

Verification of a specific microRNA target site by allele replacement in the moss *Physcomitrella patens*

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Since their discovery in *Caenorhabditis elegans* 1993 (Lee et al. 1993) miRNAs have become a very interesting item for research in all biological and medical categories. At least in between the plant kingdom particular miRNAs are highly conserved. In 2005 the first miRNAs from *Physcomitrella patens* were published (Arazi et al. 2005). In *Arabidopsis thaliana* miR160 is known to target the Auxin Response Factor 17 (ARF17). We performed BLAST searches using the miR160 sequence from *Arabidopsis thaliana* to identify putative miR160 target genes in *Physcomitrella patens*. We identified a gene encoding an auxin response factor (ARF), which showed the highest similarity to the ARF10 from *Oryza sativa*. By small Northern blotting with RNA extracted from auxin-treated wild type moss the presence of miR160 could be verified in *Physcomitrella patens* and we were able to show its induction by auxin. Also the cleavage product of the ARF mRNA was confirmed by 5'RACE-PCR. The cleavage site was mapped between position 10 and 11 of the miRNA. To elucidate the influence of miRNA regulation, we replaced five nucleotides within the miRNA binding site of the ARF gene in order to achieve miRNA resistance. The replacement was done without a change in the amino acid sequence of the protein. The perfect allele replacement was shown by PCR and sequencing of the ARF cDNA. The mutant did not show the miRNA cleavage product neither in the RACE-PCR nor in the Northern blot. An aberrant phenotype of the ARF10 mutant plant could not be detected which could be due to a redundancy between several miR160 regulated ARFs as it was shown in *Arabidopsis* for the ARFs 10, 16 and 17 (Okushima et al. 2005; Wang et al. 2005).

The expression of putative ARF target genes will be analysed in the miR160 resistant ARF mutant line.

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We gratefully acknowledge financial support from Landesstiftung Baden-Württemberg (P-LS-RNS/40).