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Sporophytic and gametophytic functions of the cell cycle-associated Mob1 gene in *Arabidopsis thaliana* L.

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ABSTRACT

Mob1 genes are primarily involved in the cell cycle progression and mitosis exit in yeasts and animals. The function of a *Mob1*-like gene (At5g45550) from *Arabidopsis thaliana* was investigated using RNAi and immunological staining. *AtMob1*-like RNAi silenced lines showed a reduced radial expansion of the inflorescence stem and a reduced elongation zone of the primary root. Morphological features of plant organs were accompanied by a reduction in cell size. The fertility of *AtMob1*-like RNAi silenced lines was very low as seed production was strongly reduced. About 2% of the progeny of *AtMob1*-like RNAi silenced plants were tetraploid. The female and male sporogenesis was affected differentially. The ovules developed irregularly and one third of the megaspores and embryo sacs degenerated prematurely. Up to 20% of the ovules produced binucleated megaspores that failed to develop further, being their degeneration likely accompanied with a delayed programmed cell death. The anthers produced about 30% of aborted pollen grains, showing also a strong variation in their size. Together, the results show that Arabidopsis MOB1-like is required to regulate cell expansion and cell division, presumably by affecting the mitotic as well as the meiotic cell cycle.

1. Introduction

Two distinct signaling networks, the mitotic exit network (MEN) and the regulator of apoptosis and cell cycle exit (HIPPO pathway), control cell proliferation and involve Mob1-like proteins in *Saccharomyces* spp., *Drosophila* and mammalian cells (Bothos et al., 2005; Edgar, 2006; Hergovich et al., 2006). Homologous sequences with significant similarity to components of the MEN network have been found in plants, but their function in cell cycle regulation remains still unclear (Van Damme et al., 2004; Citterio et al., 2005, 2006; Bedhomme et al., 2008). Functional characterization of Mob1 in *S. cerevisiae* and *S. pombe* has revealed that Mob1p is a regulator of the localization and activity of Dbf2 protein kinase (reviewed by Vitulo et al., 2008) and that Mob1p–Dbf2 interaction leads to release from the nucleolus and subsequent activation of Cdc14p phosphatase during anaphase (Luca et al., 2001; Stegmeier and Amon, 2004). The release of Cdc14 from its inhibitor complex (Shou et al., 1999; Visintin et al., 1999) promotes the inactivation of the mitotic CDK1-cyclin B complex, which is the hallmark for mitosis exit. Recent advances in *Drosophila* led to the identification of Mats (Mob as tumor suppressor, dMob1) as a factor that induces tissue overgrowth without affecting pattern formation. Along with Mats, the Hippo (Hpo), Salvador (Sav), and Lats/Warts (dNDRs) factors are part of a pathway that participates in the control of tissue growth and programmed cell death (Harvey et al., 2003; Jia et al., 2003; Pantalacci et al., 2003; He et al., 2005; Huang et al., 2005).

Differential display analyses performed on an apomeiotic mutant of *Medicago sativa* L., characterized by first and second division restitution mechanisms (Tavoletti, 1994; Barcaccia et al., 1996) responsible for 2*n* megaspore formation (Tavoletti et al., 1996; Barcaccia et al., 2000), led

Abbreviation: BSA, Bovine Serum Albumin; Cdc14p, Cell division cycle protein 14; CDK1, Cyclin dependent kinase 1; cDNA, DNA complementary to RNA; DAPI, 4',6-diamidino-2-phenylindole; Dbf2, Dumbbell former; DIC, Differential interference contrast microscopy; DMSO, Dimethylsulfoxide; FEAR, cdc-Forteen Early Anaphase Release network; Lats, large tumor suppressor; Mats, Mob as tumor suppressor; MEN, Mitotic exit network; MES, 2-(N-morpholino)ethanesulfonic acid; MMC, Megaspore mother cell; Mob, Mps one binder; MTSB, Microtubule-stabilising buffer; RNAi, RNA interference; Sav, Salvador; swi1, switch1; TNE, Two-N-Egg; UTR, Untranslated region(s).

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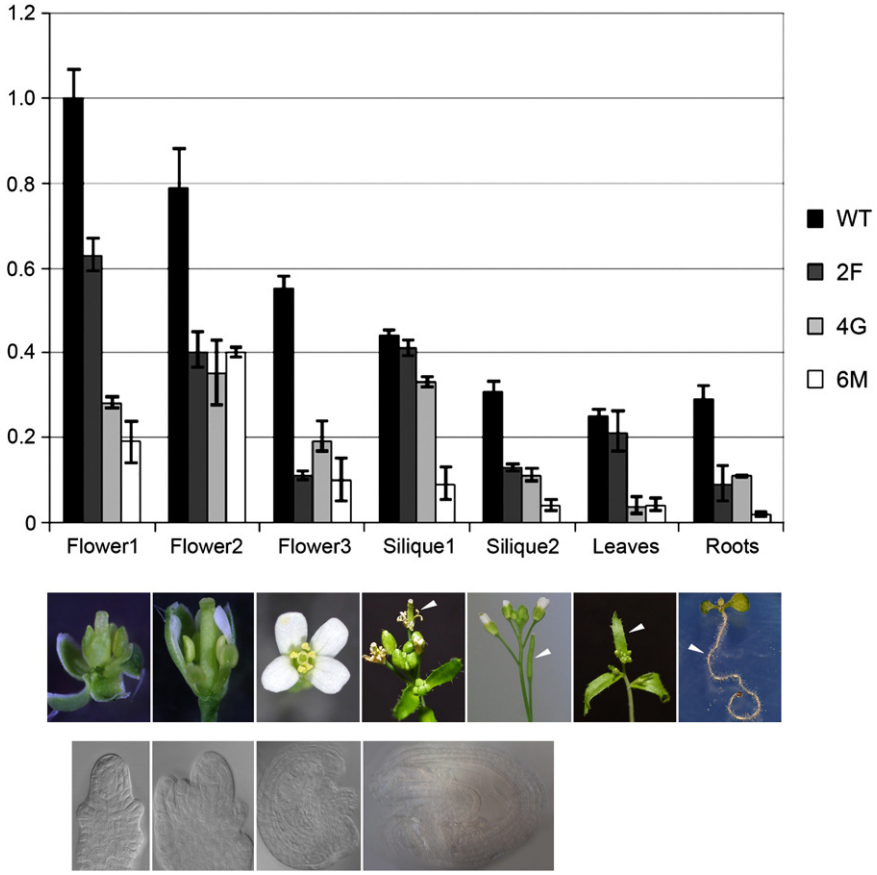

Fig. 1. Expression analysis in *AtMob1*-like RNAi lines as assessed by Real-Time PCR. Three different stages of the flower development were analyzed along with young and mature siliques, leaves and roots (bottom panels). Flower and associated ovule stages are reported below the histograms. Dark gray, light gray and white histograms refer to the expression levels recorded in 2F, 4G and 6M *AtMob1*-like RNAi lines, respectively. The black histograms refer to the wild-type. Data are expressed in arbitrary units normalized against the level of expression detected on wild type flower stage 1.

to the identification and characterization of *Mob1*-like genes, which were shown to be expressed with a contrasting transcript level and temporal pattern between mutant and wild-type plants (Barcaccia et al., 2001). In particular, of the two cloned alfalfa *Mob1*-like genes, one was constitutively expressed whereas the other was expressed only in flower buds during sporogenesis and gametogenesis. Analyses of gene expression during reproduction in alfalfa wild-types and 2*n* egg cell producers proved that *Mob1* transcripts and proteins are specifically localized in degenerating megaspores of normal ovules and in enlarged megaspore mother cells and embryo sacs of apomeiotic ovules characterized by restitutional meiosis (Citterio et al., 2005). Gene products were also found in microspore tetrads at the beginning of pollen development as well as in tapetum cells of anthers undergoing programmed cell death to allow pollen dispersal at maturity (Citterio et al., 2005). Overall results suggested that *Mob1*-like genes play a key role during the reproductive pathway in plants.

Here we report on the *Mob1*-like gene function in the model plant *Arabidopsis thaliana*. Down-regulation of the *Mob1* gene (At5g45550) affected sporophytic as well as gametophytic development, presumably because of misregulation of cell cycle progression.

2. Materials and methods

2.1. Construction of the binary vectors and plant transformation

For the production of a RNAi construct specific to the Arabidopsis *Mob1*-like gene (*At5g45550*), a unique 158 bp cDNA fragment was amplified using specific primers designed in the 3′-UTR: RNAMOB1FOR

Table 1
Statistics on morphological characterization (A) and seed production (B) of *AtMob1*-like RNAi silenced lines.

	Plant height (cm)	Leaves/rosette (no.)	Stems/plant (no.)	Branches/stem (no.)	Siliques/plant (no.)	Silique size (mm)	Days to flowering
A							
WT	$40.3^{a} \pm 2.7$	$13.4^{a} \pm 0.8$	$3.0^{b} \pm 1.1$	$2.9^{b} \pm 0.6$	$66.7^{a} \pm 4.2$	$47.9^{a} \pm 6.3$	$30.5^{a} \pm 1.4$
2F	$38.0^{a} \pm 4.0$	$13.0^{a} \pm 0.4$	$3.4^{b} \pm 0.8$	$3.4^{b} \pm 1.1$	$61.0^{b} \pm 6.2$	$33.0^{b} \pm 3.0$	$30.0^{a} \pm 1.3$
4G	$28.6^{b} \pm 2.8$	$9.0^{c} \pm 1.7$	$10.6^{a} \pm 2.5$	$5.4^{a} \pm 1.1$	$21.0^{c} \pm 7.1$	$17.8^{c} \pm 2.5$	$24.9^{b} \pm 2.1$
6M	$39.0^{a} \pm 3.7$	$12.0^{b} \pm 0.3$	$3.4^{b} \pm 1.0$	$3.0^{b} \pm 0.6$	$60.0^{b} \pm 5.8$	$31.0^{b} \pm 3.5$	$29.0^{a} \pm 1.8$

	Seeds/plants (no.)	Viable seeds/silique (no.)	Aborted seeds/silique (no.)	Aborted/total seeds (%)	Seed germinability (%)
B					
WT	$3614^{a} \pm 382$	$54.2^{a} \pm 4.8$	$3.6^{c} \pm 4.7$	6.2	98.5
2F	$2105^{b} \pm 402$	$36.0^{b} \pm 4.2$	$13.4^{b} \pm 6.1$	27.1	96.0
4G	$130^{d} \pm 74$	$10.4^{d} \pm 5.2$	$21.3^{a} \pm 6.4$	67.2	78.3
6M	$1754^{c} \pm 262$	$30.0^{c} \pm 6.7$	$15.7^{b} \pm 5.2$	34.4	94.0