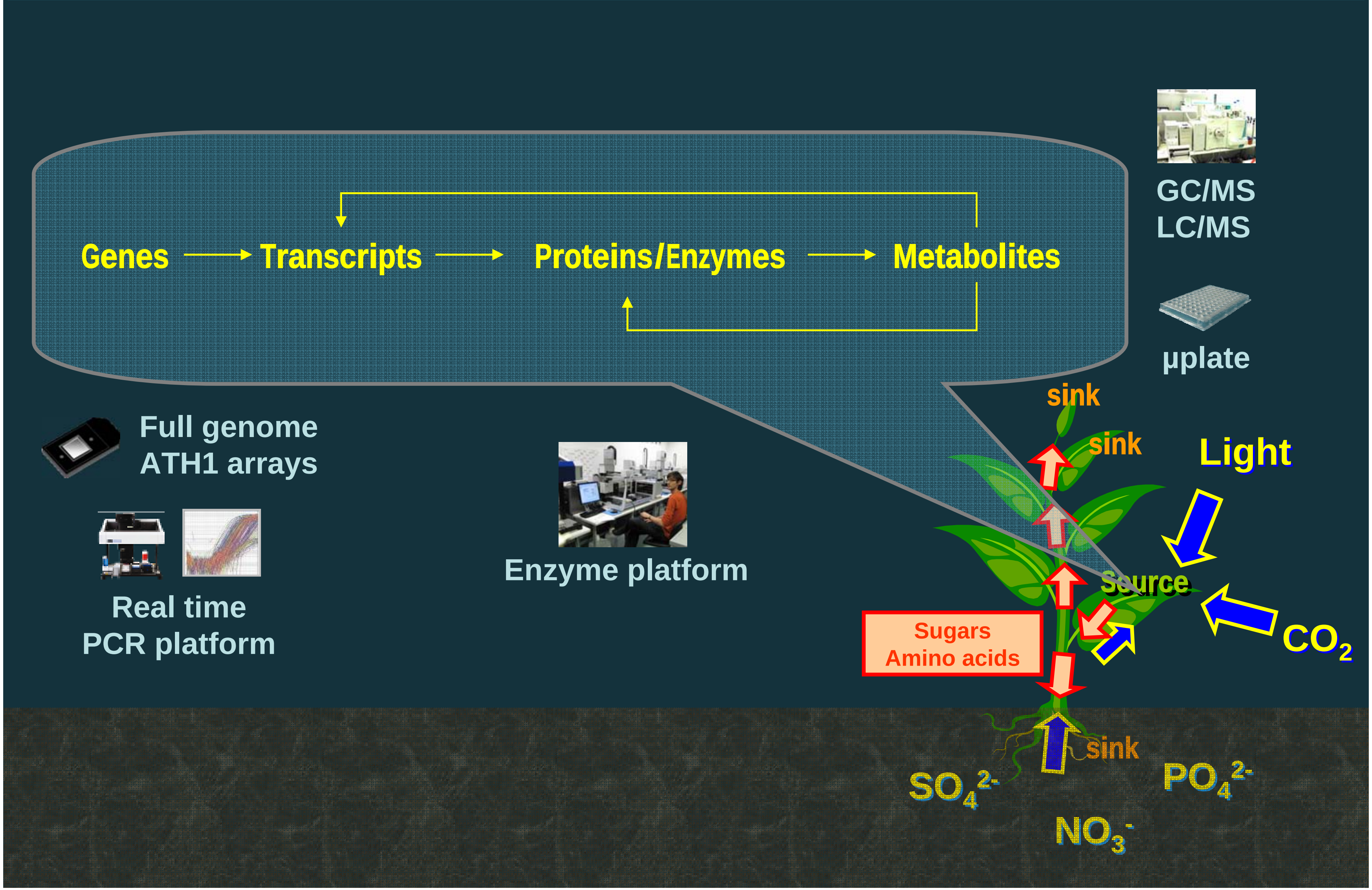


Enzyme activities from various pathways in *Arabidopsis* leaves

How is metabolism contributing to plant performance?

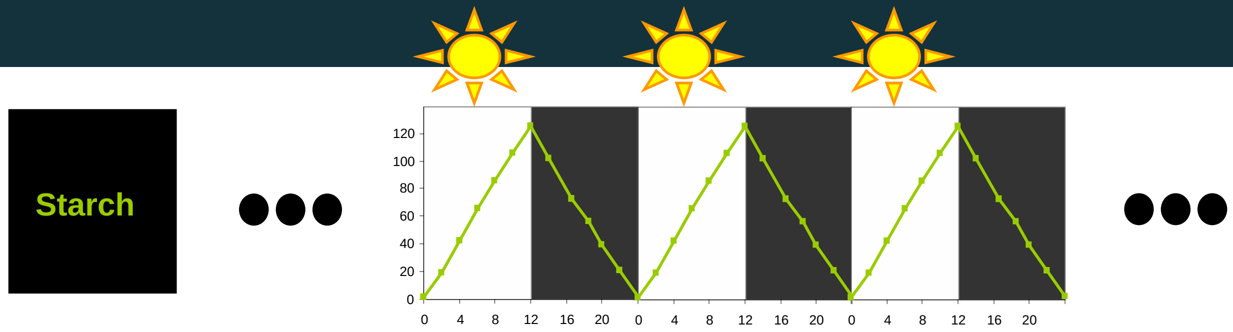


Objectives



Genes
Transcripts
Proteins/Enzymes
Metabolites
GC/MS
LC/MS
µplate
Full genome
ATH1 arrays
Real time
PCR platform
Enzyme platform
sink
sink
Light
Source
CO2
Sugars
Amino acids
sink
SO4 2-
PO4 2-
NO3 -

Every day plants experience massive changes in their environment

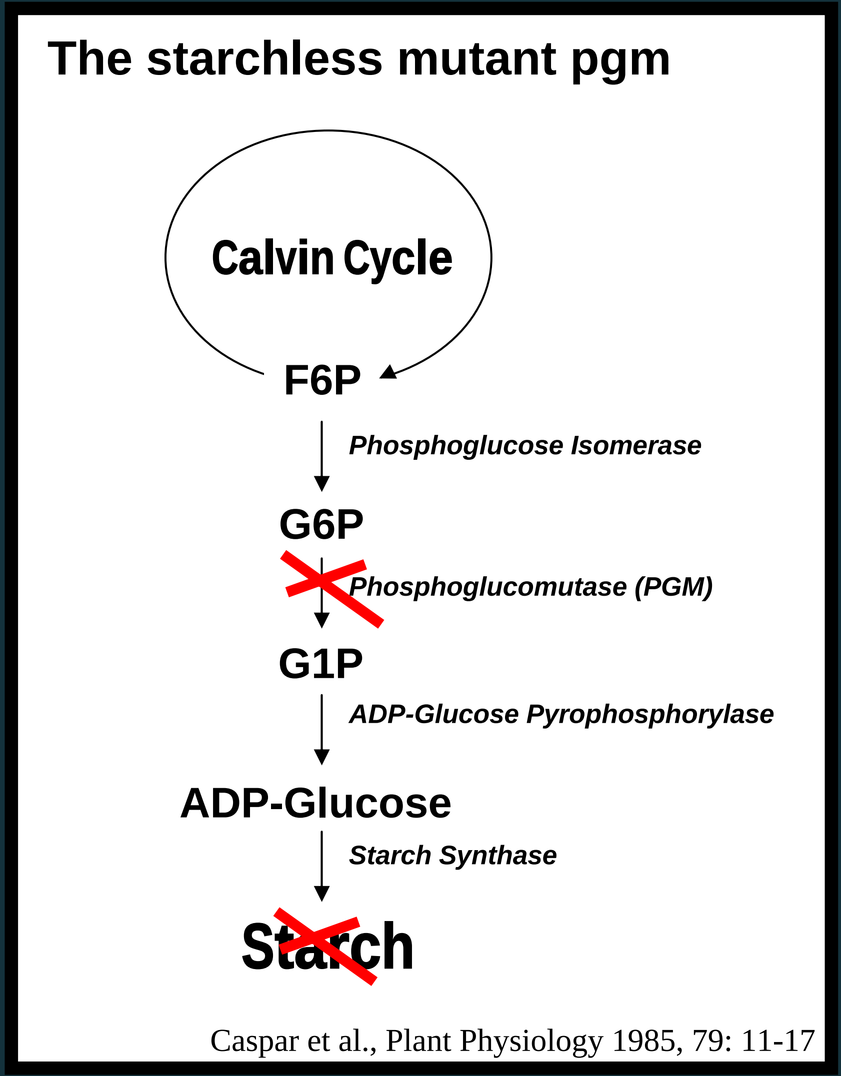


… even under controlled conditions, e.g.

… during the day, plants reduce C and during the night they become net consumers of reduced C…

… for this they evolved starch

Starch makes a difference



Starch makes a difference

6 weeks old plants after 3 weeks under different day length regimes

20 h light

16 h light

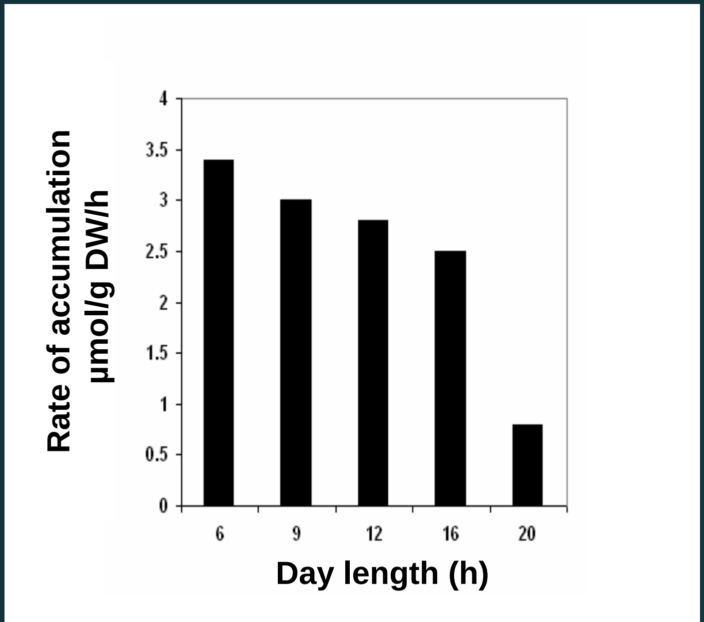
12 h light

9 h light

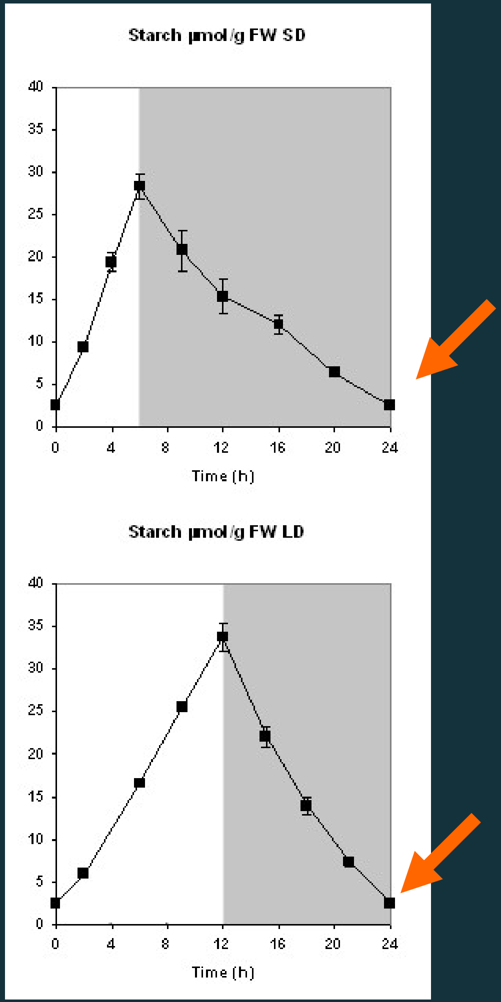
7 h light

5 h light

Transitory starch is adjusted to day length

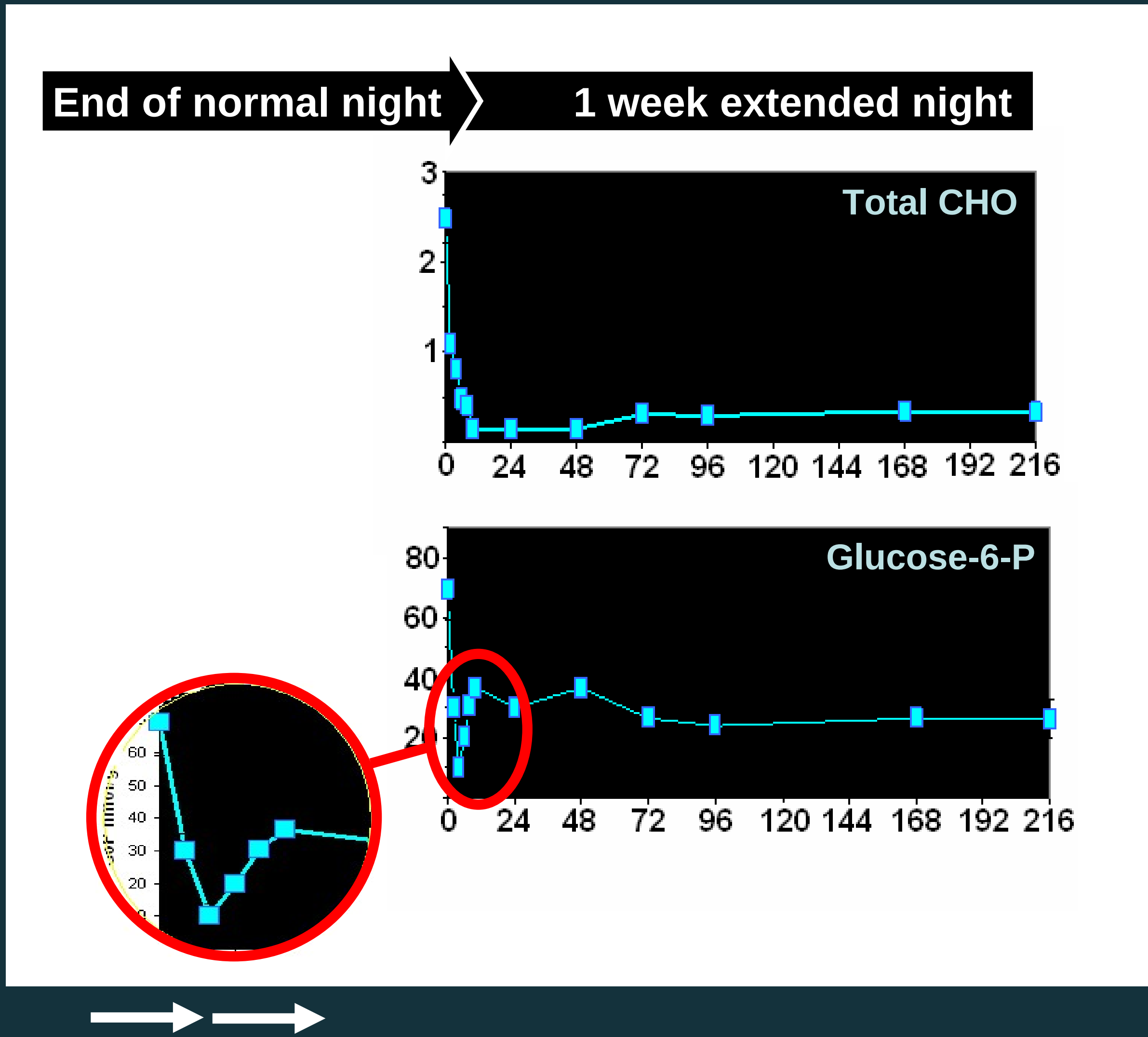


Rate of starch accumulation in the light



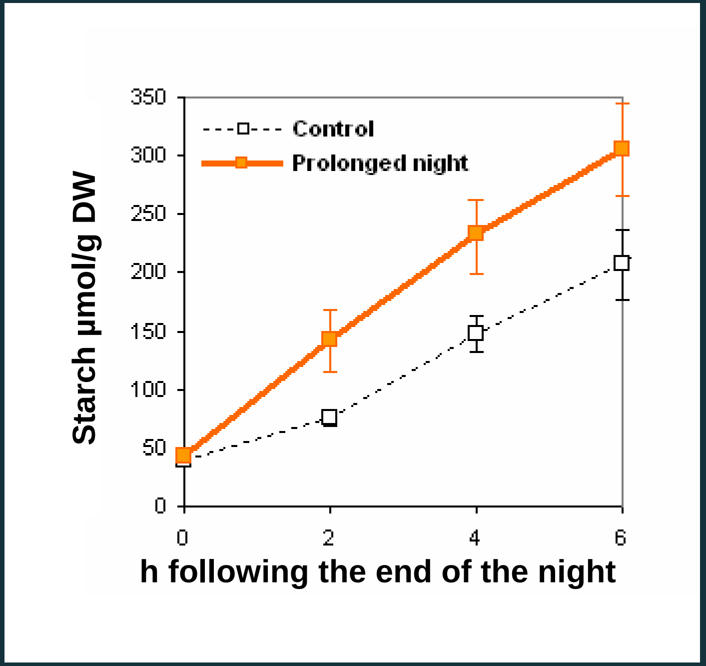
Diurnal changes in starch content in 6 / 18 and 12 / 12 day / night cycles

Plants walk a tightrope



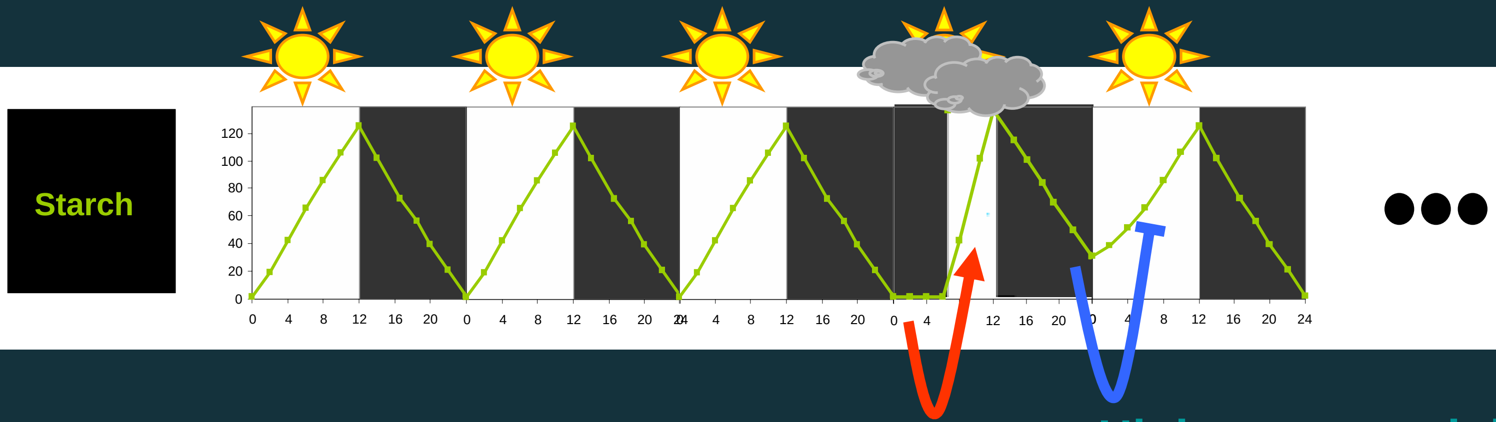
Glycolysis? Gluconeogenesis?

Low C at night promotes accumulation of starch in the light



One prolonged night is enough to enhance the rate of starch accumulation

How is metabolism reprogrammed in response to fluctuating C?



Low sugar at night stimulates starch synthesis

High sugar at night reduces starch synthesis

Investigate events during the diurnal cycle, when changes in starch metabolism are being triggered by changes in endogenous sugar levels

Carbohydrates undergo large diurnal changes

140 metabolites from various pathways

WT

Carbohydrates

Photorespiratory intermediates

Amino acids

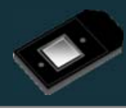
?

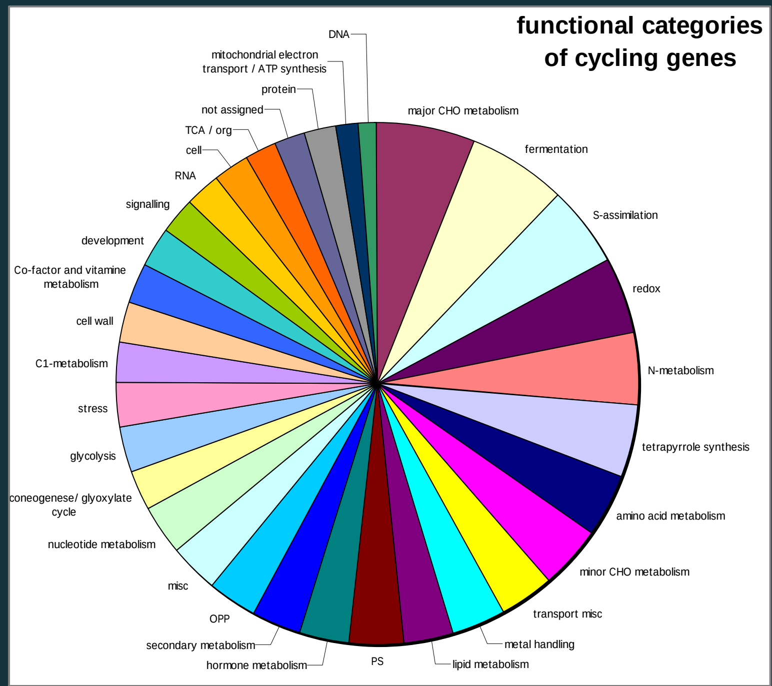
Low

High

Relative changes in metabolite levels throughout a night and day cycle

Full genome ATH1 arrays





30-50% of the expressed genes undergo a significant diurnal change

The starchless mutant pgm as a model system

140 metabolites from various pathways

WT

Carbohydrates

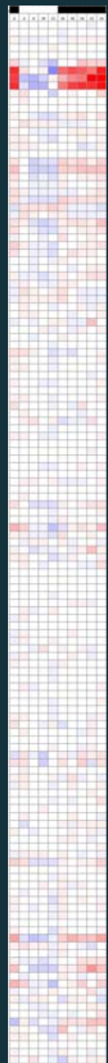
Photorespiratory intermediates

Amino acids

Low

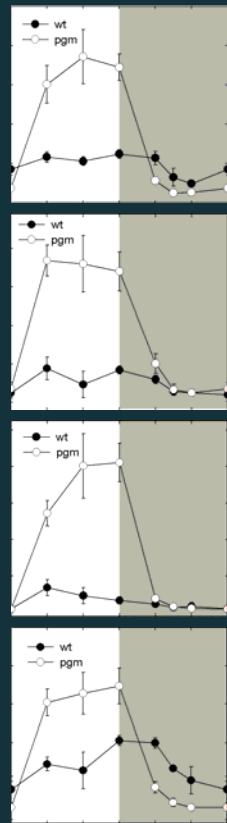
High

Relative changes in metabolite levels throughout a night and day cycle



pgm

Soluble CHO changes are amplified in the starchless mutant *pgm*
Very low at night
Very high during the day



Glucose-6P

Fructose

Glucose

Sucrose

Diurnal changes of sugar-regulated genes should be accentuated in pgm

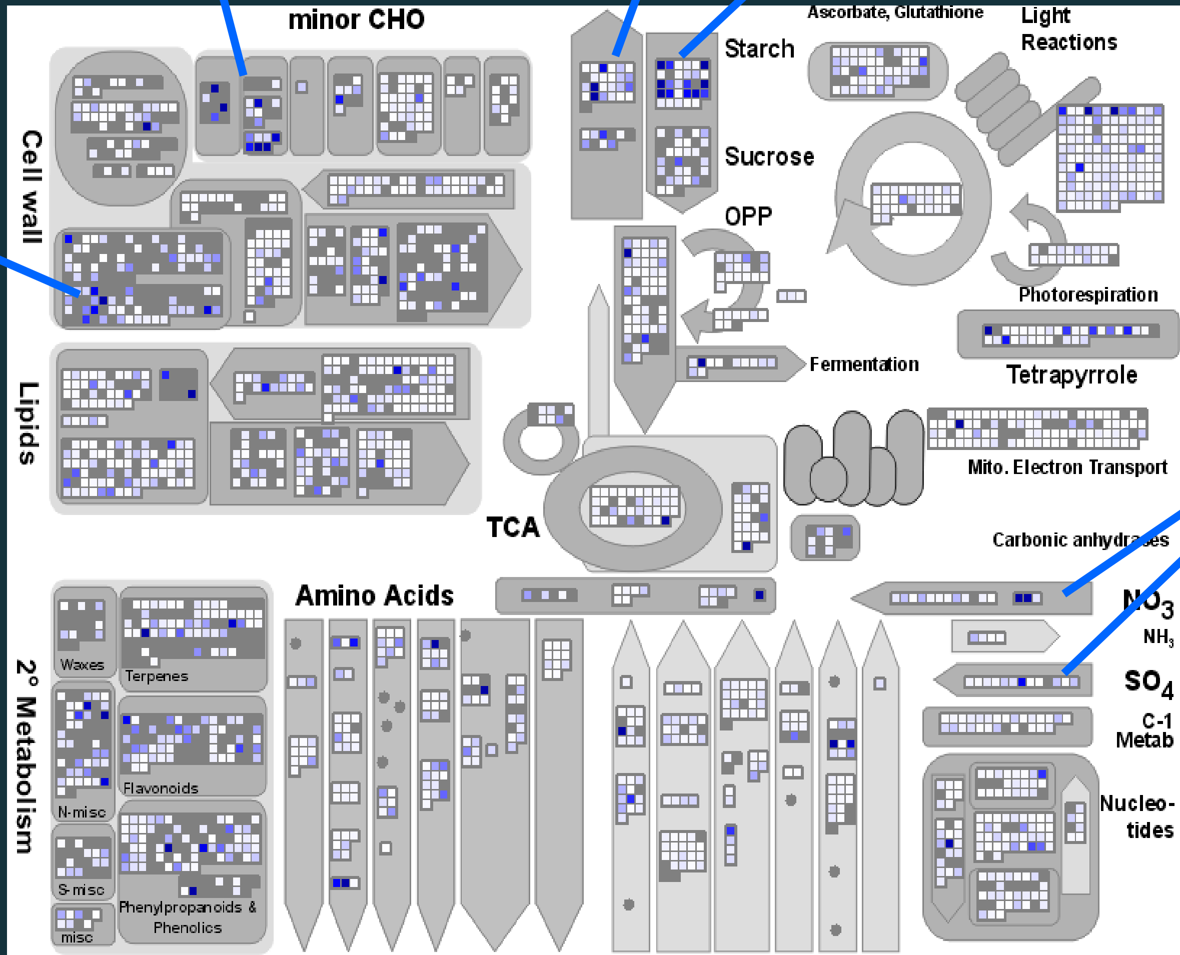
Amplitude of the diurnal changes in transcript levels

Trehalose metabolism

Starch synthesis

Starch degradation

Cell wall extension



Nitrate and sulphate reduction

WT

Genes assigned to metabolism

MapMan visualisation: increasingly large amplitude is shown as a increasingly intense blue colour

Amplitude of the diurnal changes in transcript levels

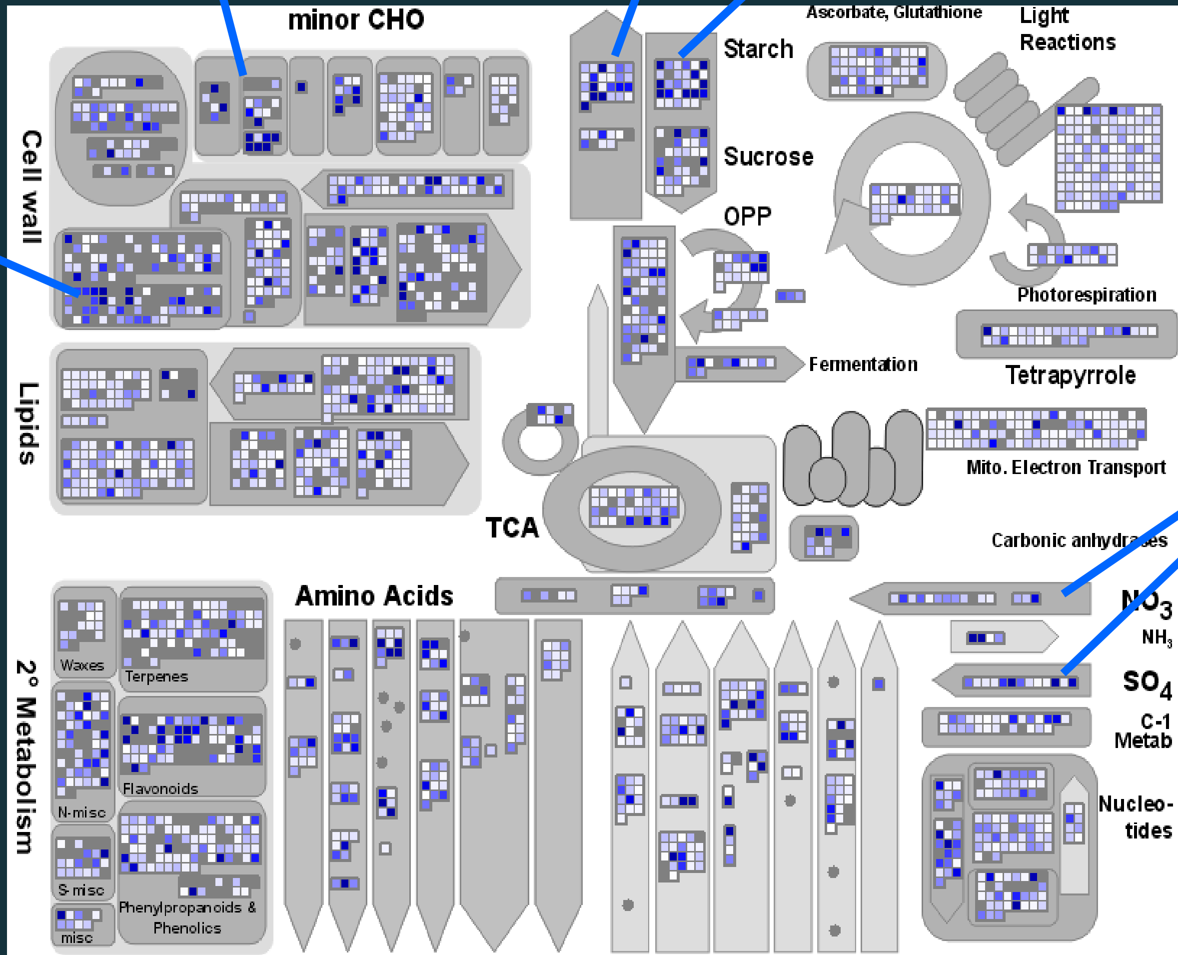
Trehalose metabolism

Starch synthesis

Starch degradation

Cell wall extension

Nitrate and sulphate reduction



pgm

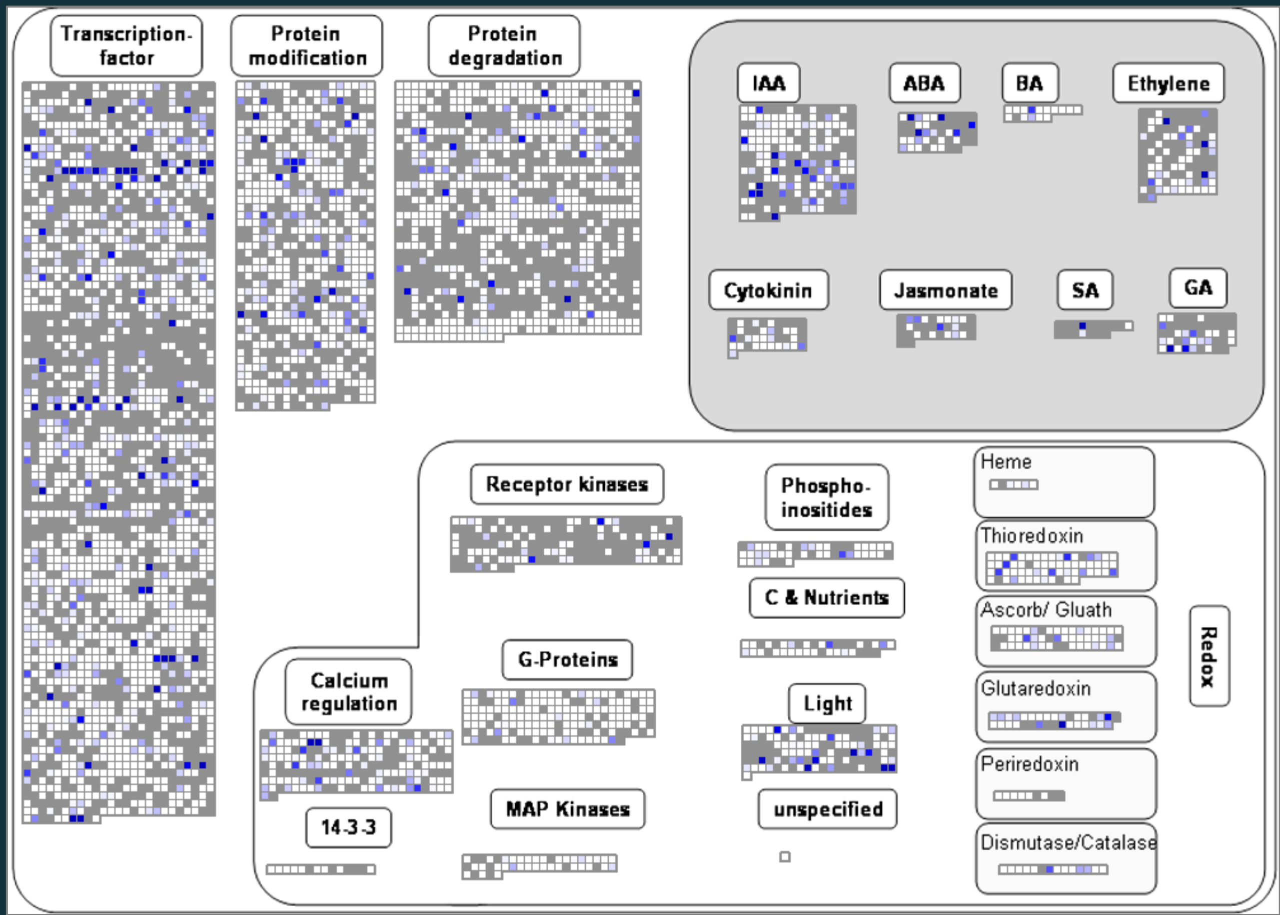
Genes assigned to metabolism

MapMan visualisation: increasingly large amplitude is shown as a increasingly intense blue colour

Amplitude of the diurnal changes in transcript levels

WT

Genes assigned to different aspects of regulation

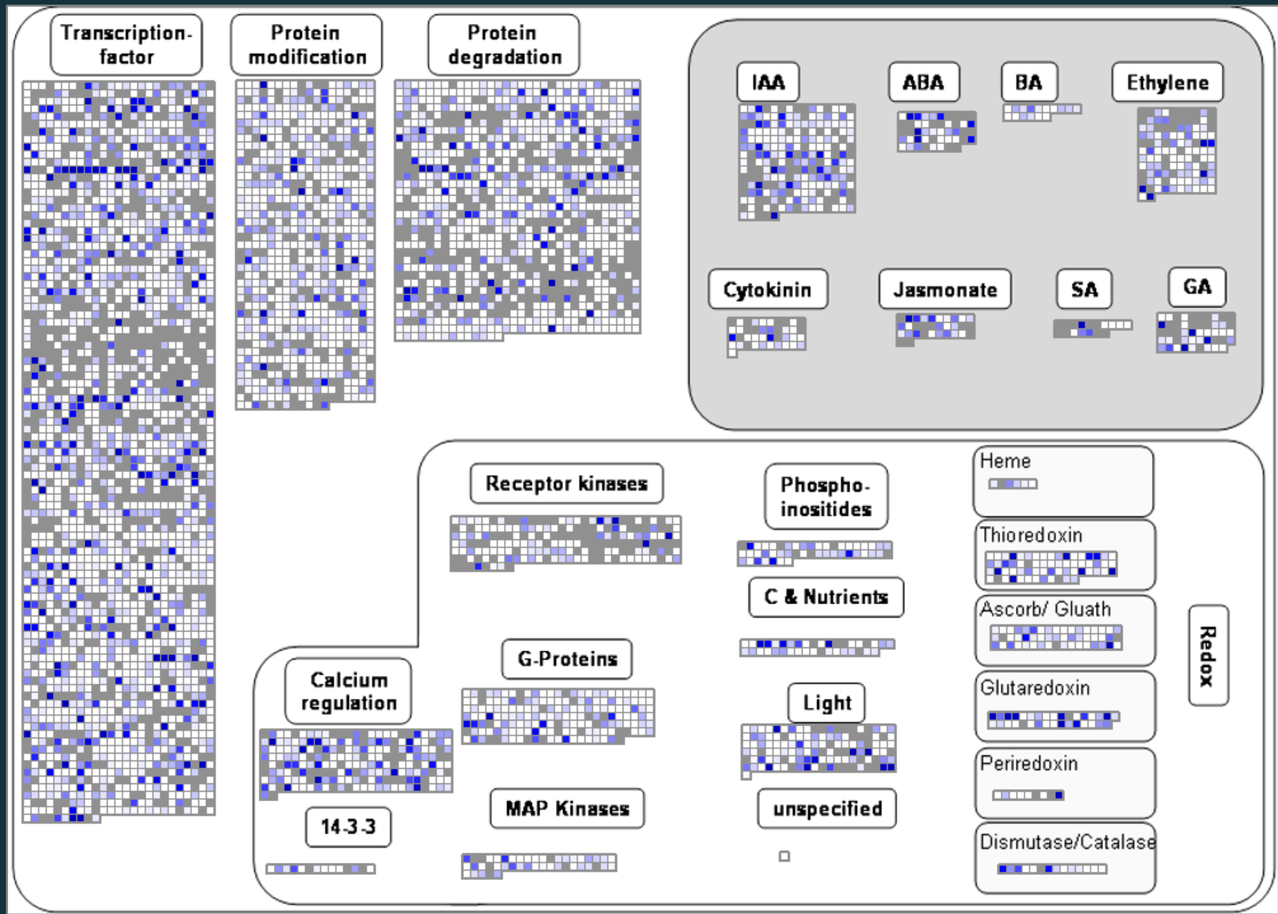


MapMan visualisation: increasingly large amplitude is shown as a increasingly intense blue colour

Amplitude of the diurnal changes in transcript levels

pgm

Genes assigned to different aspects of regulation



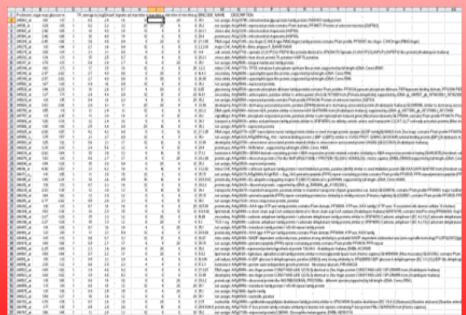
Over 4000 genes show 2-fold larger diurnal changes in pgm

MapMan visualisation: increasingly large amplitude is shown as a increasingly intense blue colour

A collection of expression arrays
to dissect multi-factorial responses

Water stress

Seedlings transferred to 100 mM Mannitol



Compare transcript profiles

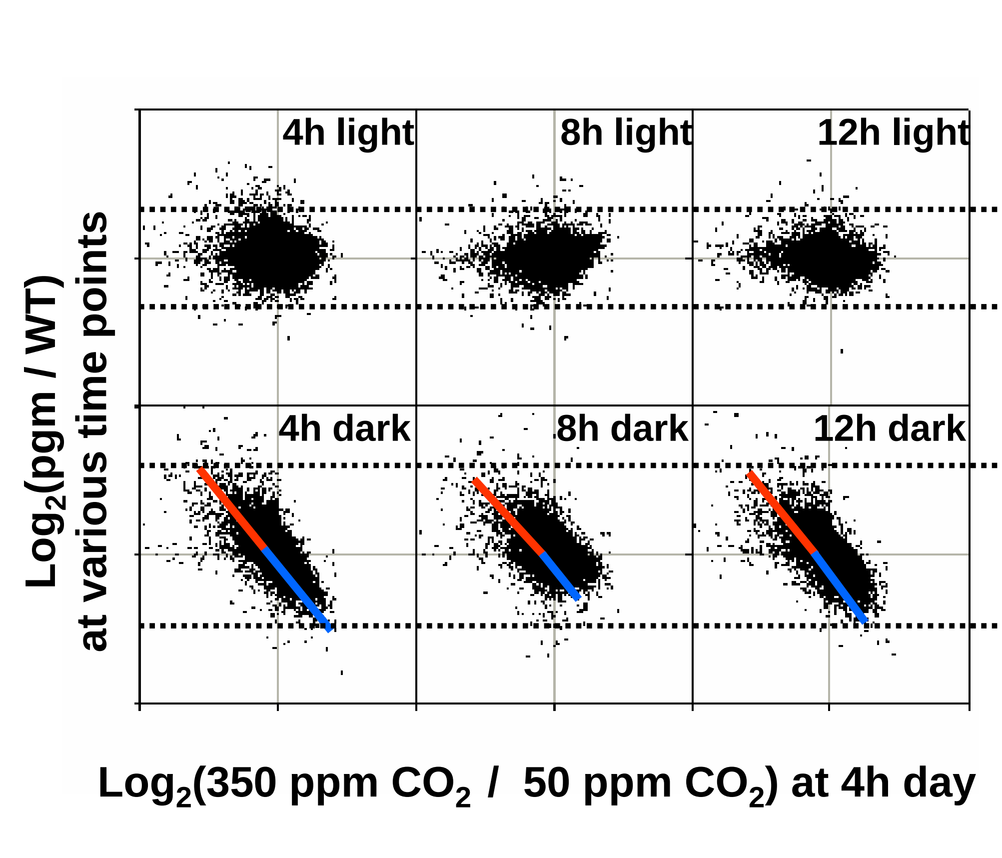
Evaluate the contribution to the total variation of sets of diagnostic genes selected for various inputs (sugars, nitrogen, light, circadian clock…)

- **200 most strongly induced genes**
- **200 most strongly repressed genes**

Low levels of sugar in the dark in pgm drive especially large changes of transcripts

Genes are induced in pgm that are repressed by sugars

Genes are repressed in pgm that are induced by sugars



Small changes during the day

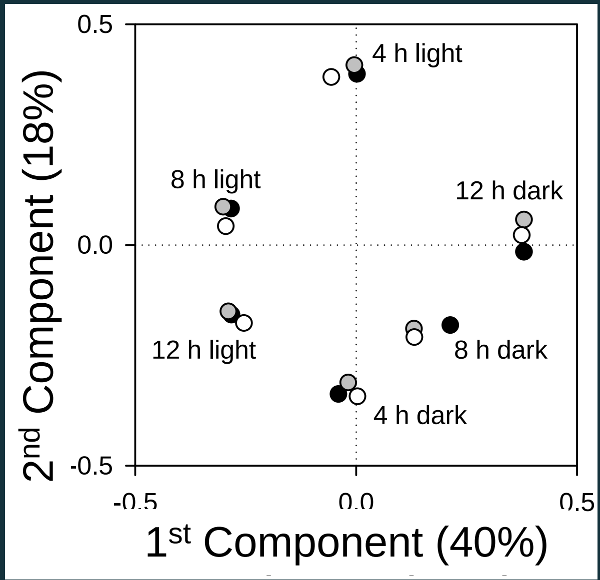
Large changes at night

Transcript for > 22,000 genes

Implies that **low sugar** is a larger and more general problem than **high sugar**

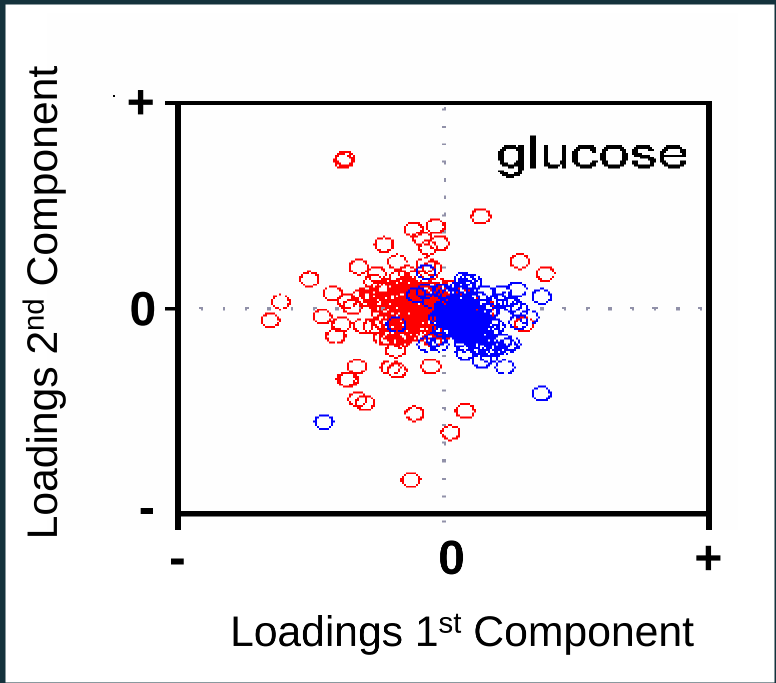
Most research on **sugar-sensing** in plants addresses high sugar responses

The contribution of sugars to the diurnal regulation of gene expression



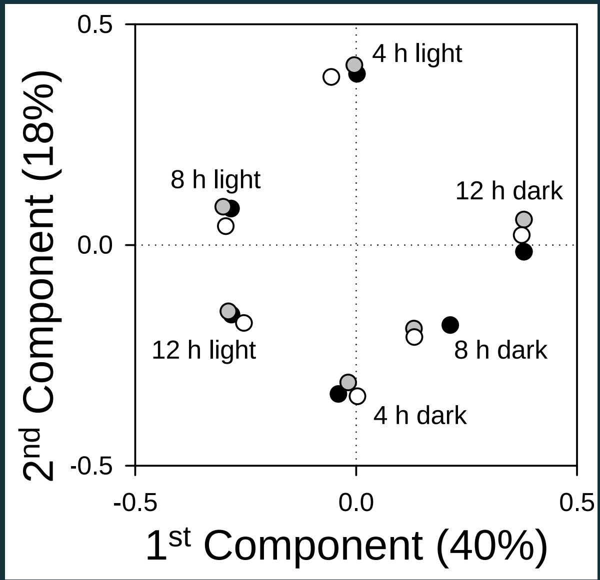
Principle component analysis of triplicate samples at 6 times during the diurnal cycle

Loadings in 1st and 2nd components



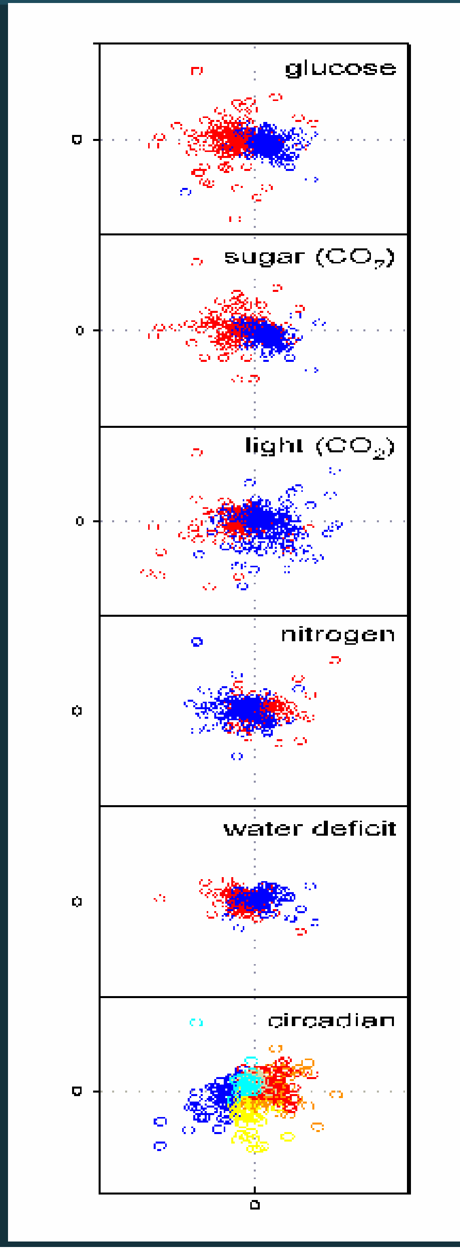
Loadings of the 200 genes that are **induced** and the 200 genes that are **repressed** most strongly by input

Sugars make a major contribution to the diurnal regulation of expression



WT

Loadings in 1st and 2nd components

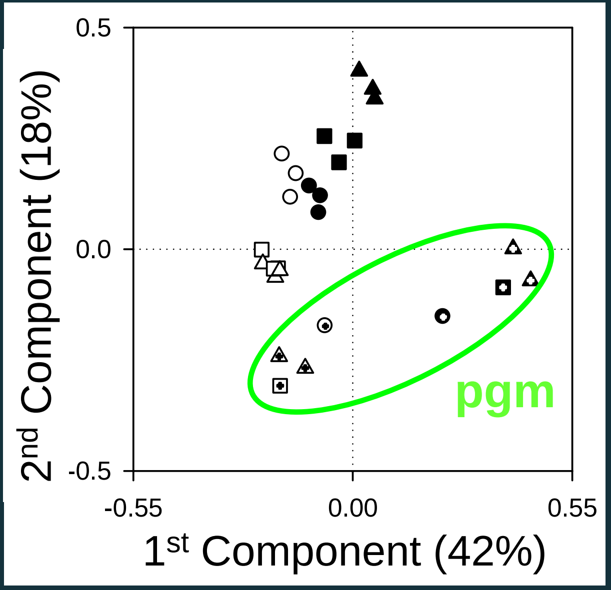


Sugars contribute strongly

Other inputs are displaced into a 2ndary role

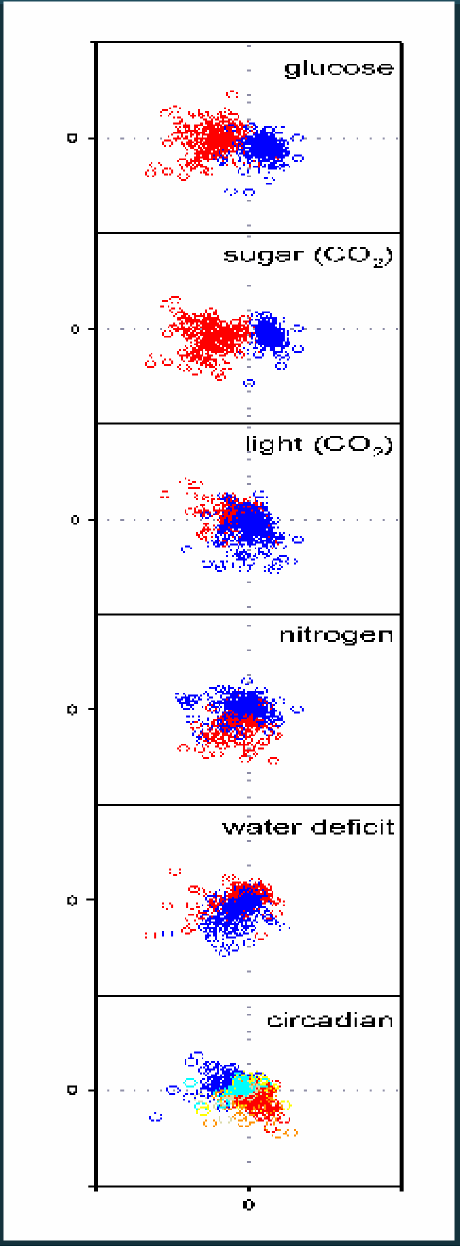
Important contribution of the circadian clock

Sugars make a major contribution to the diurnal regulation of expression



WT + *pgm*

Loadings in 1st and 2nd components



Sugars dominate

No big change

Altered contribution of the circadian clock

Bläsing et al. Plant Cell 2005

Candidate genes

We found many genes possibly involved in the daily orchestration of metabolism and growth in response to sugar availability…

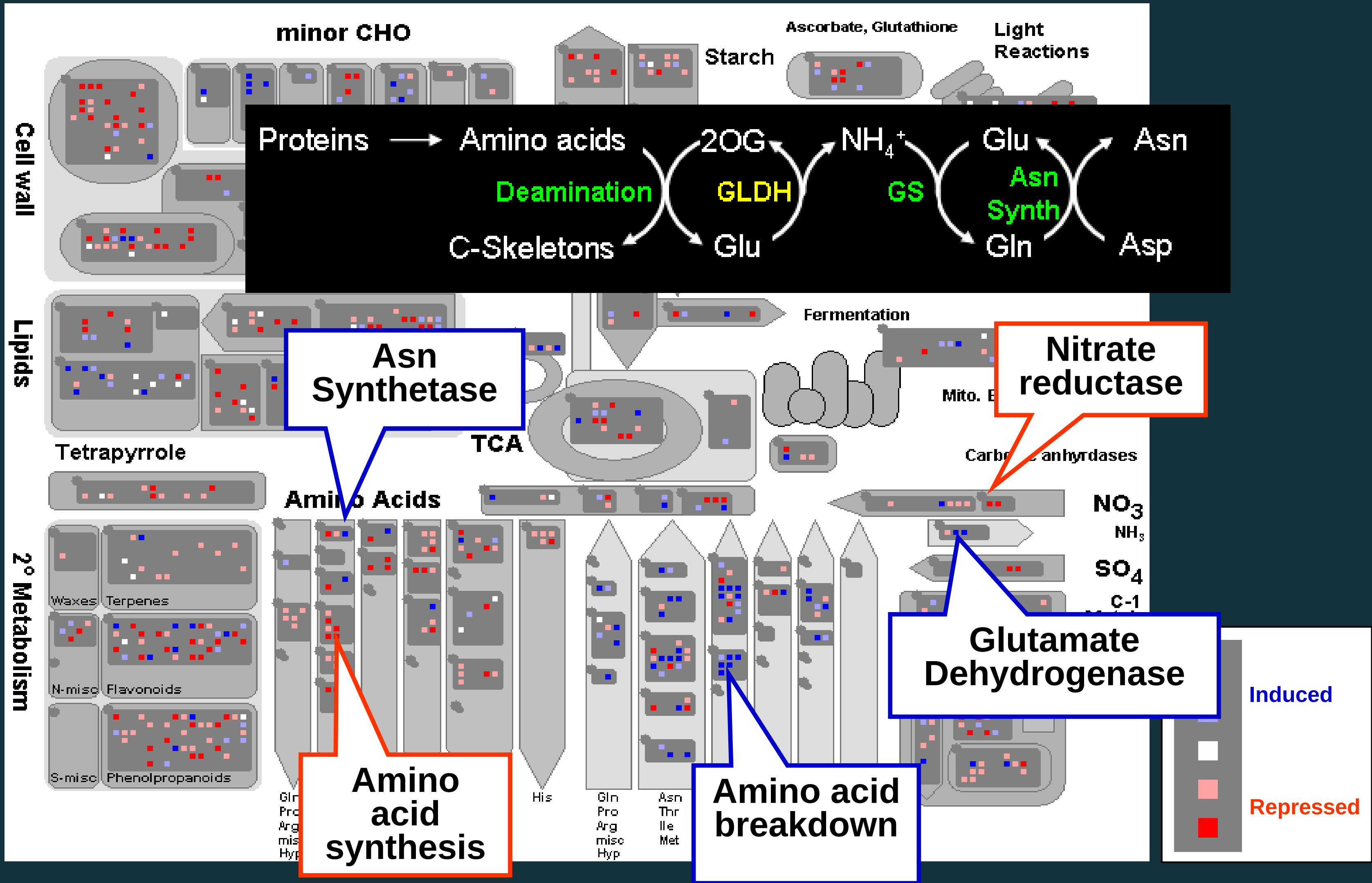
⇨ **89 genes likely involved in signalling**

- 29 Transcription factors
- Several protein kinases
- 5 E3 ligases and other UBQ system components
- Cell division
- Chromosome modelling
- Unknown proteins

… and thought we would learn from them how plants quickly respond to fluctuations in C-supply

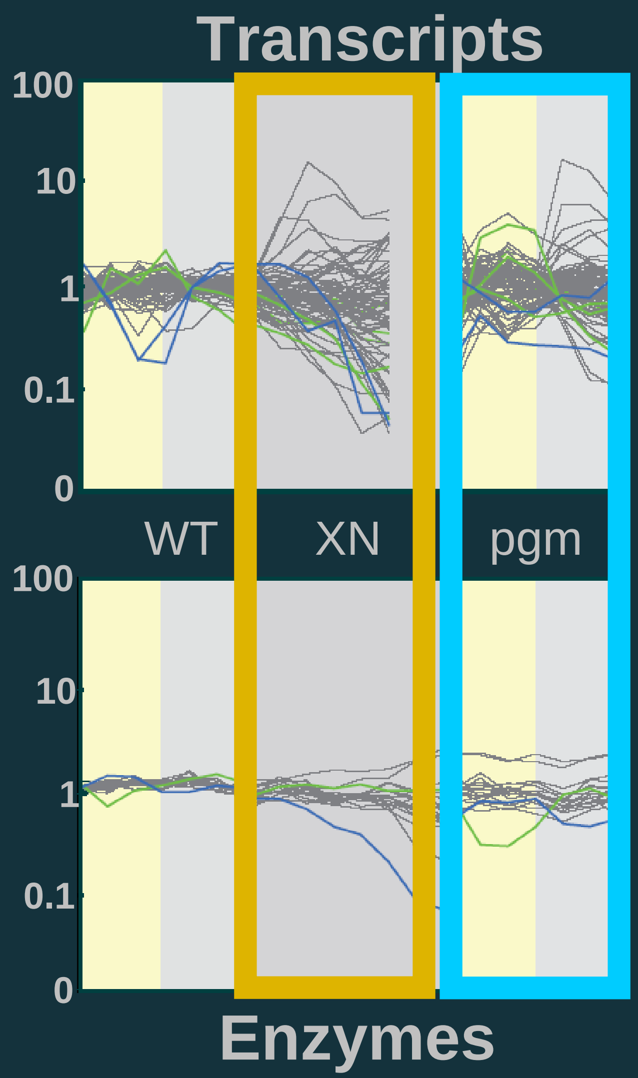
What about pathway enzymes?

Overlay plot showing responses to low C found in both WT and *pgm*



Thimm et al Plant J. 2004

Enzymes are by far more stable than transcripts encoding them



Extended darkness (XN)
Sustained carbon starvation

Rapid changes of transcripts

Slower changes of enzymes
One exception: NR

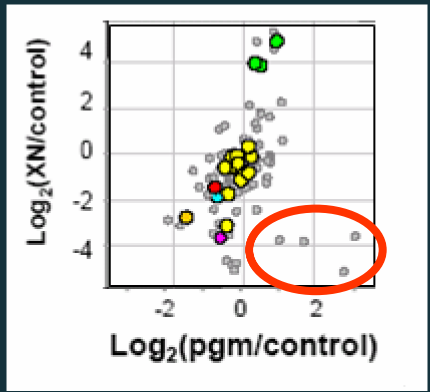
Starchless *pgm* mutants
Very high sugars each day
Very low sugars each night

Large and rapid diurnal changes of transcripts

No large diurnal changes of enzyme activities
One exception: AGPase

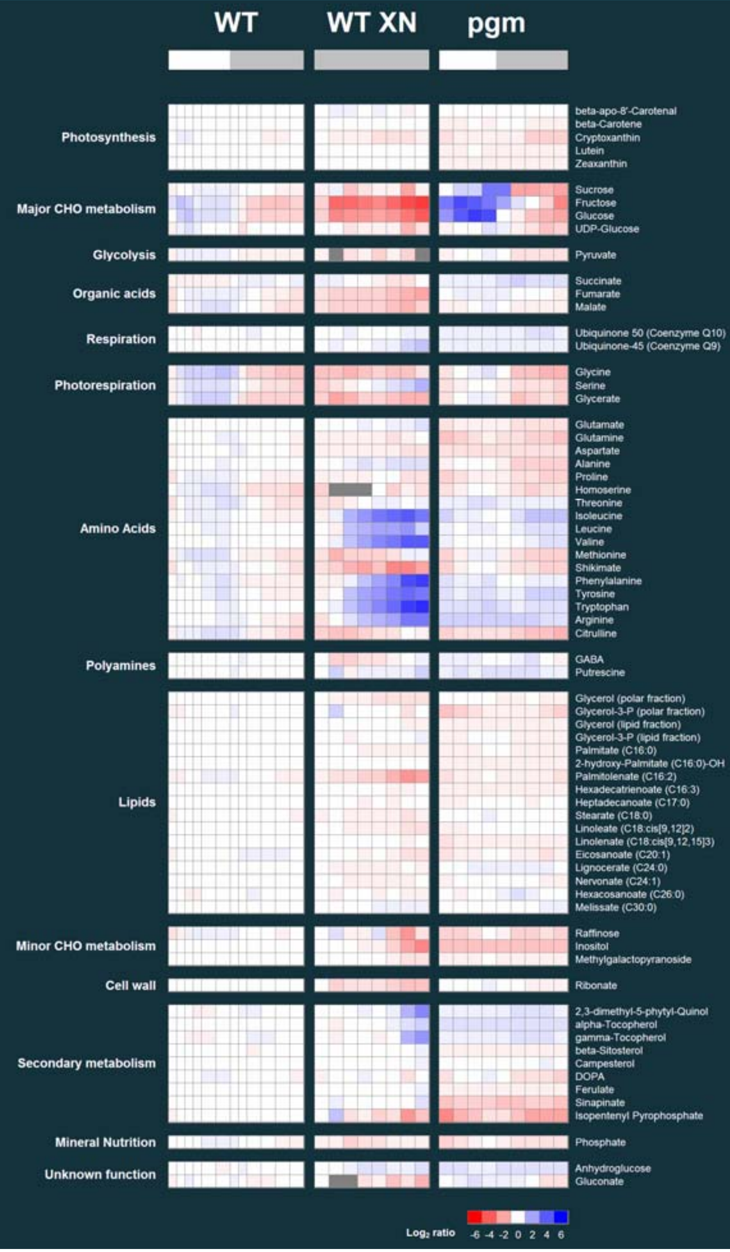
Shifts in overall levels of enzyme activities

Metabolite profiling confirms metabolic stability

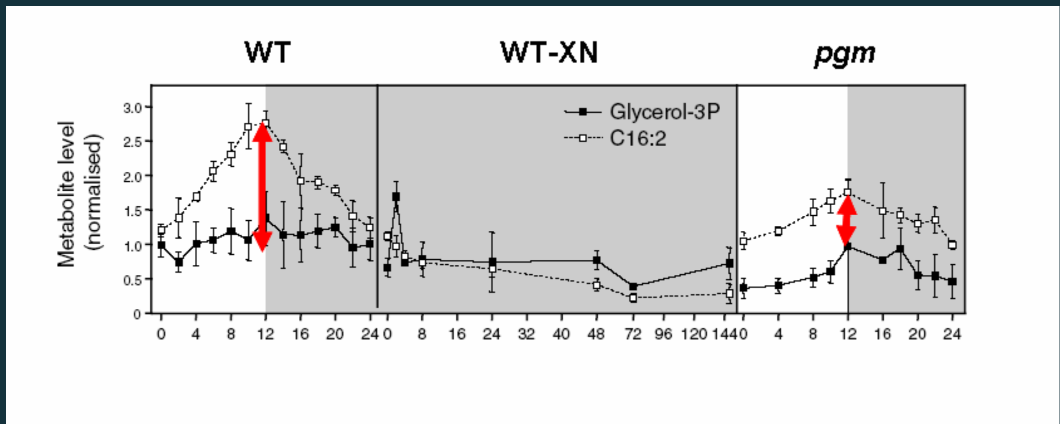


The starchless mutant *pgm* has a C-starvation metabolic phenotype

Carbohydrates



The metabolite profile integrates changes in the activities of 100's of enzymes

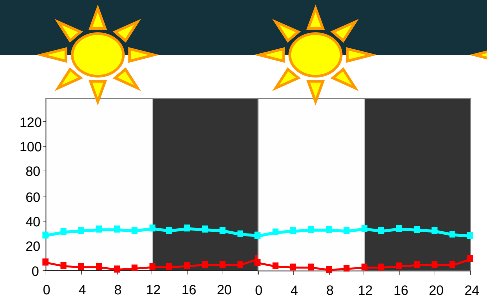


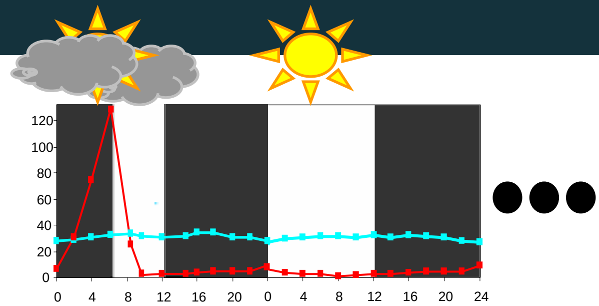
Gibon et al. Genome Biology 2006

Metabolic acclimation

mRNA

Activity



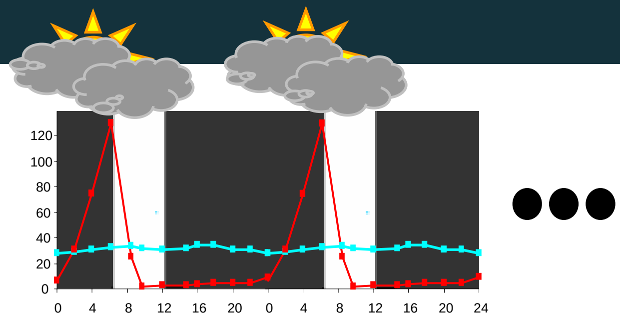


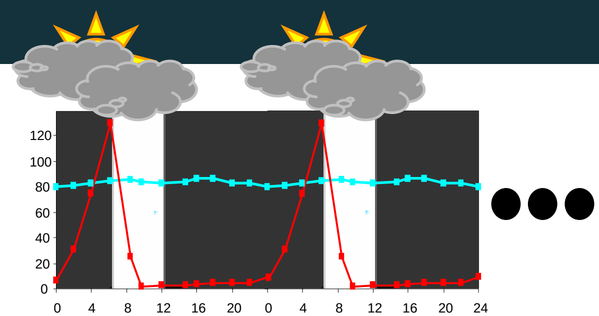
Transcripts respond early and strongly…

…enzymes don't panic

mRNA

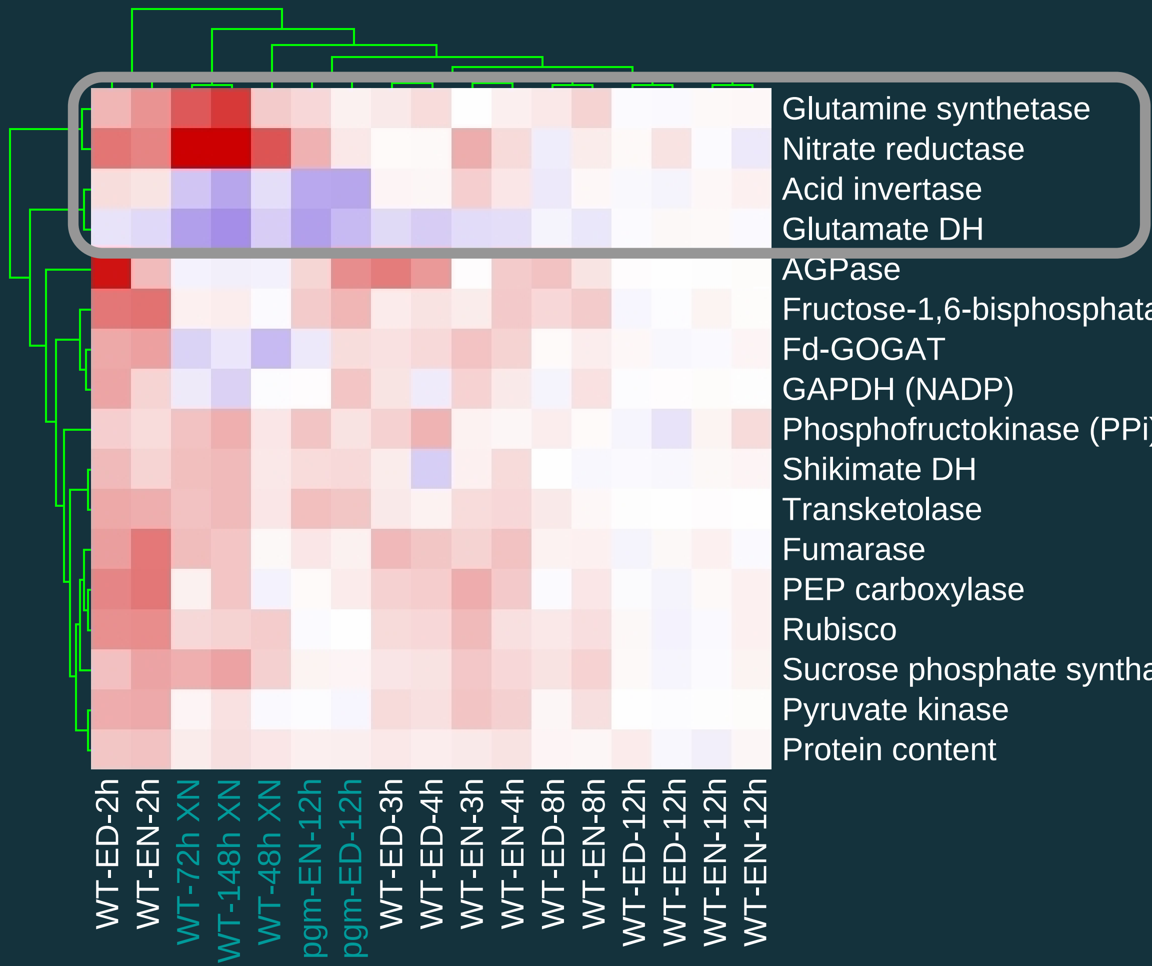
Activity





…changes are integrated over time

Diagnostic enzymes



Enzymes calling the tune?

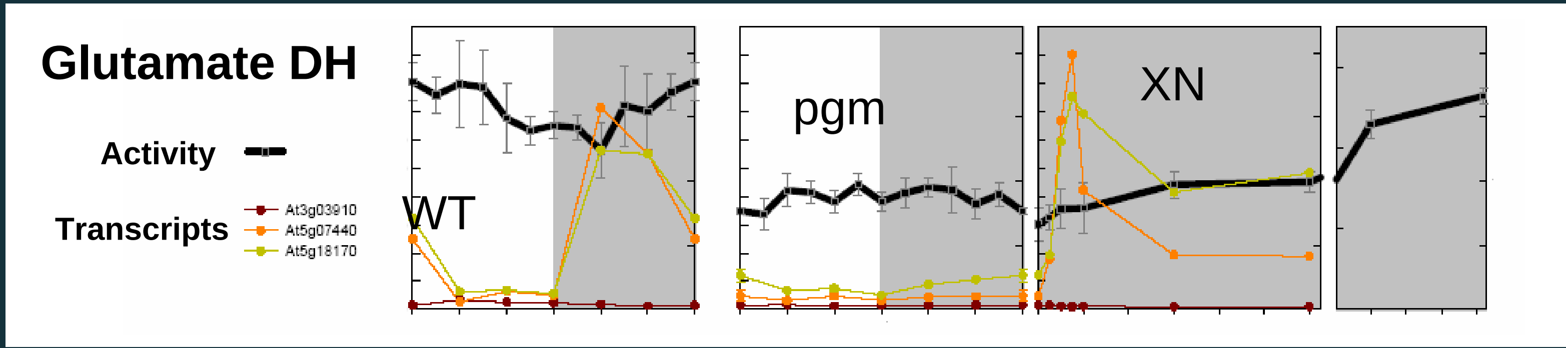
« Benchmark » - C-starvation, provoked (XN = extended night) or chronic (pgm = starchless mutant)

C-availability as related to the photoperiod length

Unsupervised clustering in TMEV

Gibon et al. In preparation

Candidate enzymes



A reduction of more than 80% of this activity exerted no effect on the response to C starvation

pgm mutants with less then 10% invertase activity left were fine !

Anyway, recent and less recent literature indicates that:

- many enzymes can by decreased by antisense without affecting plant phenotypes
  e.g. Stitt Rubisco papers; Fernie TCA-cycle papers
- most extractable activities are by far in excess when compared to fluxes
  Leegood & Ap Rees Biochem. Biophys. Acta 1978; Junker et al. Phytochemistry 2007

Enzyme variants influencing plant performance

ADPglucose pyrophosphorylase activity and starch accumulation in immature tomato fruit: the effect of a *Lycopersicon hirsutum*-derived introgression encoding for the large subunit☆

Arthur A. Schaffer [a,*], Ilan Levin [b], Ismail Oguz [b,1], Marina Petreikov [a], Felix Cincarevsky [b], Yelena Yeselson [a], Shmuel Shen [a], Nehama Gilboa [b], Moshe Bar [b,2]

[a] *Department of Vegetable Crops, Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, Israel*
[b] *Department of Plant Genetics, Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, Israel*

Introgression of a large subunit of AGPase stabilising the activity associated with a high sugar content in tomato fruit

Introgression of an invertase with higher affinity for sucrose associated with and increase in sugar yield in tomato fruits

Zooming In on a Quantitative Trait for Tomato Yield Using Interspecific Introgressions

Eyal Fridman,[1*†] Fernando Carrari,[2†] Yong-Sheng Liu,[1‡] Alisdair R. Fernie,[2] Dani Zamir[1§]

Natural variation for sulfate content in *Arabidopsis thaliana* is highly controlled by APR2

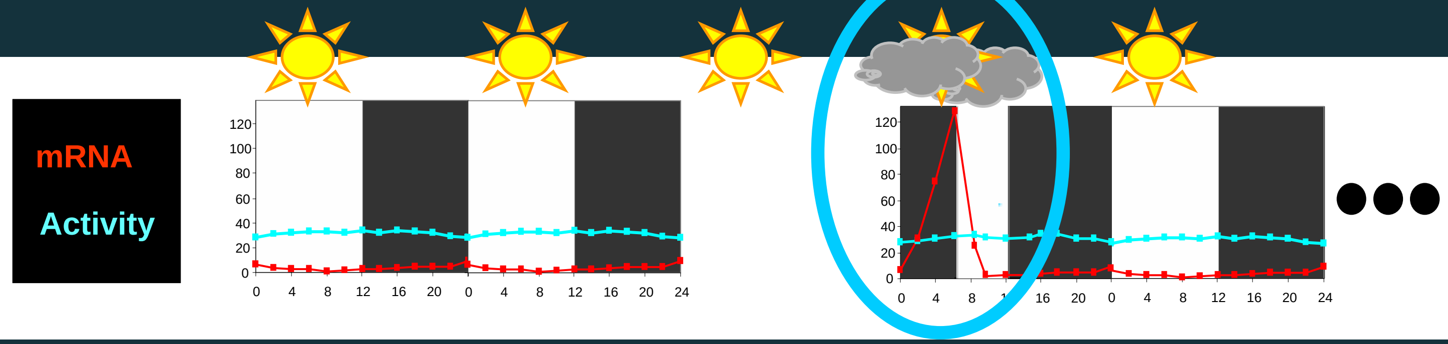
Olivier Loudet[1], Vera Saliba-Colombani[2], Christine Camilleri[1], Fanny Calenge[2], Virginie Gaudon[2], Anna Koprivova[3], Kathryn A North[3], Stanislav Kopriva[3] & Françoise Daniel-Vedele[2]

Higher sulphate content in leaves due to an adenosine-5'-phosphosulfate reductase with altered properties (V_{max} and K_m for glutathione)

Only a few QTLs for complex traits have been "resolved" (e.g. as QTN within genes). This indeed represents an intensive work. Maybe not the easiest approach for someone interested in the regulation of metabolism?

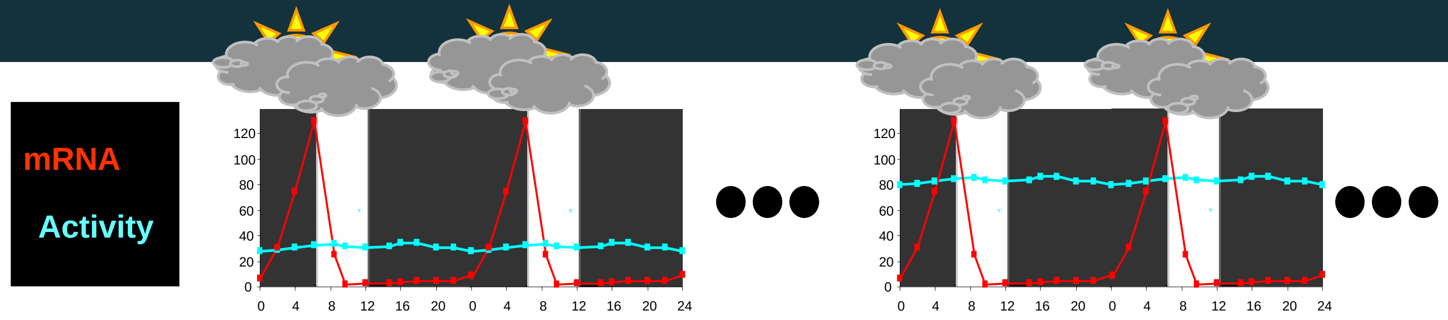
Metabolic acclimation

Post-translational mechanisms?



Transcripts respond early and strongly…

…enzymes don't panic

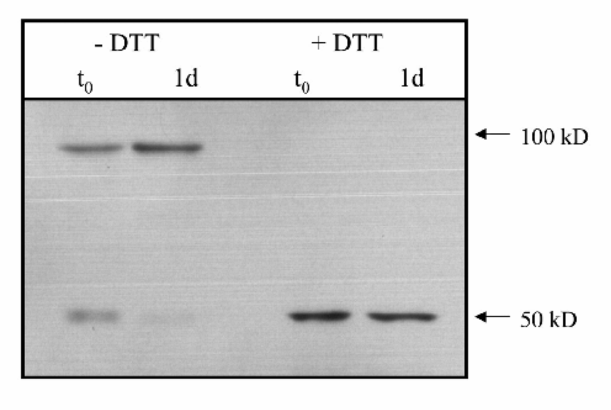


…changes are integrated over time

Post-translational redox modification of AGPase regulates starch synthesis

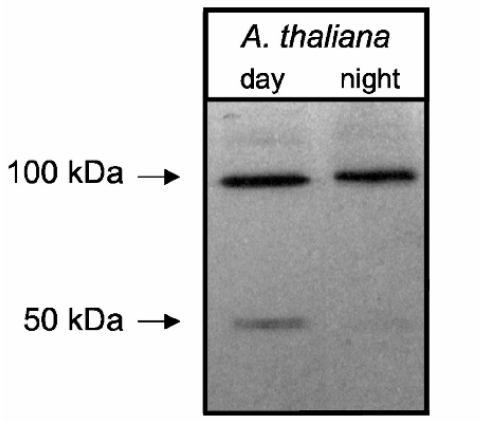
AGPase was known to be allosterically regulated by 3PGA (+) and Pi (-) (Gosh & Preiss, J. Biol. Chem.1969, 241:4491-4504)

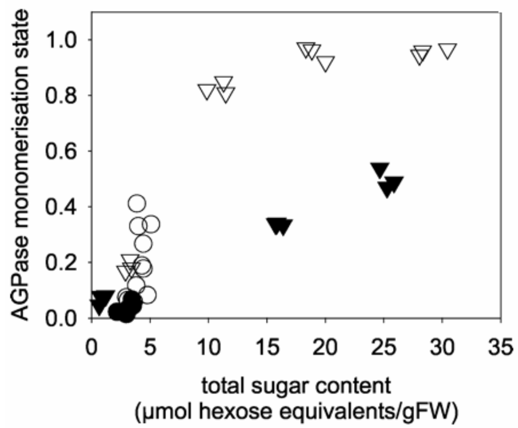
But it was also found to be activated *in vitro* through redox modification in higher plants (J. Biol. Chem. 2000, 275: 1315-1320)



In detached potato tubers, starch synthesis was found to be inhibited, but 3PGA and Pi levels were not modified. In contrast AGPase is oxidised *in vivo*. This leads to its inactivation, which can be reversed *in vitro* in the presence of DTT.

Tiessen et al. (2002) Plant Cell 14: 2192-2213



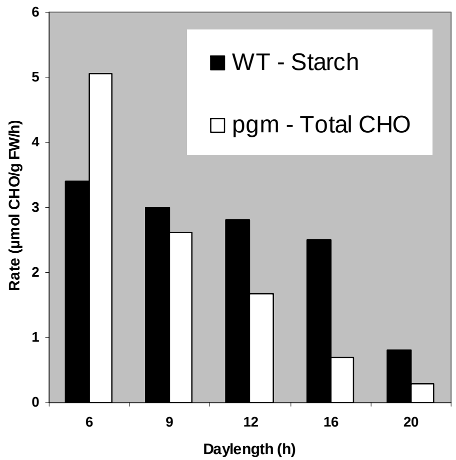


AGPase is redox regulated *in vivo* in leaves of various species.

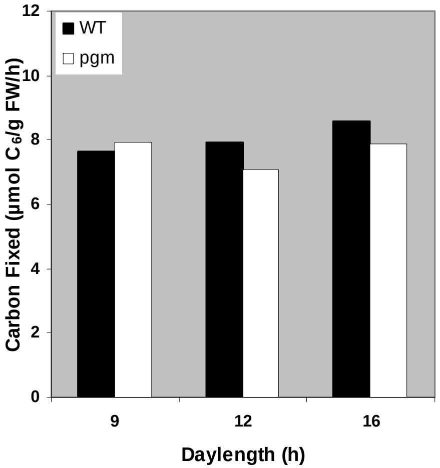
Reduction of AGPase is related to the sugar content.

Hendriks et al. (2003) Plant Physiology 133: 838-849

Plants accumulate starch more rapidly in the light when C is limiting at night...



Source leaves retain more CHO in the light when C is limiting at night...



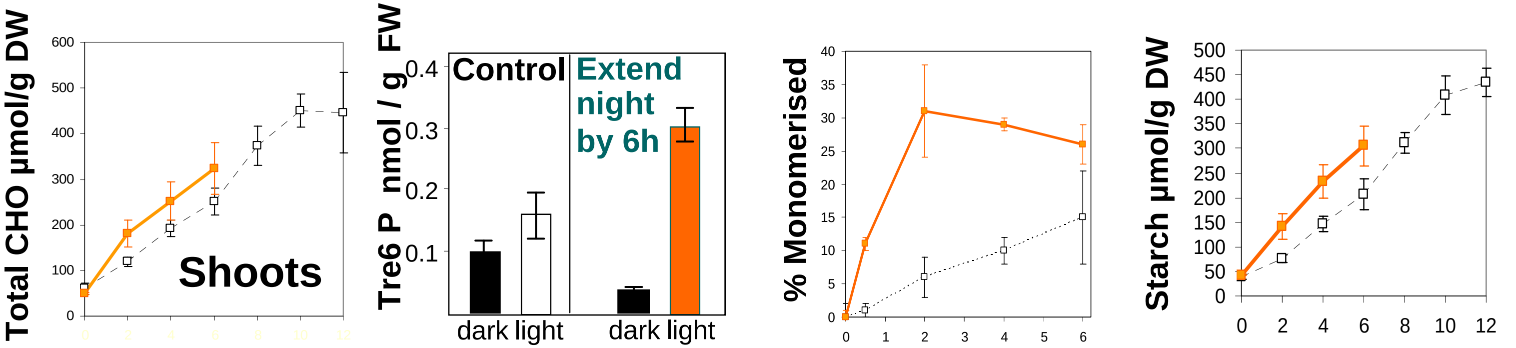
...while C assimilation remains unchanged

Carbon shortage during the night limits growth and thus, the use of CHO during the day!

The accentuated increase of Tre6P promotes AGPase activation and starch accumulation

Prolonged night **Growth decreased**

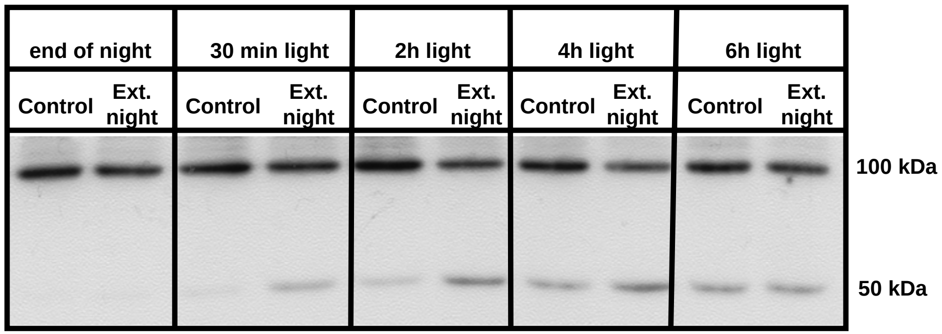
End of the normal night



1. Sugars accumulate → **2. Trehalose-6P increases** → **3. AGPase is activated** → **3. Stimulation of starch synthesis**

Control

Extended night



Gibon et al. Plant J 2004 / Lunn et al. Biochem J 2006

Conclusion: What strategy to identify metabolic hot spots?

Sugars can exert a major effect on the transcriptome. We identified a number of candidate genes.

Diurnal changes of transcripts are integrated over time as changes in enzyme activities and metabolites. Several enzymes responded to C-starvation

We could not understand these responses using reverse genetics approaches

Screen for alterations in properties such as V_{max}, K_m or K_i …) depending on:

- growth conditions
- genotypes

Acknowledgements

And also: metanomics, Ally Fernie, Thomas Altmann, Ed Buckler & Amit Gur
Financing: Max Planck Society & BMBF - German Plant Genome Initiative (GABI)