Mitteilungen und Nachrichten

4th Sulphyton Workshop, 5th – 8th September 2013 in Athens, Greece

In September 2013 the 4th Sulphyton workshop[1] took place in Athens, organized by the Agricultural University of Athens. The international plant sulfur research community has set up the Plant Sulfur Network[2], which organizes scientific meetings on a regular basis covering all topics of plant sulfur research. The International Plant Sulfur Workshop series are organized every 3 years. In the between years the Sulphyton Workshops take place, which provide an open platform for the presentation and discussion of ongoing plant sulfur research. The main topics of the meetings are:

- plant sulfur nutrition
- sulfur and crop quality
- post genomic technologies
- cross-talk of metabolic pathways interacting with sulfur
- managing sulfur nutrition
- diagnosing sulfur deficiency
- sulfur in plant stress response
- regulation of sulfur assimilation pathways and sulfur metabolism
- interaction between sulfur and nitrogen metabolism or other nutrients

The 4th Sulphyton workshop focused on plant sulfur metabolism and its regulation, the effects of sulfur nutrition on sulfur secondary metabolism, the role of the enzyme sulfite oxidase in plants and the effects of stress such as light stress, high salt and heavy metals on sulfur metabolism. Extended abstracts of the talks are presented here along with a brief description of the background of the workshop's scientific excursion, during which the highly contaminated heavy metals and sulfides mining sites of Lavrion were visited.

The 9th and 10th International Plant Sulfur Workshops will be held in Freiburg, Germany (April 14–17, 2014) and in Goslar, Germany (September 2–5, 2015) respectively, whilst the 5th Sulphyton Workshop will be held in Groningen, The Netherlands (September 1–4, 2016).

[1] www.aua.gr/sulphyton4
[2] www.plantsulfur.org

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Abstracts:

1) Systems biology of plant sulphate metabolism: The OAS module

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O-acetyl-L-serine (OAS) provides the carbon backbone for cysteine synthesis. OAS is formed through the activity of serine acetyltransferase (SERAT). Upon sulfate starvation most of the intermediate metabolites of the sulfate assimilation pathway are present in reduced amounts while OAS accumulates. Additionally, resupply of sulfate quickly reverts OAS accumulation to its normal very low level. Thus, OAS appears to be a suitable candidate for a signaling molecule for the sulfur status of a plant. Further, support for a signaling function of OAS comes from sulfur (S) assimilation by enteric bacteria. OAS accumulates under S depletion and autocatalytically converts to N-acetylserine (NAS), which is sensed and activates the sulfur assimilation operon. In plant systems OAS accumulation from sulfate starvation or OAS application induces genes of the sulfur assimilatory pathway, namely SULTR1.1 and SULTR1.2 (encoding sulfate transporters) and APR1-3 (encoding 5'-adenylylsulfate reductases). However, previous studies showed an induction of sulfate starvation-responsive SULTR family 1 members before accumulation of OAS in the respective tissues. Therefore OAS has been assumed to integrate sulfur metabolism with carbon and nitrogen metabolism. The difficulty in all prior investigations is that the concentrations of various potential signaling molecules within sulfate metabolism change upon sulfate depletion, making it difficult to assign functions to distinct metabolites. Cellular sulfate, sulfite and sulfide as well as cysteine, glutathione (GSH), and *S*-adenosylmethionine are reduced, while inversely OAS and reactive oxygen species (ROS) increase.

Database evaluations revealed two experiments in which OAS accumulation occurred independently of changes in sulfate availability to the plants. First, *Arabidopsis thaliana* plants grown under a normal day light cycle transiently accumulated OAS during the night. And second, plants transferred from constant light to darkness exhibited a short transient OAS peak within minutes after transfer, while sulfate, sulfide, sulfite, cysteine and GSH levels remained unaltered. In both experiments, a set of genes was identified which apparently respond to OAS accumulation based on their slightly time shifted induction relative to OAS. In order to experimentally test this assumption, transgenic Arabidopsis plants were generated with a SERAT gene under the control of a dexamethasone inducible promoter. Dexamethasone induction of the SERAT transgene resulted in accumulation of OAS, while within a time window of up to 6 hours after induction no other sulfur related metabolites changed in concentration. Later, cysteine and GSH accumulated, as is well known from previous experiments. When applying very stringent selection conditions in all above mentioned experimental conditions, the following genes consistently accumulated: adenosine 5'-phosphosulfate reductase 3 (APR3; At4g21990), sulfur deficiency induced 1 (sdi1, previously termed MS5-1; At5g48850), sulfur deficiency induced 2 (sdi2, previously termed MS5-2; At1g04770), low sulfur induced 1 (LSU-1; At3g49580), serine hydroxymethyltransferase 7 (SHM7; At1g36370) and ChaC-like protein (ChaC; At5g26220). These genes are known to be among the most strongly induced genes from the transcriptome studies of sulfate starvation mentioned above.

Using these genes to query about 1400 transcriptome datasets (www.attedII.jp) resulted in the identification of a stable cluster of co-expressed genes. In addition to the above mentioned six core genes, the OAS cluster contained further sulfur pathway related genes, i.e. the vacuolar sulfate transporters (SULTR4;1, At5g13550, and SULTR4;2, At3g12520), and APR1 (At4g04610) and APR3 (At1g62180). Further members of this OAS cluster are LSU2 (At5g24660), a beta glucosidase with putative myrosinase function (BGLU28; At2g44460) and an unknown protein, which responds strongly to sulfate starvation (At1g12030). Interestingly most of these genes are also induced under selenium treatment, which mimics sulfate starvation, or under conditions inducing ROS species, such as menadione treatment or cadmium stress.

In conclusion, it can be assumed that OAS plays a role as a signaling molecule in the plant's response to sulfate deprivation, though it must be clearly stated that OAS is not the only necessary signal. Further, we can assume that OAS acts as sig-

naling molecule in various other, seemingly unrelated physiological responses to environmental signals or stresses, such as dark-light shifts, diurnal rhythms, and responses to ROS inducing conditions. Speculatively the OAS cluster genes are mobilized as part of a response module to various stimuli or stresses.

2) Arabidopsis cytoplasmic serine acetyltransferase interacts with putative transcription factor SCL11

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Cysteine biosynthesis represents the final step of sulfate assimilatory reduction and the sole *entry point* of reduced sulfur into an organic form in plants. Cysteine is not only a protein constituent, but a substrate for methionine biosynthesis, and a precursor for the synthesis of essential molecules such as glutathione, thiamine, biotin and coenzyme A. Cysteine biosynthesis in higher plants involves the cysteine synthase complex (CSC), which consists of serine acetyltransferase (SAT) and O-acetylserine(thiol)lyase (OAS-TL) enzymes. SAT transfers an acetyl moiety of acetyl-CoA to serine, producing *O*-acetylserine (OAS), which in turn accepts sulfide in reaction catalyzed by OAS-TL, releasing cysteine. The Arabidopsis genome contains five SAT genes and nine OAS-TL genes, with SAT1, 3 and 5 as well as OAS-TLA1, B and C being the predominantly expressed isoforms. The formation of CSC alters the activities of both enzymes and serves SAT activation but OAS-TL deactivation. The stability of CSC is controlled by OAS and sulfide concentrations. When sulfate is limiting CSC dissociates turning SAT inactive; however, when there is surplus of sulfate CSC is stabilized by sulfide and the subsequent synthesis of cysteine is very efficient. SAT and OAS-TL isoforms are present in multiple cellular compartments; however, the relevance of CSC formation in each compartment for flux control of cysteine synthesis remains controversial.

Based on the findings that CSC formation controls cellular sulfur homeostasis, we hypothesized that activity of either SAT or OAS-TL either alone or complexed may be additionally modulated by interactions with other proteins. It is also possible that such an interaction would transfer the sulfur status message to the other proteins to alter their function. There are already several reports showing the ability of CSC proteins to interact with other proteins. It has been demonstrated that cytoplasmic isoform of OAS-TLA1 interacts with plasma-membrane sulfate transporter SULTR1;2. The binding of OAS-TLA1 inhibits the activity of the transporter and coordinates the internalization of sulfate with the energetic/metabolic state of the cell. In another report cyclophilin CYP20-3 (also known as "ROC4") of the chloroplast stroma was found to function *in vivo* in assisting the folding or assembly of SAT1 enzyme to form the CSC in chloroplast facilitating the biosynthesis of cysteine. Along with other antioxidants, the newly formed cysteine is essential for the biosynthesis of glutathione that enables the chloroplast of the buildup of cellular reduction potential mitigating detrimental effects of ROS. An alternate and more direct regulatory link between sulfur metabolism and cellular redox state is a putative interaction of thioredoxin with OAS-TL in mitochondria as indicated by proteomic studies.

Here, we report the application of a yeast two-hybrid method (Y2H) that used the entire cytoplasmic CSC of *Arabidopsis thaliana* to search for interacting proteins. The cytoplasmic CSC was chosen because it may play rather regulatory then biosynthetic function, as the mitochondrial SAT/OASTL was shown to be most important for cysteine synthesis. The region encoding SAT5 was fused with the GAL4 binding domain and introduced on the same vector with OAS-TLA1 coding region into the yeast cell. The searched cDNA library was made from 5-week old *Arabidopsis thaliana* plants starved for sulfur for two days. This approach let us to identify, among four other putative partners of the CSC, Scarecraw-like11 (SCL11) encoded by At5g59450. The protein belongs to the GRAS protein family, which is unique to plants. GRAS proteins are typically composed of 400–770 amino acid residues and exhibit considerable sequence homology to each other in their respective C-termini with the highly divergent N-termini. Although the Arabidopsis genome encodes at least 33 GRAS protein family members only a few have been characterized so far. These proteins play a crucial role in diverse plant growth and development processes, ranging from gibberelin signaling, root radial pattering, light signal transduction, and axillary shoot meristem formation. It is generally believed that GRAS proteins could be involved in transcriptional regulation; however, for many of the proteins with SCL11 among them, the exact function has yet to be determined. *SCL11* was reported to be expressed strongly in roots, whereas in flower its expression was detected mainly in sepals and upper region of carpel.

We were interested whether the formation of the CSC is a prerequisite for the interaction with SCL11. More detailed studies using Y2H revealed that SCL11 binds to SAT5 but not to OAS-TLA1. Additionally, the interaction domain is likely present in a structurally conserved region of SAT because SCL11 was also able to interact with chloroplastic SAT1 and mitochondrial SAT3. The physical interaction between SAT5 and SCL11 was next independently validated using two techniques: pull-down of the proteins overexpressed in *Escherichia coli* and Bimolecular Fluorescence Complementation (BiFC) of the proteins overexpressed in the leaves of *Nicotiana benthamiana*. BiFC revealed the cytoplasmic localization of the interaction with the clear exclusion from the nucleus. The independent studies on the cellular localization of SCL11-GFP fusion in *Nicotiana benthamiana* leaves demonstrated the protein can localize to cytoplasm and nucleus, although *in silico* studies revealed no NLS present in SCL11 sequence. The nuclear localization might suggest the function of SCL11 in transcription. Additional hint comes from the results of Y2H. When fused with the GAL4 binding domain, SCL11 was able to activate the reporter genes even without interaction with SAT5 (auto-activation), suggesting it could act as transcription activator in yeast, as it was similarly noted for another 20 GRAS family members. We did not observe any phenotypic abnormalities for the Arabidopsis knock-out mutant *scl11*. All *scl11* seedlings were indistinguishable from the wild-type when grown for ten days in different conditions (sulfur deficient media, osmotic stress, high glucose, addition of abscisic acid or 1-aminocyclopropane-1-carboxylic acid). Also the adult *scl11* plants show normal morphology, despite smaller or delayed flowering appearance. However, more careful observation of these mutant plants in comparison with the allelic mutant plants revealed that phenotypes in different alleles fell into the range of variation as observed among wild-type plants grown simultaneously.

Based on the findings discussed above, we hypothesize that the interaction between SAT5 and SCL11 might serve to transfer the sulfur status signal to the transcriptional machinery. SCL11 does not have to directly bind to DNA but could also act indirectly as coactivator, as it has transactivation abilities, or could interact with transcription factors to modulate gene expression. Another possibility, as the precise role of SCL11 is not