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Plasma Membrane-Associated SCAR Complex Subunits Promote Cortical F-Actin Accumulation and Normal Growth Characteristics in *Arabidopsis* Roots

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ABSTRACT The ARP2/3 complex, a highly conserved nucleator of F-actin polymerization, and its activator, the SCAR complex, have been shown to play important roles in leaf epidermal cell morphogenesis in *Arabidopsis.* However, the intracellular site(s) and function(s) of SCAR and ARP2/3 complex-dependent actin polymerization in plant cells remain unclear. We demonstrate that putative SCAR complex subunits BRK1 and SCAR1 are localized to the plasma membrane at sites of cell growth and wall deposition in expanding cells of leaves and roots. BRK1 localization is SCAR-dependent, providing further evidence of an association between these proteins in vivo. Consistent with plasma membrane localization of SCAR complex subunits, cortical F-actin accumulation in root tip cells is reduced in *brk1* mutants. Moreover, mutations disrupting the SCAR or ARP2/3 complex reduce the growth rate of roots and their ability to penetrate semi-solid medium, suggesting reduced rigidity. Cell walls of mutant roots exhibit abnormal structure and composition at intercellular junctions where BRK1 and SCAR1 are enriched in the adjacent plasma membrane. Taken together, our results suggest that SCAR and ARP2/3 complex-dependent actin polymerization promotes processes at the plasma membrane that are important for normal growth and wall assembly.

Key words: cell expansion; cell morphogenesis; cytoskeleton; root biology; Arabidopsis.

INTRODUCTION

Plant cells acquire their shapes according to the patterns in which their walls expand during cell growth (Mathur, 2004). Actin filaments are thought to influence the expansion pattern of the cell wall by helping to direct the delivery of secreted wall material and membranes to sites of cell growth (Smith and Oppenheimer, 2005; Hussey et al., 2006). Numerous studies have established a role for the ARP2/3 complex in the spatial regulation of epidermal cell growth in plants (Szymanski, 2005). The animal Arp2/3 complex, consisting of seven subunits including the actin-related proteins Arp2 and Arp3, nucleates actin polymerization mainly by initiating new branches on the sides of existing actin filaments (Goley and Welch, 2006). Arp2/3 complex activity depends on nucleation promoting factors including members of the Scar/WAVE family (Stradal and Scita, 2005). In mammalian cells, Scar/WAVE is found in a complex with four other proteins: Sra1, Nap1, Abi1, and Hspc300 (Eden et al., 2002; Gautreau et al., 2004). In response to upstream regulators Rac and Nck, Scar/WAVE is activated via a mechanism that has been proposed to involve either dissociation of the complex or recruitment of the intact complex to sites of F-actin nucleation (reviewed, Takenawa and Suetsugu, 2007). Components of the Scar/WAVE complex localize to plasma membranes at the leading edge of animal cells where they nucleate cortical F-actin that promotes lamellipodial extension (Miki et al., 1998; Nozumi et al., 2003; Kunda et al., 2003; Rogers et al., 2003; Stovold et al., 2005).

Homologs of all mammalian Arp2/3 and Scar/WAVE complex subunits have been identified in *Arabidopsis*, including a family of four proteins distantly related to animal Scar/WAVE proteins (AtSCAR1 to AtSCAR4; Szymanski, 2005). Three of the

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four *Arabidopsis* SCAR proteins have been shown to stimulate mammalian Arp2/3 complex-dependent actin polymerization in vitro (Frank et al., 2004; Basu et al., 2005; Uhrig et al., 2007; Zhang et al., 2008). Although neither ARP2/3 nor SCAR complexes have been purified from plant extracts or reconstituted from plant subunits to date, numerous in-vitro and in-vivo binding interactions between putative complex subunits support the existence of ARP2/3 and SCAR complexes in plants (Szymanski, 2005; Uhrig et al., 2007). Mutations in genes encoding nine different subunits of the putative ARP2/3 or SCAR complexes dramatically perturb the growth patterns of epidermal hairs called trichomes and also reduce lobe outgrowth and intercellular adhesion in epidermal pavement cells (reviewed, Szymanski, 2005; Smith and Oppenheimer, 2005; also see more recent studies by Basu et al., 2005; Zhang et al., 2005; Djakovic et al., 2006; Le et al., 2006). Plants lacking all SCAR function are phenotypically equivalent to ARP2/3 complex subunit mutants (Zhang et al., 2008), and double mutants lacking both SCAR and ARP2/3 complex function are no more severe than either type of single mutant (e.g. El-Assal et al., 2004; Djakovic et al., 2006), suggesting that SCAR is the primary if not the sole activator of the ARP2/3 complex in *Arabidopsis*. Surprisingly, no severe loss of F-actin has been observed in ARP2/3 or SCAR complex subunit mutants. Instead, excessive bundling and spatial disorganization of cytoplasmic actin strands have been observed in expanding mutant trichomes, and alterations in cortical F-actin distribution have been observed in expanding mutant pavement cells (e.g. Mathur et al., 2003a; Le et al., 2003; Li et al., 2003; Deeks et al., 2004; Brembu et al., 2004; Djakovic et al., 2006).

In the moss *Physcomitrella patens*, ARP2/3 and SCAR complex subunit mutants have severe defects in filament elongation associated with loss or disruption of the F-actin 'cap' structure normally found at the apical growth site (Harries et al., 2005; Perroud and Quatrano, 2006, 2008; Finka et al., 2007). Moreover, ARPC4 (a subunit of the putative ARP2/3 complex) and BRK1 (the plant homolog of the Hspc300 subunit of the animal Scar/WAVE complex) are both specifically localized at the filament apex (Perroud and Quatrano, 2006, 2008). Thus, tip-localized activity of the SCAR–ARP2/3-dependent actin nucleation pathway plays a critical role in promotion of tip growth in *P. patens*. The ARP2/3 complex also promotes tip growth in elongating root hairs of *Arabidopsis*, but its role is minor and largely redundant with those of other actin regulatory proteins (Mathur et al., 2003b; Deeks et al., 2007). Although the genes encoding ARP2/3 and SCAR complex subunits are ubiquitously expressed (e.g. El-Assal et al., 2004; Basu et al., 2005; Uhrig et al., 2007), very little is known about roles for this pathway in vascular plants outside the context of expanding leaf epidermal cells and tip growing cells. Even in these cells, it remains unclear where SCAR and ARP2/3 complex-dependent actin nucleation occurs and what cellular processes are disrupted to cause the cell shape and adhesion defects observed in mutants.

Here, we use complementary methods to demonstrate that SCAR complex subunits are associated with the plasma membrane in various tissues of *Arabidopsis.* Analysis of roots of SCAR complex subunit mutants demonstrates an important role for SCAR and ARP2/3 complexes in accumulation of cortical (plasma membrane-associated) F-actin and in certain aspects of root growth and cell wall assembly. Together, these results provide new perspectives on the role(s) played by SCAR and ARP2/3 complex-dependent actin polymerization in plant cell growth.

RESULTS

BRK1 and SCAR1 Are Localized to the Cell Periphery

To gain insight into where SCAR- and ARP2/3-complex-dependent actin polymerization occurs in plant cells, we studied the localization of BRK1 and SCAR1 fluorescent fusion proteins in transgenic plants. BRK1::YFP expressed from the *BRK1* promoter is functional as indicated by its ability to complement the distorted trichome phenotype of *brk1* mutants (Figure 1C). In contrast to *scar2* mutants, which display a mild distorted trichome phenotype (Basu et al., 2005; Zhang et al., 2005), *scar1* mutants have normally shaped trichomes and lack any obvious phenotypes, although a redundant role for SCAR1 in trichomome morphogenesis is suggested by a slight enhancement of the *scar2* trichome phenotype in *scar1;scar2* double mutants (Zhang et al., 2008). Thus, to determine whether GFP::SCAR1 is functional in vivo, we tested its ability to rescue the *scar2* trichome phenotype when driven from the *SCAR2* promoter (since the wild-type *SCAR1* gene is not sufficient for normal trichome morphogenesis in *scar2* mutants, we reasoned that *SCAR1* promoter-driven expression of SCAR1::GFP would not rescue the *scar2* trichome phenotype). Indeed, normal trichome morphology was restored in *scar2* mutants transformed with *SCAR2p*::GFP::SCAR1 (Figure 1E). Thus, GFP::SCAR1 is functional because it can substitute for SCAR2 and we reasoned that the localization of *SCAR2p*::GFP::SCAR1 in complemented *scar2* mutants could provide information regarding the intracellular sites of SCAR1 and/or SCAR2 function.

Both BRK1::YFP and GFP::SCAR1 localized to the periphery of expanding trichomes (Figure 2A–2H). In young trichomes, BRK1::YFP and GFP::SCAR1 were enriched at the tips of newly initiated branches (Figure 2A–2D), presumably corresponding to sites of active cell growth. In partially expanded branches of older trichomes, BRK1::YFP and GFP::SCAR1 both remained peripherally localized but were spread along the full length of the elongating branch (Figure 2E–2H). At this stage, growth is also distributed along the entire length of the branch (Schwab et al., 2003). Thus, BRK1::YFP and GFP::SCAR1 appear to be localized at growth sites in expanding trichomes. GFP::SCAR1 was also associated with medium-sized intracellular organelles of unknown identity in expanding trichomes (Figure 2D). However, this component of the GFP::SCAR1 localization pattern was not observed for BRK1::YFP (Figure 2B).