

The Plant Specific WRKY Transcription Factor Family in *Physcomitrella patens*

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WRKY proteins are a class of transcription factors that are confined to the plant kingdom. The WRKY domain, a 60 amino acid region, can be divided into the N-terminal conserved amino acid sequence WRKYGQK and a C-terminal zinc-finger-like motif. WRKY proteins have been identified due to their specific binding to a conserved promoter element, the W Box, with its consensus sequence (T)TGAC(C/T). In higher plants, W boxes were identified as functionally important elements in the promoter regions of several senescence and defense response genes.

The WRKY transcription factors in *Arabidopsis thaliana* comprise a large gene family of 74 members. Up to now they have been shown to be involved in various processes, including development and senescence as well as responses to stress and pathogen attack. Therefore, WRKY transcription factors are thought to play an essential role in senescence, stress and pathogen-triggered signal transduction cascades.

WRKY proteins can be structurally divided into three groups based on the number and type of their WRKY domains: Proteins with two WRKY domains belong to group I and those with one WRKY domain belong either to group II or III. Group II and III WRKY proteins can be distinguished from each other mainly by differences within their zinc-finger motif.

A subfamily of group II WRKY proteins contains a second highly conserved amino acid motif outside the WRKY domain, the so called HARF domain. Although no defined function has been assigned to this domain, the absolute conservation of the motif GHARFRR within this subfamily suggests a functional significance. Outside the conserved regions the overall sequence homology of the WRKY proteins is low.

We used a PCR-based approach with degenerate primers to isolate WRKY genes from *Physcomitrella*, opening up the possibility of targeted disruption of the gene sequence by homologous recombination. In addition, seven EST sequences corresponding to WRKY genes were found in GenBank, that seem not to overlap with known PpWRKY sequences. In total, 16 different WRKY gene fragments have been obtained.

This offers an excellent opportunity to study the role of different WRKY transcription factors in diverse processes conserved between lower and higher plants.

In addition, the highly conserved amino acid sequences within the WRKY and HARF domains of all Group II / HARF proteins, even across plant orders, suggest a conserved function of this subfamily of transcription factors: Sequence comparison of the WRKY/HARF domains between the *Physcomitrella* and *Arabidopsis* counterparts revealed that they are more closely related to each other than the *Arabidopsis* subfamily to any other *Arabidopsis* WRKY genes.

PpWRKY1 is the only Group II / HARF protein studied that is expressed both in protonema and the leafy gametophyte. A knock-out mutant for PpWRKY1, generated via homologous recombination, did not reveal any phenotypic change. All the other Group II / HARF genes studied so far were shown to be not expressed either in Wt or in mutant moss protonema.

Hence, functional redundancy may exist within the group meaning that more than one related WRKY gene will have to be disrupted before gene function can be determined.