RNAi-mediated gene silencing in legumes

L. HANDSCHUH, B. FLOREK, M.M. SIKORSKI, M. FIGLEROWICZ

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland
e-mail: luizahan@ibch.poznan.pl
e-mail: beti@ibch.poznan.pl

The major goal of the presented studies is the elaboration of an effective method of RNAi-mediated gene silencing in legumes. Their objects are three lupine species: *Lupinus angustifolius*, *Lupinus luteus* and *Lupinus albus*. The target sequences, *pds* gene coding for phytoene desaturase and *Llpr-10* genes encoding small pathogenesis-related proteins of class 10, were introduced to plant tissue by agroinfiltration method. *Agrobacterium tumefaciens* was transformed with a binary vector pAWo59 containing a fragment of the target gene or a whole coding sequence inserted in 3 orientations: sense, antisense and both together in a hairpin-type construct.

The lack of phytoene desaturase expression disrupts the carotenoid biosynthesis pathway, which results in photobleaching. Most of the *pds*-silenced plants (i.e. *Nicotiana benthamiana*, *Pisum sativum*, *Nicotiana tabacum*) show characteristic white spots on the leaf surface. However, our preliminary results show completely different visual effects: the *pds*-hairpin construct treated plants have light green closed leaves in comparison to dark green flat leaves of the control plants.

*Llpr-10* genes are members of multigene family expressed in all plant tissues but at different levels. Some are induced/suppressed by plant hormones, especially cytokinins and plant defense signaling molecules such as salicylate, but their biological function still remains unknown. We succeeded to efficiently silence *Llpr-10.2b* gene with a hairpin construct. However, the target gene silencing was not followed by any phenotypic effects. Presumably, the missing gene function was substituted by homologous genes which were not suppressed despite the high level of identity.

Identification and expression of miR159 in * Pharbitis nil*

P. NOWAKOWSKA, W. WOJCIECHOWSKI, J. KOPCEWICZ

Department of Physiology and Molecular Biology of Plants, Institute of General and Molecular Biology, Nicolaus Copernicus University, Torun, Poland
e-mail: pnowa@uni.torun.pl

Introduction: MicroRNAs are noncoding RNAs ~21nt in length that have been identified in both animals and plants. They function by silencing the activity of genes. It occurs both on the level of transcription and translation, as well as during the remodeling of chromatin. They are in-
involved in growth and development control and differentiation. In plants miRNA take part in such processes as the control of the polarity of leaves, the unequal fission of the cells of stomatal apparatuses or the development control of inflorescence. In [http://www.sanger.ac.uk/Software/Rfam/mirna/](http://www.sanger.ac.uk/Software/Rfam/mirna/) we found two microRNAs in *Arabidopsis thaliana* that are connected with flowering processes: miR159 and miR172. MiR159 directs the cleavage of mRNA encoding GAMYB-related proteins. These proteins are thought to be involved in the GA-promoted activation of the floral meristem-identity gene *LEAFY*. The miR172 is involved in regulation of flowering time and floral organ identity by repressing translation of *APETALA2*-like genes. The aim of this work was to identify miR159 homolog and examined its expression in the obligatory short-day plant *Pharbitis nil*.

**MATERIAL AND METHODS**

Low molecular mass RNA were isolated from different tissues of seedling and flower buds of mature *Pharbitis nil*, precipitated, separated and subjected to blot-hybridization analysis.

**RESULTS AND CONCLUSION**

RNA blot analysis using a probe antisense to the miR159 identified 21–25 nucleotides RNA accumulating in all tissues. Strong signal was showed especially in vegetative and flower buds. There were no significant changes in accumulation of miR159 transcripts between control and after a 16-hour inductive night plants. These observations indicate that the sequence of miR159 is highly conserved and could be involved in *Pharbitis nil* buds development.

References:
we found that mitochondria are inherited from *S. girgensohnii*. However this species was excluded as a donor of chloroplasts for *S. russowii*.

These data show that there is a general uniparental mode of organellar transmission in Bryophytes, with some exceptions, as in the case of flowering plants. Further studies are necessary to determine whether the transmission in Bryophytes is maternal or paternal.

**RNA interference-mediated silencing of two *Arabidopsis thaliana* genes encoding nuclear cap-binding proteins**

P. PIONTEK, A. KASPROWICZ, M. SAWCZAK, J. CICHOCKA, A. JARMOLOWSKI

Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznan, Poland
e-mail: ppiontek@amu.edu.pl

Nuclear cap-binding protein complex (CBC) consists of two subunits cap-binding proteins (CBP): CBP20 and CBP80 and is involved in several aspects of RNA metabolism. The CBC binds to the cap structure of all RNA polymerase II transcripts and promotes efficient splicing of pre-mRNA, nuclear export of U-rich small nuclear RNAs, mRNA 3' end formation and also plays a role in nonsense-mediated mRNA decay (NMD). In our laboratory we have previously characterized CBC from *Arabidopsis thaliana* (AtCBC), which is also composed of two subunits, AtCBP20 and AtCBP80. An increasing number of genetic mutations that contribute to plant hormones signaling have been characterized. Two of them – T-DNA insertion mutations in cbp20 and cbp80 have been described recently that affect response to abscisic acid (ABA).

ABA regulates several physiologically important stress and developmental responses throughout the life cycle of plants. During seed development, ABA is responsible for the acquisition of nutritive reserves, desiccation tolerance, maturation and dormancy. During vegetative growth, ABA is a central signal that enables plant responses to various adverse environmental conditions such as drought, salt and cold stresses.

Cbp20 and cbp80 insertion mutants are recessive and show ABA hypersensitivity in early steps of ABA signaling. We have obtained mutants of *Arabidopsis* with silenced expression of cbp20 or cbp80, using RNAi phenomenon. RT-PCR and Real-time PCR assays showed strongly decreased expression level of both genes studied in silenced plants. The RNAi and knockout mutants of AtCBP20 and AtCBP80 have been compared in the context of phenotype features, drought stress tolerance and reaction on ABA presence. Our observations confirmed high similarity between these plants. Contrary to the wild type, all of them show slightly delayed development and serrated rosette leaf margins, fail to germinate on medium containing 0.3 μM ABA and display an increased tolerance to water deprivation. Our data are consistent with previous observations and strongly suggest that the whole CBC may participate in the transduction of stress signals mediated by ABA.
Symbiotic nitrogen fixation in narrow-leafed lupin investigated with differential screening and cDNA arrays

A. ZMIENKO, A.B. LEGOCKI, M. FIGLEROWICZ, J. PODKOWINSKI

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland
e-mail: akisiel@ibch.poznan.pl

Symbiotic interactions with nitrogen-fixing rizobia is (with one known exception) a unique feature of legumes. Establishing symbiosis leads to formation of root nodules where atmospheric nitrogen is fixed by the microsymbiont. Following perception of chemical signals produced by bacteria (Nod factors), proper activation or suppression of specific plant genes is required at every step of this complex process. Until now, several plant genes specific for- or induced during symbiosis have been described, with only a few being characterized functionally. Some of them show homology to genes involved in other processes like arbuscular mycorrhiza, lateral root formation or pollen tube growth, suggesting that those common mechanisms have been recruited and modified during evolution of symbiotic nitrogen fixation.

Our objective was to identify *Lupinus angustifolius* genes induced or suppressed during symbiosis with *Bradyrhizobium* sp. (*Lupinus*) and to compare their expression patterns during symbiosis to other conditions (stress, pathogenesis, hormone induction). We selected 59 candidate genes by differential screening of 5000 recombinants from a cDNA library prepared from roots with nodules. PCR-amplified cDNA fragments representing those pre-selected genes were used to prepare cDNA arrays, together with probes for 67 randomly selected clones from the same library and about 20 probes representing genes – markers of symbiotic nitrogen fixation and different metabolic pathways. With those arrays gene expression profiling during symbiosis (from 0 to 34 days post inoculation with microsymbiont) and in other physiological states was performed. Results of this work will be presented and discussed.
The cortex and pericycle cells start to dedifferentiate, and the nodule primordium is formed on this stage of symbiosis. The pool of subtracted cDNA molecules (SNF 5 dpi versus root) was cloned and 768 clones picked up by random were organized in an array. SNF (Symbiotic Nitrogen Fixation) specific genes were identified in two different screens: 1 – hybridization of cDNA arrays with subtracted probes (forward versus reverse) and 2 – hybridization of the arrays with unsubtracted, complexity probes (probe representing transcriptome of infected root 5 dpi versus probe uninfected root).

The same technique – hybridization of cDNA arrays with complexity probe was applied to profile expression of the isolated clones.

**Root hair morphogenesis in two mutant Arabidopsis thaliana plants carrying disrupted genes encoding nuclear cap-binding proteins**

M. SAWCZAK, P. PIONTEK, A. JARMOLOWSKI, Z. SZWEYKOWSKA-KULINSKA

Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznan, Poland

e-mail: msawczak@op.pl

The CBC is nuclear cap-binding complex which consists of two subunits CBC20 and CBC80. CBC participates in RNA-processing events probably by regulation of: intron splicing, nuclear export, transcript stability and RNA degradation. CBP20 and CBP80 insertion mutants show ABA hypersensitivity and few developmental changes like serrated leaf margin or delayed growth. Abscisic acid plays a primary role in plant responses to many stress conditions for example drought and cold stress. It also participates in many growth and developmental processes.

Root hairs are tubular outgrowths from root epidermal cells. They are crucial for plant nutrition and water uptake. Root hair development can be subdivided into several stages: selection of a site for hair formation, swelling formation, the transition to tip growth and tip growth maintenance. Root hair growth is modulated by many hormonal, developmental, and environmental signals. In the case of wild type plants the response to phosphorus or iron deficiencies root hair length and density increase. These morphogenetic changes in roots lead to a higher surface-to-volume ratio. Various hormone-related Arabidopsis mutants exhibit differences in response to P and Fe deficiency stress leading the conclusion that some plant hormones participate in root hair morphogenesis. Little is known about ABA participation in this process.

In our laboratory we investigated whether mutants CBP20 and CBP80 show differences in root hair morphogenesis. Our observations revealed that root hairs appearance in the case of both mutants grown in control medium slightly differs from wild type plants in shape and length. Unexpectedly, dramatic changes at almost every stage in root hair development were observed in both mutants grown in medium without P or Fe. In the case of phosphorus deficiency, mutants have a significant portion of root hairs, that apparently do not make the transition from swelling to tip growth producing thick short hairs. Exposure of mutants to medium without iron causes multiple tip formation on individual swelling that do not follow elongation.
Analysis of HYL1 Arabidopsis thaliana gene expression

B. SZARZYNSKA, J. LESICKA, J. CICHOCKA, A. JARMOLOWSKI, Z. SZWEYKOWSKA-KULINSKA
Department of Gene Expression, Instytute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznan, Poland
e-mail: bogna-szarzynska@wp.pl

The HYL1 (Hyponastic Leaves 1) gene is present in the Arabidopsis genome in a single copy, on the first chromosome. HYL1 protein seems to be a part of a macromolecular complex that is involved in miRNA metabolism. Not only does hyl1 mutation cause developmental abnormalities, but also it impairs responses to plant hormones, among others it increases sensitivity to abscisic acid (ABA) [1]. It is known that ABA has strong influence on ontogenetic development as well as plant responses to environmental stresses, such as drought, high salinity, cold stress and pathogen attack.

The HYL1 gene is expressed in different tissues of A. thaliana at similar levels and the abundance of HYL1 transcripts is downregulated by ABA. It is consistent with the fact that the basal amounts of transcripts of two ABA-inducible genes: COR47 and KIN2 are higher in hyl1 homozygous mutants than in wild type plants [1]. Those data suggest that the HYL1 protein is a negative regulator at the transcript level or a component of a complex which play such role.

In our laboratory we have obtained lines of A. thaliana wild type and homozygous hyl1 mutant plants (with T-DNA insertion from the SALK Institute) transformed with pCAMBIA binary vector containing the HYL1-GFP sequence expressed from the natural promotor of HYL1 and cauliflower mosaic virus promoter (35S). We are testing whether the presence of HYL1-GFP fusion protein reverts the A. thaliana hyl1 mutants to the wild type phenotype. We are also compare effects of the influence of ABA on wild type plants, mutants with no expression of HYL1 and those exhibiting overexpression of this protein. We have previously observed that in transfected protoplasts of Nicotiana tabacum and Arabidopsis thaliana both GFP- HYL1 and HYL1-GFP proteins concentrate in a nucleus and cytoplasm and their ability to form subnuclear bodies seems not to depend on the presence of exogenous abscisic acid. However, one should keep in mind that the abscisic acid receptors has not been identified yet. To gain further insight into the matter of correlation between the action of abscisic acid and activation of HYL1 gene regulatory sequence we acquired plants, which express GFP-GUS and GUS- GFP fusion sequences from the natural promotor of HYL1.

References:
A new subgenomic RNA of brome mosaic virus

A. URBANOWICZ1, R. WIERZCHOSLAWSKI2, A. DZIANOTT2, M. FIGLEROWICZ1, J.J. BUJARSKI1,2

1Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland
2Plant Molecular Biology Center and the Department of Biological Sciences, Northern Illinois University, USA
e-mail: aniau@man.poznan.pl

Brome mosaic virus (BMV) is a tripartite, single-stranded model (+)RNA virus of plants. Its genome is composed of monocistronic RNA1 and RNA2 and dicistronic RNA3. Previously it was postulated that subgenomic RNA4, responsible for expression of coat protein from the dicistronic RNA3 segment is the only subgenomic RNA of BMV. In the course of our research we have observed a second subgenomic RNA (RNA3a) that accumulates in BMV-infected plants. In addition, ultracentrifugation of BMV virus preparation revealed encapsidation of RNA3a in BMV virions. Cloning and sequencing demonstrated that RNA3a corresponds to the 5' portion of BMV RNA3 (the movement protein 3a), and resembles messenger RNA in carrying the capped 5' end and a short polyA tail at the 3' end. The in vitro replication reaction demonstrated an efficient production of RNA3a during copying of negative RNA3 strands. We propose a mechanism of discontinuous RNA3 synthesis, where RNA3a arises by pausing of BMV replicase at the polyU tract. Overall, we report a novel mechanism engaged in the formation of subgenomic RNAs in plus RNA viruses and explain the function of the internal polyA tract during BMV RNA replication.