

## FUNCTIONAL ANALYSIS OF *MOB1*-LIKE GENES USING *ARABIDOPSIS* T-DNA TAGGED MUTANTS

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A novel family of proteins (Mob1, Mps-one-binder) has been demonstrated to be important for both mitosis completion and cell plate formation in yeast. This family includes a group of cell cycle-associated proteins highly conserved in eukaryotes, whose founding members are implicated in mitotic exit and co-ordination of cell polarity with cell cycle progression. Mob1-related proteins have been also found in animals. Although there are data to suggest that Mob1 proteins act as kinase activating subunits in higher eukaryotes, their function remains to be proved. Present findings imply animal and yeast Mob1 have similar functions. The key role of Mob1 in plants is poorly understood.

Recently, we cloned two *Mob1*-like genes from alfalfa (*Medicago sativa*). Overall results proved that Mob1-like proteins are involved in cell proliferation and are localized in the cell division plane during cytokinesis. In addition, *Mob1*-like genes showed to play a key role during the reproductive pathway in plants: localization of their transcripts and proteins was associated to meiotic division abnormalities and programmed cell death within reproductive organs. The involvement of *Mob1* genes in cell cycle control and programmed cell death is also supported by recent data collected in *Arabidopsis thaliana* and *Drosophila melanogaster*.

The aim of this work is to elucidate the role of *Mob1* genes in plants. To gain further insights on their function, two *Arabidopsis Mob1*-like genes were bioinformatically, genetically and molecularly characterized. Reproductive organs of *Arabidopsis Mob1*-like mutants (loci At5g45550 and At4g19050) were also investigated by means of cytohistological techniques.

Protein database searches disclosed ovary-specific Mob1 gene products in nematodes, human, rat and mouse organisms. Moreover, it has not escaped our notice that Mob1 can be a component of multi-domain proteins. The Mob1/phocein domain (pfam03637) can be combined (*e.g.* in *Arabidopsis* and rice) in complex proteins with elements of the NB-ARC domain (pfam00931), a signaling motif shared by cell death gene regulators, as well as with motifs of the LRR domain (pfam00560). Proteins containing a highly conserved Mob1 domain include also receptors for ubiquitination targets (F-box), Seryne/Threonine and Tyrosine kinases as well as CBL-interacting kinases.

Functional analysis of *Mob1*-like genes was attempted by using T-DNA tagged Mob1 mutants of *Arabidopsis* obtained from the SALK Institute. A number of selfed progeny plants were used for genetic analysis and assayed by PCR with specific primer combinations. Segregation patterns were not significantly deviating from the expected ratios for both *Mob1*-like genes. *Arabidopsis Mob1*-like genes proved to be highly expressed in roots and siliques and less expressed in stems and leaves. The amount of transcripts was intermediate in flowers although both genes were shown to

be upregulated during meiosis and gametogenesis. A closer inspection of the quantitative data showed that gene expression of At5g45550 was higher than At4g19050 (from 2 to 3-fold times). Plants that displayed a double dose of the allele carrying the T-DNA were investigated on the basis of plant morphological traits and cytohistological observations of meiosis and gametogenesis. Neither sporophytic nor gametophytic alterations were observed. Therefore, single insertional homozygous mutants were crossed to generate double mutants with either T-DNA tagged genes. Results of Arabidopsis Mob1-like gene segregation and expression are reported and the potential role of plant Mob1 genes discussed.