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

yet known, is that it may modulate (positively or negatively) the activity of SAT5 in certain conditions. However, the confirmation of any of these hypotheses needs further studies.

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3) Analysis of the tobacco *UP15* gene induced during sulfur and nitrogen deficiency

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Sulfur is an essential macro-element, which in many geographical regions is a limiting factor for plant biomass production. Low sulfur availability in soils resulting from decreased atmospheric pollution or usage of S-free fertilizers decreases quantity and quality of plant yield. Since the importance of sufficient supply of S on plant yield has become apparent, much greater emphasis is put on studies focused on adaptation, tolerance and changes in metabolic pathways in plants exposed to S deficiency stress, as well as on regulatory aspects of plants response to S limitation. Despite several years of intensive studies, the details of these regulatory mechanisms are still unknown and many questions concerning signaling networks and significant regulatory factors that take a part in response to S-deficit remain unanswered. Analyses of genes encoding proteins of unknown function seem to be a reasonable approach to help clarify these problems.

The *UP15* gene from tobacco (*Nicotiana tabacum* cv LABurley21) was identified in our laboratory as induced by short term S starvation. Two independent clones corresponding to *UP15* were found during screening for differentially regulated genes by suppression subtractive hybridization (SSH) method. Regulation of *UP15* gene by S deficiency in young and mature leaves of tobacco was verified by quantitative RT-PCR. Moreover, *UP15* appeared to be up-regulated even stronger than by S-deficiency in the conditions of nitrogen deficiency. *UP15* encodes a small (168 amino acids) Gly-rich protein of unknown function. Near the C-terminal part of this protein the sequence for nuclear localization signal (NLS) can be found, however, *in silico* analysis revealed that several other subcellular localizations of the UP15 protein should be considered, including chloroplasts. To determine function of UP15 protein and to identify protein partners that would be able to interact with this protein *in vivo* under S-deficit, the yeast-2-hybrid (Y2H) system was applied with cDNA library prepared from two-month-old tobacco plants maintained for 2 days in S deficient conditions. One of the candidate clones identified in this experiment encoded part of amidophosphoribosyltransferase (ATase) called also glutamine phosphoribosylpyrophosphate amidotransferase (GPAT). This enzyme is localized in stroma of chloroplast and is responsible for the first step of purine biosynthesis by transforming glutamate into glutamine. Interaction between UP15 and full length ATase was confirmed by Y2H and *in vitro* by pull-down assay of the proteins expressed in bacterial cells. However, it still remains to be demonstrated if both proteins interact in plant cells. The, preliminary results indicated that the full length ATase fused to fluorescent protein (YFP or CFP) could be detected, as expected, in close proximity or inside the chloroplasts. However, the UP15 protein seems to be unstable and several independent trials failed to demonstrate presence of the UP15-YFP fusion proteins *in planta*. Thus, it is tempting to speculate that UP15 is a regulatory unit quickly degraded in plant cell.

The detected interaction might suggest that UP15 plays a role in adaptation of plant metabolism to the imbalanced nitrogen-sulfur homeostasis due to reduced availability of sulfur or nitrogen source. However, the hypothetical role of UP15 in this regulatory network remains to be determined.

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4) Using metabolic engineering to improve the nutritive quality of rice

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Rice is a staple food for 3 billion people and can account for a significant proportion of their dietary protein, especially in the developing nations of Asia. However, rice protein is deficient in several amino acids essential in diets of non-ruminant animals, including lysine, tryptophan, and methionine. Unless the diet is supplemented with other protein sources, these amino acid deficiencies can result in significant health consequences for humans and significantly stifled growth in animals. Traditional rice breeding programs have developed high yielding varieties with higher seed protein levels (e.g. IR64), but these varieties are still deficient in several essential amino acids. A more targeted approach to improve the amino acid balance in rice is seed-specific expression of exogenous storage proteins to 'pull' essential amino acids into the seed. Sunflower seed albumin (SSA), with its very high methionine and cysteine contents (16% and 8% respectively), is one of the most sulfur-rich storage proteins known. In addition, SSA is rumen stable, meaning that the improved amino acid profile of SSA rice would be bioavailable, even to ruminant animals like sheep, goats, and cattle. Despite achieving high SSA expression in transgenic rice seed, overall levels of essential amino acids remained nearly unchanged. This suggests that free amino acid pools, in particular methionine and cysteine, limit protein expression in this context, and that limiting amino acids are redirected into SSA expression from endogenous seed proteins. An alternative targeted approach to improve the nutritive quality of rice seeds is to increase the biosynthesis of cysteine and methionine. Extensive work in model organisms such as Arabidopsis and tobacco and crops such as potato suggests that overexpression of serine acetyltransferase and feedback-insensitive cystathionine-gamma-synthase would have the potential to increase the synthesis of cysteine and methionine to the point where these free amino acids are no longer limiting to storage protein expression. These 'push' approaches proved to be somewhat successful in increasing seed protein incorporated methionine, but the gains fell short of producing greatly improved nutritive quality. Since neither manipulation of sink nor source strength proved to be entirely sufficient on its own, we are combining these two approaches in one rice line. We anticipate that this "Push plus Pull" approach will result in rice with greatly improved nutritive quality for human and animal consumption.