

MADS about MOSS

Stacy D. Singer¹, Naden T. Krogan² and Neil W. Ashton^{1*}

¹Department of Biology, University of Regina, Regina, Saskatchewan, S4S 0A2, Canada

²Department of Botany, University of Toronto, Toronto, Ontario, M5S 3B2, Canada

*Email: physcomitrella@gmail.com Tel: +1 306 585 4252 Fax: +1 306 337 2410

Classic *MIKC*-type MADS-box genes (*MIKC*^c genes) are indispensable elements in the genetic programming of pattern formation, including the segmental organisation of angiosperm flowers, in seed plants. Since little is known about the functions of *MIKC*^c genes in non-seed plants, a functional analysis of moss *MIKC*^c homologues was performed using the genetically amenable, simple model plant, *Physcomitrella patens*.

Expression of moss homologues was knocked down using an antisense RNA approach or abolished by generating transformants with single gene knockouts.

The knocked down (“antisense”) transformants displayed a multifaceted mutant phenotype comprising abnormal leaf morphogenesis, delayed gametangia formation and abnormal sporophyte development, suggesting that *MIKC*^c genes are involved in diverse aspects of the moss developmental program. Conversely, knocked out transformants were phenotypically normal, consistent with the existence of functional redundancy within the small group of *MIKC*^c genes in the *P. patens* genome. Consequently, we have been unable to assign specific functions to particular genes.

In situ gene expression studies revealed that the moss *MIKC*^c genes are generally expressed fairly ubiquitously in gametophytic and sporophytic tissues. This observation lends support to the hypothesis that broad expression patterns represent the ancestral state of MADS-box genes in land plants and that organ-specific expression, which characterises many *MIKC*^c genes in extant spermatophytes including those that specify floral organ identity, corresponds to a derived condition that evolved in this lineage following its separation from the lineage that led to ferns and fern allies.

One of the functions of MADS-box genes in *P. patens* appears to be an involvement in gametangia formation (the differentiation of reproductive organs from non-reproductive tissues at the gametophore apex). Interestingly, expression studies of *MIKC*^c genes in charophycean algae suggest that they too are involved in haploid reproductive cell differentiation. This function assumes a special significance because it is analogous to that of C-function genes in spermatophytes.

However, in view of the broad expression patterns of the moss *MIKC*^c genes, as well as the pleiotropic effects of *MIKC*^c gene knock-down mutations, it seems more plausible that the genes described here are not organ identity genes. Instead, these genes probably play a more general role in the transcriptional control of development or cell differentiation, as has been proposed previously for ancestral *MIKC*^c genes in land plants. In fact, the abnormally large cells and irregular leaf margins and midribs observed in the moss *MIKC*^c knock-down strains hint that these genes could function in an extensive manner through involvement in the regulation of cell growth and/or division.

We conclude that *MIKC*^c genes play significant roles in morphogenetic programming of *P. patens*. Functional redundancy characterises some members of the gene group.

Our findings provide clues concerning the ancestral roles of at least some *MIKC^c* genes that may be represented in the genomes of diverse extant plant taxa.