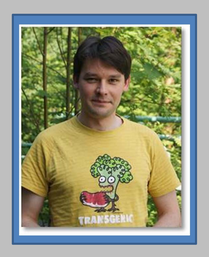
The Yang cycle in higher plants

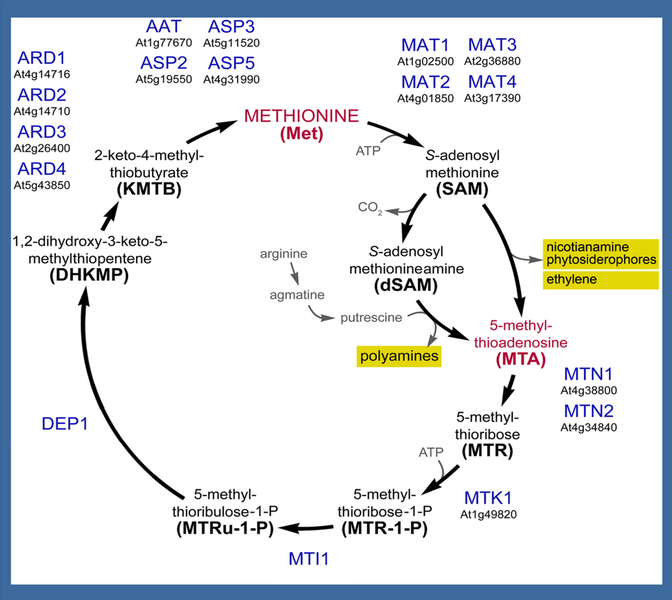


Benjamin Pommerrenig[1], Wolfgang Zierer[1], Franz Klebl[1], Kirsten Feussner[2], Ivo Feussner[2], Norbert Sauer[1]

1 Molekulare Pflanzenphysiologie, FAU Erlangen-Nürnberg, Staudtstraße 5, 91058 Erlangen, Germany
2 Abteilung Biochemie der Pflanze, Albrecht-von-Haller-Institut für Pflanzenwissenschaften, Justus-von-Liebig-Weg 11, 37077 Göttingen, Germany

1 Introduction and overview

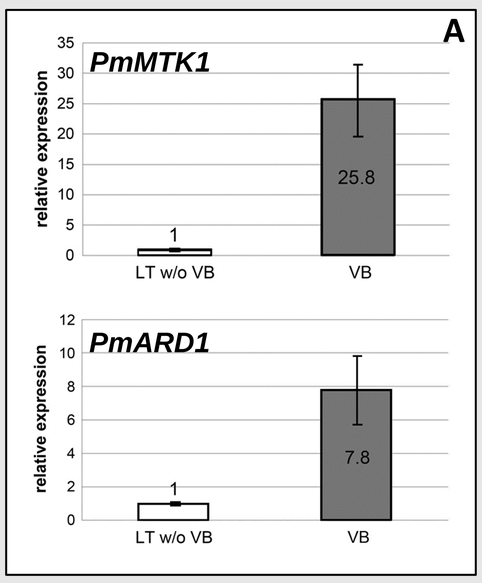
The Yang cycle [also named Methionine cycle, Methionine salvage pathway or 5'-Methylthioadenosine (MTA) cycle] is a set of reactions that recycle MTA to methionine. MTA is produced either from S-Adenosyl methionine (SAM) or decarboxylated SAM (dSAM) in the biosynthetic pathways leading to either ethylene, polyamines or phytosiderophores. With quantitative RT-PCR and Promoter-GUS analyses we investigated the expression of genes coding for enzymes of the Yang cycle in Arabidopsis and *Plantago major*. To analyze distribution of SAM and MTA in isolated vascular tissue and mesophyll from Plantago source leaves, comparative analyses by UPLC-TOF were performed in these tissues. Additionally we identified so far uncharacterized genes coding for enzymes that catalyze two important steps in the Yang cycle, namely the conversion of MTR-1-P to MTRu-1-P and from MTRu-1-P to DHKMP (see Yang cycle overview below for abbreviations).

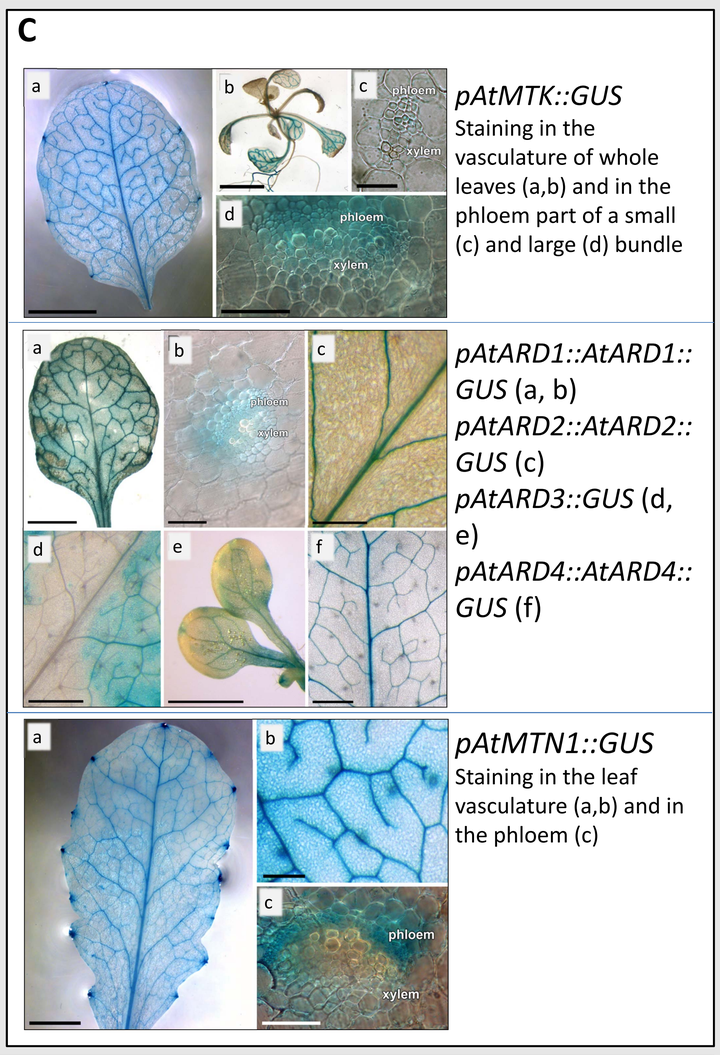


The Yang cycle in plants

In a series of reactions MTA, produced as a byproduct from e.g. polyamine synthesis is converted back to methionine. A nucleosidase (MTN), a kinase (MTK), an isomerase (MTI), a dehydratase-enolase-phosphatase complex (DEP) and a acidoreductone-dioxygenase (ARD) are all involved in this back-conversion. In the overview on the left, metabolites are printed in **black**, MTA producing biosynthetic pathways are highlighted in yellow and catalyzing enzymes are printed in **blue**. MIPS numbers are given for corresponding genes in Arabidopsis.

2 Vascular specific expression of *MTN*, *MTK* and *ARD* genes





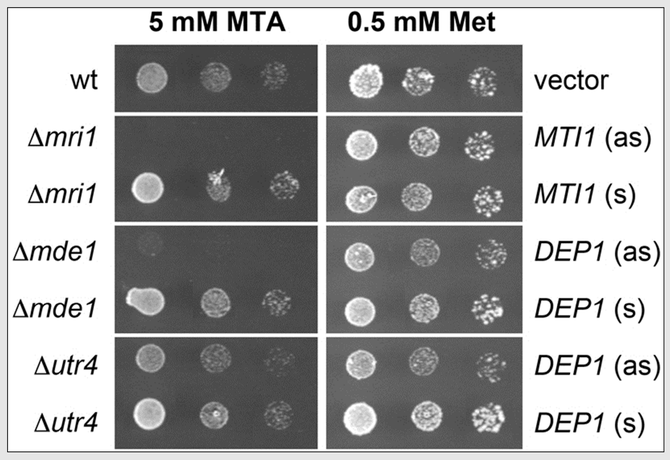
pAtMTK::GUS
Staining in the vasculature of whole leaves (a,b) and in the phloem part of a small (c) and large (d) bundle

pAtARD1::AtARD1::GUS (a, b)
pAtARD2::AtARD2::GUS (c)
pAtARD3::GUS (d, e)
pAtARD4::AtARD4::GUS (f)

pAtMTN1::GUS
Staining in the leaf vasculature (a,b) and in the phloem (c)

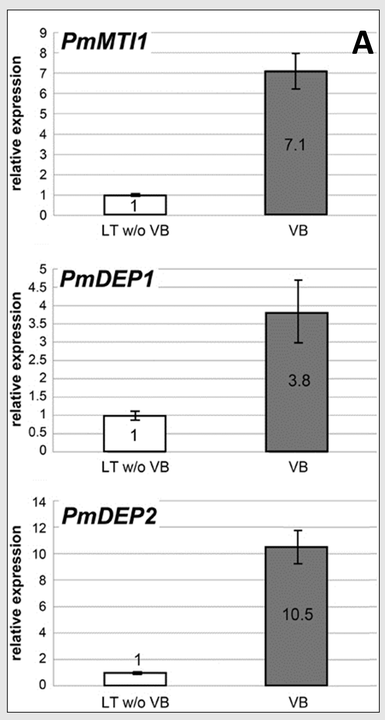
(A) qPCR with cDNA from isolated vascular bundles (VB) and leaf tissue without VB from *Plantago major*. mRNA levels of *MTK1* and *ARD1* transcripts are highly enriched in the VB fraction.
(B) Vascular tissue can easily be separated from Plantago leaves. Bundles and remaining leaf tissue can be used for RNA isolation and for metabolite analyses as well. White arrowheads point to minor veins, which cannot be separated from the mesophyll.
(C) Staining of Arabidopsis plants expressing GUS fusions under the control of promoters from known Yang cycle genes is specific for the vascular tissue.

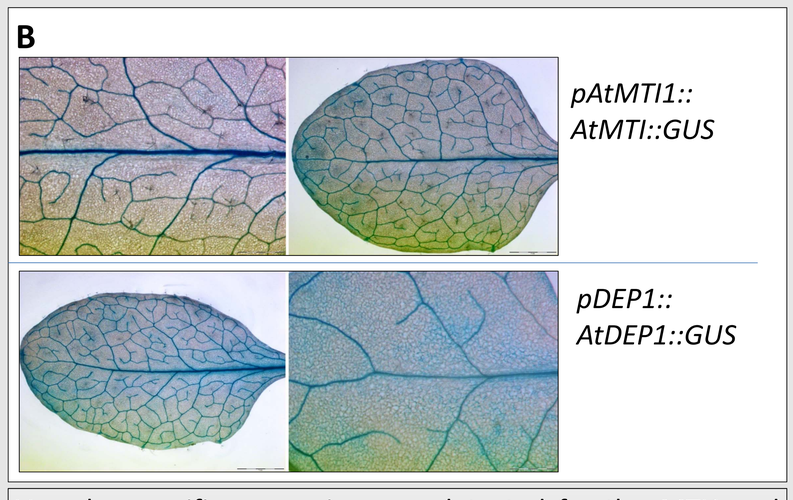
3 *MTI1* and *DEP1*: Two newly identified Yang cycle genes



Yeast mutants defective in the Yang cycle genes *MRI1*, *MDE1* or *UTR1* transformed with the genes for the predicted Arabidopsis homologs either in sense (s) or in antisense (as) orientation. Expression of *MTI1* in (s) but not in (as) complements the growth defect of the Δ*mri1* mutant on MTA medium. Expression of *DEP1 (for DEHYDRATASE-ENOLASE-PHOSPHATASE COMPLEX1)* complements the growth defects of Δ*mde1* and Δ*utr4* mutants.

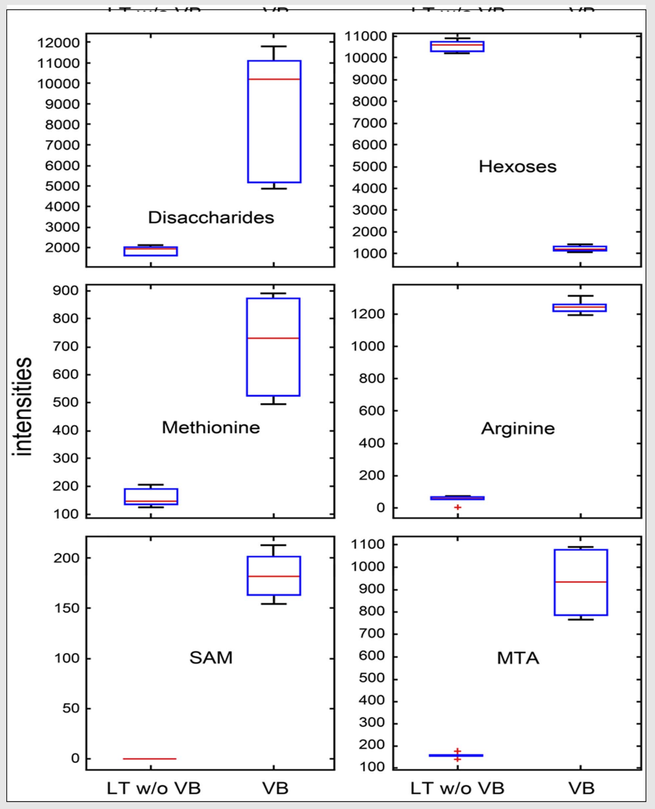
4 Vascular specific epression of *MTI* and *DEP* genes





Vascular specific expression was detected for the *MTI1* and the *DEP1* genes both with qPCR with cDNA from vascular against non-vascular tissue from Plantago **(A)** and with Promoter-GUS analyses in Arabidosis **(B)**. In Plantago, two homologous *DEP* genes, named *PmDEP1* and *PmDEP2* were identified.

5 Detection of Yang cycle metabolites in vascular bundles



Box-Whisker plots of 6 metabolites that were measured by UPLC-TOF. Disaccharides were 8-fold enriched in vascular bundles, whereas hexoses were 10-fold higher in the mesophyll.
Three Yang-cycle metabolites, Met, SAM and MTA, could be identified in our preparations. Met and MTA were about 6-fold enriched in the vasculature, the enrichment of SAM was about 200-fold. This high value for SAM is the consequence of low intensities in vascular preparations and intensities below the detection limit in preparations from non-vascular tissue.
Surprisingly, arginine, a precursor of putrescine biosynthesis in Arabidopsis was also more than 100-fold enriched in the vasculature.

6 Summary

We identified all genes coding for enzymes of the plant Yang cycle to be expressed exclusively or predominantly in the phloem part of the vascular tissue. We could show this for the so far known Yang cycle genes coding for MTN, MTK and ARD enzymes as well as for the yet missing genes coding for MTI and DEP proteins. Comparative metabolite analyses of both SAM and MTA in isolated vascular tissue and mesophyll from Plantago source leaves could show a clear accumulation of these two Yang cycle products in the vascular bundles.