Visualization of unconventional Myosin VIII isoforms in plant cells via GFP fusion constructs of the tail domains

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Abstract: Myosins comprise a large superfamily of motor proteins which move along actin filaments. Their exact roles in actin-based processes in plants are not yet fully understood. Myosins have three domains in common; a motor domain that hydrolyzes ATP and interacts with actin filaments, a neck domain that binds calmodulin light chains, and a tail domain that is thought to interact with the cargo. The structure of the tail domain varies between classes and even between members in the same class. In the Arabidopsis thaliana genome 17 myosin genes have been identified - class VIII myosins with four isoforms, and class XI myosins with 13 isoforms. With the intention to identify the cargo organelles, we tried to visualize the tail domains of the four class VIII isoforms in plant model systems via GFP-fusion constructs.

To this end, we fused GFP N-terminally to the tail domains of ATM1, ATM2, VIIIa, VIIIb and expressed these constructs transiently in Allium cepa and Vicia faba and by stable transformation in tobacco BY-2 cells and A. thaliana plants. Our data suggest, that ATM1 and VIIIa are localized to the plasmamembrane with particularly strong labelling at the plasmodesmata and the maturing cell plate in BY-2 and A. thaliana, corroborating our earlier immunofluorescence study (Reichelt et al.,1999). However, ATM2 and VIIIb are most strongly localized in the nucleoli and only weakly at the plasmamembrane, suggesting their involvement in nuclear functions possibly in the transport of nucleolar components. These results indicate that the two subgroups of class VIII myosins seems to have distinctive roles in plant cells.

Conclusions:

ATM1 and VIIIa are found at the plasmamembrane and at the membrane of the forming cell plate and young transversal walls in stably transformed BY-2 cells and Arabidopsis seedlings. The root hair tips in Arabidopsis also show a strong accumulation of the two myosin isoforms. They might be involved in actin-dependent transport processes close to membranes or for the attachment of actin filaments to membranes. No significant differences are visible between the two isoforms.

ATM2 and VIIIb are found in nucleoli and to some degree in the karyoplasm of all cells, in stably transformed BY-2 cell lines, in Arabidopsis seedlings and in transiently expressing Allium and Vicia epidermal cells. The two isoforms might play a role in nucleolus-specific functions and transport of ribosomal precursors. Again, no significant differences are visible between the two isoforms.

All four isoforms are expressed roughly on the same level in major tissues of Arabidopsis seedlings.

Fig. 1A Domain structure of A. thaliana class/VIII myosins (A): Motor domain in red, IQ domains in yellow, coiled coil domains in blue (Reddy and Day, 2001)

Fig. 1B Structure of the CAT-GFP fusion construct containing the 35S double enhancer promoter from CaMV, the trans-criptionalleader from TEV (TL) and a polyadenylation site (pA).

Fig. 2 Stably transformed BY-2 cells (A,B) GFPATM1tail, (C,D) GFPVIIIAtail, note strong labelling of cell plate and young cross walls.

Fig. 3 Stably transformed BY-2 cells (A,B) GFPATM2tail (C) GFPVIIIAtail note prominent fluorescence in the nucleoli and weak labelling of the karyoplasm

Fig. 4 Transient expression after particle bombardment (A-D) Vicia faba: GFPATM1tail (A), GFPVIIIAtail (B). (C-D) Allium cepa: GFPATM1tail (C), GFPVIIIAtail (D). note bright fluorescence of the nucleoli

Fig. 5 A. thaliana stably transformed with GFPATM1tail (A) tip labelling of roothair (B) prominent label in transversal wall of rhizodermis (C) stomatal cells, motile activity of fluorescent particles at labelled cross walls

Fig. 6 A. thaliana stably transformed with GFPVIIIAtail (A) root hair zone, nuclei visible in each cell (B) Cells of the apical meristem with nuclei. (C) roothair and epidermal cell, bright fluorescence in nuclei

Fig. 7 Expression of the four different Myosin/VIII genes monitored by RT-PCR amplification of the respective tails from organ-specific cDNA of A. thaliana wildtype. All four isoforms are strongly expressed in all organs.

PCR-Product size: ATM1tail-686bp ATM2tail-720bp VIIIAtail-680bp VIIIItail-670bp

References:
