Short communication

Unconventional myosins of the plant-specific class VIII: endocytosis, cytokinesis, plasmodesmata/pit-fields, and cell-to-cell coupling

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AtATM1 myosin of \textit{Arabidopsis thaliana} was the first plant myosin to be identified and sequenced (Knight and Kendrick-Jones, 1993). Based on its N-terminal motor domain and C-terminal sequences, it was assigned into its own class VIII (Reichelt et al., 1999). Currently, the sequences of seven myosins of this class can be found in the genomic databases (Baluška et al., 2001a; Liu et al., 2001; Reddy and Day, 2001; Reichelt and Kendrick-Jones, 2000). They are characterized by a unique C-terminal tail region showing no apparent homologies to any other known myosins. This region, however, is highly conserved amongst individual members of this myosin class and contains multiple potential phosphorylation sites (Baluška et al., 2001a). Besides this tail region, AtATM1 myosin VIII contains an unusual N-terminal extension with a PEST domain prior to their conserved motor domain, 4 IQ motifs, and a short region of predicted \( \alpha \)-helical coiled coil domain which maybe responsible for dimerization (Baluška et al., 2001a; Reichelt and Kendrick-Jones, 2000). At the very end of C-terminus, clusters of basic residues are present which might be involved in binding to phospholipids (Knight and Kendrick-Jones, 1993). A polyclonal antibody was raised against the whole tail domain of AtATM1 myosin VIII (predicted coiled coil domain and unique C-terminal region) (Reichelt et al., 1999) which localizes these plant-specific myosins to the plasma membrane at subcellular structures only observed in plant cells: namely cytokinetic cell plates and plasmodesmata (Baluška et al., 2000, 2001a; Reichelt et al., 1999). In addition, immunofluorescence spots of variable size are found distributed throughout the cytoplasm. Detailed analysis of subcellular distributions of myosin VIII and actin in cells of root apices suggest that myosin VIII maybe less important for intracellular motility and more involved in anchoring of actin filaments at cell peripheries (Baluška et al., 2000; Reichelt et al., 1999; Volkmann and Baluška, 1999). For example, abundant myosin VIII is highly localized at actin-enriched cross-walls, which anchor spindle poles in mitotic cells and longitudinal F-actin bundles in elongating cells. These patterns of subcellular distributions are dramatically altered during strong osmotic stress when almost all myosin VIII associates with callosic pit-fields of plasmolysing cells (F. Baluška and D. Volkmann, preliminary data). Additional possible roles for myosin VIII may include the following: organizing F-actin at cell peripheries, stabilizing the structurally delicate callosic cell plates during cytokinesis, and as structural supports for the cortical ER elements tightly underlying the plasma membrane both outside and within plasmodesmata. Moreover, our preliminary data suggest that myosin VIII might also drive invagination of the plasma membrane during fluid-phase endocytosis (Baluška et al., 2002) and interconnect the cytoskeleton with the plant extracellular matrix via putative binding of myosin VIII to the callose synthase complexes.

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The plasmodesmata of plant cells provide direct communication pathways within plant tissues for trafficking of diverse proteins and nucleic acids. These cell-to-cell channels are equipped with central ER elements and are supported by the dynamic actin cytoskeleton and are nonspecifically open (Jackson, 2000; Wu et al., 2002; Zambryski and Crawford, 2000) and that this active gating process requires an intact actin cytoskeleton (Ding et al., 1996) which maybe controlled by actomyosin-based contractile forces (Baluska et al., 2001a; Overall et al., 2000). In addition to actin and myosin VIII, several myosin-like proteins were localized to plasmodesmata (Blackman and Overall, 1998; Overall et al., 2000; Radford and White, 1998; White et al., 1994). Moreover, Arp3-like protein (Van Gestel et al., submitted), ER-based calreticulin (Baluska et al., 1999), calcium-dependent protein kinase (F. Baluska and D. Volkmann, preliminary data; Yahalom et al., 1998), and centrin (Blackman et al., 1999) also localize to plasmodesmata and might participate in their active gating. Microinjection of the polyclonal antibody raised against the tail domain of myosin VIII (Reichelt et al., 1999) into living root epidermis cells of Arabidopsis roots and mesophyll cells of tobacco leaves resulted in dilatation of plasmodesmata, as evidenced by enhanced cell-to-cell coupling monitored by comicroinjected dextran FITC and Lucifer Yellow dye (Table 1). These findings strongly suggest that myosin VIII-based forces are required for the maintenance of the structural integrity of plasmodesmata and their immunodepletion causes nonspecific opening of plasmodesmata. This would be in agreement with findings that depletion of cellular ATP, either with azide or due to anaerobiosis, induces opening of plasmodesmata (Cleland et al., 1994; Tirlapur and König, 1999). In plant tissues, there are two well-known examples where the cell-to-cell coupling is shut off on a developmental basis. First, root trichoblast become symplastically isolated during root hair formation (Duckett et al., 1994; Tirlapur and König, 1999). Secondly, stomatal plasmodesmata are closed, and later even truncated, during development of guard cells of functional stomata (Palevitz and Hepler, 1985; Wille and Lucas, 1984). Our recent experimental data obtained using two-photon near-infrared femtosecond laser scanning microscopy (Tirlapur and König, 2002a,b), which allows non-invasive laser-assisted propidium iodide loading into target cells (Tirlapur and König, 1999, 2002a,b), have revealed that postmitotic root cap statocytes are also symplastically isolated (U. Tirlapur, K. König, F. Baluska, and D. Volkmann, in preparation). This surprising result may be related to their well-known role as the gravising statocytes (Sack, 1991). Such speculation is strongly supported by our observations that gravitostimulation of roots resulted in differential opening of statocyte plasmodesmata depending on their position within root caps (U. Tirlapur, K. König, F. Baluska, and D. Volkmann, in preparation). As postmitotic root cap statocytes, like trichoblasts initiating root hairs, do not show abundant myosin VIII at their plasmodesmata and pit-fields (Baluska et al., 2000), myosin VIII might be expected to be abundant within those plasmodesmata which are engaged in extensive cell-to-cell trafficking. In fact, plasmodesmata and pit-fields in the inner cortex cells of maize root apices, located within the transition root apex zone (Baluska et al., 2001b) where phloem-unloading takes place, are equipped with abundant F-actin and myosin VIII molecules (Baluska et al., 2001a). Intriguingly, these callosic and actomyosin-enriched domains act as selective platforms specialized for fluid-phase endocytosis (Baluska et al., submitted). All this suggest that unconventional myosins of the plant-specific class VIII act as a multifunctional molecules at callose-enriched subcellular domains of higher plant cells like cytokinetic cell plates, plasmodesmata, and pit-fields.

References


