ALFALFA MOB1-LIKE PROTEINS ARE INVOLVED IN CELL PROLIFERATION AND LOCALIZE TO THE DIVISION PLANE DURING CYTOKINESIS


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Progression through the cell cycle is central to the proliferation of cells and fundamental to the growth and development of all organisms, including higher plants. The spatial control of cell division is largely dependent on plant-specific cytoskeletal structures, such as the pre-prophase band, which marks the division plane before mitosis and the phragmoplast, which is required to lay down the new cell wall during cytokinesis. Mob1 proteins, a novel group of eukaryotic proteins, seem to play an important role in the cell cycle control, being involved in chromosome separation and completion of cytokinesis. An EST with significant similarity to the yeast Mob1 genes was isolated by mRNA profiling in a reproductive mutant of alfalfa and its full-length was obtained by RACE. Sequence-tagged site amplification and Southern blot hybridization revealed a multiple gene family in alfalfa with at least three separate members. Sequencing analysis of genomic DNA and cDNA clones enabled to find out two introns in all Mob1-like members. The organization of the genomic clone with information on CDS and indication of position and composition of regulating motifs and splicing sites is available in the EMBL database as accession number AJ635582. For mRNA expression analysis, a 400 bp specific cDNA fragment of the Mob1-like gene was transcribed in vitro to obtain DIG-UTP labelled RNA sense and antisense probes. Moreover, to produce an antibody for the detection of the Mob1-like protein, a peptide of 15 amino acids from the N-terminal region of the protein was selected on the basis of its predicted structure. The quantitative comparison of both transcripts and proteins detected in dividing and non-dividing root and leaf cells, as assessed by flow cytometry analysis, proved that Mob1-like is a gene mainly involved in cell proliferation. Nevertheless, the presence of Mob1-like transcripts and proteins in quiescent cells suggests an added function for the members belonging to the Mob1 family. Analysis of gene expression was performed in root tips by means of in situ hybridization in order to localize Mob1-like transcripts in alfalfa apical meristems. The hybridization signal was detectable in the cells of the root apex where periclinal cell divisions mainly occur and the protoderm differentiates. As expected, the signal was not detectable in the root cap. Western blotting of alfalfa proteins isolated from plantlet roots and cotyledons revealed a doublet of proteins of about 28 kDa and 47 kDa, providing that Mob may be a component of multi-domain proteins. Immulocalization in synchronized cells with the anti-Mob1 polyclonal antibody allowed to determine that Mob1-like proteins are expressed in a cell cycle-dependent manner. Along with a cortical localization late in interphase, the Mob1-like proteins were visualized during mitosis in the cytoplasm around the daughter nuclei as grain
clusters from which fibrillar structures radiate in all directions, preferentially toward the cell midplane. At the end of mitosis, Mob1-like proteins localized in the cell plate during cytokinesis by marking the progressive formation of the phragmoplast from the inside to the cell periphery. Grains likely correspond to sites in which microtubules are reorganized during cell cycle progression, the yeast SPBs and animal MTOCs.