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.