Abstract

During the transition from vegetative to reproductive growth severe changes take place at the apical meristem of plants. The undetermined vegetative meristem changes slowly to the determined flower meristem, and the floral organ primordia are initiated in a whorl like manner, followed by the process of organogenesis of the flower organs. This transitions is due to the spatial and temporal expression of a variety of highly specialized genes. Many of these genes involved in these processes have been identified by the analysis of their respective mutants. The first floral identity gene to be cloned was *DEFICIENS* (DEF) from the model plant *A. majus* (Sommer et al., 1990).

DEF determines, together with GLOBOSA (GLO), the fate of the second and third whorl organs of the *A. majus* flower. Mutations in the *DEFICIENS* gene lead to severe phenotypic changes. In homeotic mutants the second whorl petals are converted to sepals, whereas in the third whorl stamens are replaced by carpeloid organs. The expression of *DEF* and *GLO* are switched on independently but than act together in up-regulating and maintaining their own expression by autoregulation, and controlling the expression of target genes. Their expression is tightly regulated in an organ and tissue specific fashion (Tröbner et al., 1992). Almost nothing is known about the molecular mechanisms that underlie this tight regulation.

The aim of this work was to elucidate the regulation of *DEFICIENS*. A yeast one-hybrid (Y1H) experiment was performed with the *DEF*-promoter as bait. In this way AmTCP1 was isolated. AmTCP1 is a DNA binding protein and belongs to the TCP family of transcription factors (Cubas et al., 1999). In yeast one-hybrid (Y1H) experiments and in EMS-Assays (electrophoresis mobility shift) AmTCP1 binds to the TCP-motif of the *DEF*-promoter at position –1337. Furthermore, EMS-assays with mutated *DEF*-TCP-motives and TCP-motives from other plant promoters showed that AmTCP1 binds specifically and with high affinity to DNA containing the core sequence GGNCCC.

To check the biological relevance of these interactions we isolated plants carrying a mutation in the *AmTCP1* gene. By screening a transposon-tagged snapdragon population, a plant line was identified with a Tam3-element inserted in the promoter of *AmTCP1*. The analysis of this line is still going on. The heterologous over-expression of AmTCP1 in *A. thaliana* and the transient over-expression in *Arabidopsis* leaves (in the presence of a *DEF*::GFP reporter construct) was not able to establish the biological relevance of the interaction. It was suspected that AmTCP1 needs special flower specific co-factors to perform its task as transcription factor. Therefore, by performing a yeast two-hybrid screen, we were able to isolate interactors whose interaction with AmTCP1 is currently being investigated.

In parallel to the interaction studies we were interested in the biochemical properties of AmTCP1. Since AmTCP1 is insoluble in *E. coli* and usually precipitates in the inclusion bodies we developed strategies to produce recombinant protein in large amounts. With the full length recombinant protein and truncated versions the domain structure of AmTCP1 was analysed.

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