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Immunological evidence for the presence of plant homologues of the actin-related protein Arp3 in tobacco and maize: subcellular localization to actin-enriched pit fields and emerging root hairs

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Summary. The actin-nucleating and -organizing Arp2/3 protein complex is well known to be conserved throughout the eukaryotic kingdom. For higher plants, however, only limited evidence is available for the presence of the Arp2/3 complex so far. Using heterologous antibodies against the *Dictyostelium discoideum* and *Schizosaccharomyces pombe* proteins and a bovine peptide, we found immunological evidence for the presence of Arp3 homologues in plants. First, proteins with a molecular mass of about 47–50 kDa were clearly recognized in extracts of both a dicotyledonous plant (tobacco) and a monocotyledonous plant (maize) in immunoblots with the anti-Arp3 antibodies. Second, immunolocalization with these Arp3 antibodies was performed on different plant cells, selected for their diverse actin organizations and functions. On isolated plasma membrane ghosts derived from tobacco leaf protoplasts, a putative Arp3 was localized along cortical actin filaments. In the inner cortex of maize roots, Arp3 was localized to actin-rich plasmodesmata and pit fields and to multivesicular bodies in the cytoplasm. During root hair formation, distinct site-specific localization was found at the protruding apical plasma membrane portions of these tip-growing cells.

Keywords: Actin; Actin-related protein 3; *Zea mays*; *Nicotiana tabacum*; Immunodetection.

Introduction

Actin polymerization in vivo is dependent on free barbed ends. Barbed ends (or plus ends) are the fast-growing ends of actin polymers, while the pointed ends (or minus ends) are slowly growing. Three mechanisms are believed to generate barbed ends in vivo: first, de novo nucleation of actin filaments from a nucleation template; second, uncapping of preexisting barbed ends by removal of a capping protein; and third, severing of preexisting filaments (Condeelis 2001). Typically, actin polymerization is closely linked to signal perception and transduction at the plasma membrane (Machesky and Insall 1999; for plant cells, see Volkmann and Baluška 1999, Staiger 2000).

De novo nucleation of actin filaments by a templating mechanism has been proposed as a function of the Arp2/3 protein complex (Kelleher et al. 1995). This protein complex, first purified from *Acanthamoeba castellanii* by Machesky et al. (1994), consists of seven subunits: two actin-related proteins (Arp2 and Arp3) and five novel proteins. One well-described example of its role in promotion of actin nucleation is the ActA-mediated formation of actin comet tails on the surface of the motile intracellular pathogen *Listeria monocytogenes* (Welch et al. 1997a). Studies on the Arp2/3 complex in a range of species have shown its abundant localization to subcellular sites with high actin dynamics, such as the cell cortex in amoeba (Kelleher et al. 1995), cortical actin patches in yeasts (Moreau et al. 1996, Winter et al. 1997), and protruding lamellipodia in vertebrate cells (Welch et al. 1997b). Recently, other functions for the Arp2/3 complex have been reported, namely, control of actin-based mitochondrial movement in yeasts (Boldogh et al. 2001) and formation of cometlike tails on the surface of endocytic vesicles and endosomes in animal cells (Merrifield et al. 1999, Taunton et al. 2000, Southwick et al. 2003). Very recently, the Arp2/3 complex has also been implicated in actin assembly

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in nonmotile cells of multicellular organisms (Hudson and Cooley 2002, Miller 2002).

The Arp2/3 complex seems to be highly conserved in eukaryotes as all seven subunits are found in amoebas, yeasts, and mammals (for an overview, see Machesky and Gould 1999). For higher plants, only limited evidence for the presence of a functional Arp2/3 complex have been reported yet (Blanchoin 2002). Genes encoding an Arp2 homologue (AtARP2, GenBank accession nr. AB026649) and an Arp3 homologue (AtARP3, GenBank accession nr. AC007357) of *Arabidopsis thaliana* were identified (see also McKinney et al. 2002). Expression of these genes was analyzed in whole plants (Klahre and Chua 1999, McKinney et al. 2002). Other *A. thaliana* entries consist of sequences highly homologous to the p21 (GenBank accession nr. AAC24070) and p41 (GenBank accession nr. AAD20675) subunits of the Arp2/3 protein complex.

Currently, antibodies raised against plant Arp2 and Arp3 proteins are not available. Thus, heterologous antibodies provide the only choice for the analysis of subcellular distributions of plant homologues of these proteins. The data presented here are based on immunoblotting, in situ immunofluorescence, and immuno-electron microscopy with antibodies against *Schizosaccharomyces pombe* and *Dictyostelium discoideum* Arp3 and against a peptide derived from the bovine C-terminal sequence of bovine Arp3. They reveal the presence of Arp3 homologues in tobacco as well as in maize. Our study suggests the involvement of plant Arp3 in the organization and dynamics of the actin cytoskeleton at the plasma membrane.

Material and methods

Plant material

Plants of *Nicotiana tabacum* L. cv. Petite Havana SR1 were grown from seeds under sterile conditions. Elongated tobacco cells were regenerated from protoplasts (Verbelen et al. 1992) which were isolated (Potrykus and Shillito 1986) from sterile leaves.

Maize seeds were soaked for 6 h and germinated in well moistened rolls of filter paper for 4 days in darkness at 20 °C. Young seedlings with straight primary roots, 50–70 mm long, were selected for immunolabelling studies.

Antibodies

Affinity-purified polyclonal antibodies against a peptide derived from the bovine C-terminal sequence of Arp3, described in David et al. (1998), were a kind gift of Dr. Pascale Cossart (Institut Pasteur, Paris, France). Polyclonal antibodies directed against Arp3 from fission yeast, described in McCollum et al. (1996), were a kind gift of Dr. Kathleen L. Gould (Vanderbilt University, Nashville, Tenn., U.S.A.). The polyclonal antibody directed against Arp3 from *D. discoideum* was a kind gift of Dr. Robert H. Insall (University of Birmingham, U.K.).

Gel electrophoresis and immunoblotting

A protein extract was made from tobacco leaves basically as described by Kermode et al. (1995). Maize root tips were processed for extraction and immunoblotting as described in detail previously (Baluška et al. 2001b). The root homogenate was centrifuged at 10,000 **g** for 20 min to remove cellular debris, large organelles, and nuclei. The supernatant was spun again at 30,000 **g** for 50 min, resulting in a microsomal fraction (pellet) and a soluble fraction (supernatant), which were both subjected to electrophoresis. After protein electrophoresis (Laemmli 1970), gels were wet-blotted onto nitrocellulose membranes. For immunoblotting, the anti-Arp3 antibodies were used in combination with horseradish peroxidase-labeled anti-rabbit immunoglobulin G (IgG) (Jackson Immunoresearch) and alkaline phosphatase-coupled anti-rabbit IgG (Promega). Detection was done with a chemiluminescence kit (Amersham Pharmacia Biotech) and with a Fast-Red kit (Sigma Chemicals), respectively.

Tobacco membrane ghosts and indirect immunofluorescence labelling

Plasma membrane ghosts were prepared from tobacco leaf-derived protoplasts, basically as described by Collings et al. (1998), but with another buffer (50 mM piperazine-N,N′-bis (2-ethanesulfonic acid) [PIPES], 5 mM EGTA, 2 mM $MgSO_4$, pH 6.6, and 300 μM phenylmethylsulfonyl fluoride) for protoplast lysis. Immunolocalization on the ghosts was done as described by Collings et al. (1998). The anti-fission yeast Arp3 antibodies were used in combination with goat anti-rabbit IgG labeled with fluorescein isothiocyanate (FITC) (Sigma). For double-labelling, mouse monoclonal anti-actin (clone C4; ICN) and FITC-labeled goat anti-mouse IgG (Sigma) were included in the respective antibody solutions and Texas red-labeled donkey anti-rabbit IgG (Jackson Immunoresearch) was used instead of FITC-labeled goat anti-rabbit IgG. All antibodies were used in 1 : 100 dilution.

Maize roots and indirect immunofluorescence labelling

Maize root tips were fixed, embedded in Steedman's wax, and sectioned, as described in detail previously (Baluška et al. 2001b). Median longitudinal wax sections (7 μm thick) were placed on slides coated with glycerol-albumen (Serva) and dewaxed. Immunolabelling used antibody against *D. discoideum* Arp3 and antibody against fission yeast Arp3 as primary antibodies and FITC-conjugated anti-rabbit IgG as secondary antibody (Sigma). All antibodies were used in 1 : 100 dilution.

Maize roots and immunogold electron microscopy

Apical 2 mm long segments of maize root tips were excised and fixed in 3.7% formaldehyde in stabilizing buffer (50 mM PIPES buffer, 5 mM $MgSO_4$, 5 mM EGTA, pH 6.9) for 1.5 h. After washing, the root segments were dehydrated in graded ethanol-phosphate-buffered saline series and embedded in London Resin White resin (hard grade; BioCell) which was polymerized for 5 days at 36 °C. Ultrathin sections were collected on formvar-coated Ni grids. The grids were further processed for immunogold localization, postfixation and postcontrasting as described previously (Reichelt et al. 1999). Immunolabelling used antibody against *D. discoideum* Arp3 as primary antibody and goat anti-rabbit IgG conjugated to 10 nm diameter gold (BioCell) as secondary antibody. Antibodies were used in 1:100 dilution.

Microscopy

One-wavelength observations on tobacco membrane ghosts were done with a Bio-Rad MRC 600 confocal system mounted on a Zeiss Axioskop microscope. Imaging of double-labelling on the ghosts was performed on a Zeiss Axiovert combined with a Zeiss LSM 410 confocal system. All micrographs represent single optical sections.

Fluorescence on wax sections of maize roots was examined with a Zeiss Axiovert 405M inverted light microscope equipped with epifluorescence and standard FITC exciter and barrier filters (BP 450–490, LP 520). Photographs were taken on Kodak T-Max films. Ultrathin sections were examined in a Zeiss EM 10 electron microscope at 60 kV.

Results

Immunoblotting of tobacco leave extracts

Immunodetection with the antibody against a peptide derived from the bovine C-terminal sequence of bovine Arp3 revealed a protein with a molecular mass around 50 kDa in extracts of tobacco leaves (Fig. 1, lane 1). In a cytoplasmic extract of rat C6 glioma cells, the antibody recognized a protein slightly larger than 50 kDa (Fig. 1, lane 2). Clearly, the antibody did not recognize pure human platelet actin (Fig. 1, lane 3). The nonpurified anti-fission yeast Arp3 antibodies also detected a protein slightly heavier than 50 kDa (Fig. 1, lane 4). But this antibody neither recognized pure human platelet actin (Fig. 1, lane 5).

Immunoblotting of maize root extracts

Both the soluble (Fig. 2, lane 1) and microsomal (Fig. 2, lane 2) protein fractions of a maize root extract were blotted and probed with the affinity-purified antibody raised against *D. discoideum* Arp3. In both fractions, strongly focused bands at about 47 kDa were detected.

Immunofluorescence localization of an Arp3 homologue on tobacco membrane ghosts

On membrane ghosts made from tobacco leaf protoplasts, the antibody against the C-terminal peptide of bovine Arp3 gave no significant localization signal (data not shown). However, confocal microscopy analysis of preparations obtained with the serum against the whole Arp3 protein of fission yeast revealed filamentous and punctate structures (Fig. 3A). A limited punctate labelling pattern was however also present on the control treated with nonimmune rabbit serum and thus could be unspecific (Fig. 3B). The filamentous structures, labeled by the antibody, resemble actin filaments. A double-labelling with monoclonal anti-actin antibody (Fig. 3C) and polyclonal anti-Arp3 serum (Fig. 3D) showed an overlap of the labeled filamentous structures.

Immunofluorescence localization of an Arp3 homologue in maize roots

With antibodies against *D. discoideum* Arp3, a strong localization was detected in root hairs and at pit fields of inner cortical root cells (Fig. 4). In the very early stage of the root hair formation, both a prominent diffuse signal and distinct punctate signals were observed within the cytoplasm (Fig. 4A). The nucleus was not labeled, while smaller organelles around the nucleus were conspicuously labeled. At the plasma membrane of emerging root hairs (Fig. 4B), the punctate Arp3 signal shows progressive increase in abundance towards the growing tip. In the cytoplasm of the emerging root hairs, labelling is associated with very small organelles of unknown identity (Fig. 4B). On radial (Fig. 4C) and tangential (Fig. 4D) longitudinal sections through root inner cortical cells, pit fields of the sidewalls are prominently labeled. Antibodies raised against fission yeast Arp3 gave

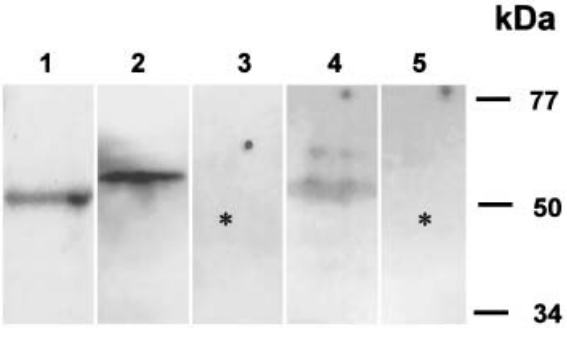

Fig. 1. Immunoblotting with purified bovine peptide Arp3 antibody (*1–3*) and fission yeast Arp3 antibody (*4* and *5*) on extract from tobacco leaves (*1* and *4*), extract from rat C6 glioma cells (*2*), and pure human platelet actin (*3* and *5*, asterisks mark the expected position of the signal). Molecular-mass protein standards (in kDa) are indicated on the right

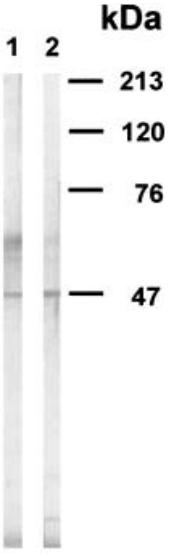

Fig. 2. Immunoblotting with anti-*D. discoideum* Arp3, on the soluble (*1*) and the microsomal (*2*) fraction of a maize root extract. Molecular-mass protein standards (in kDa) are indicated on the right

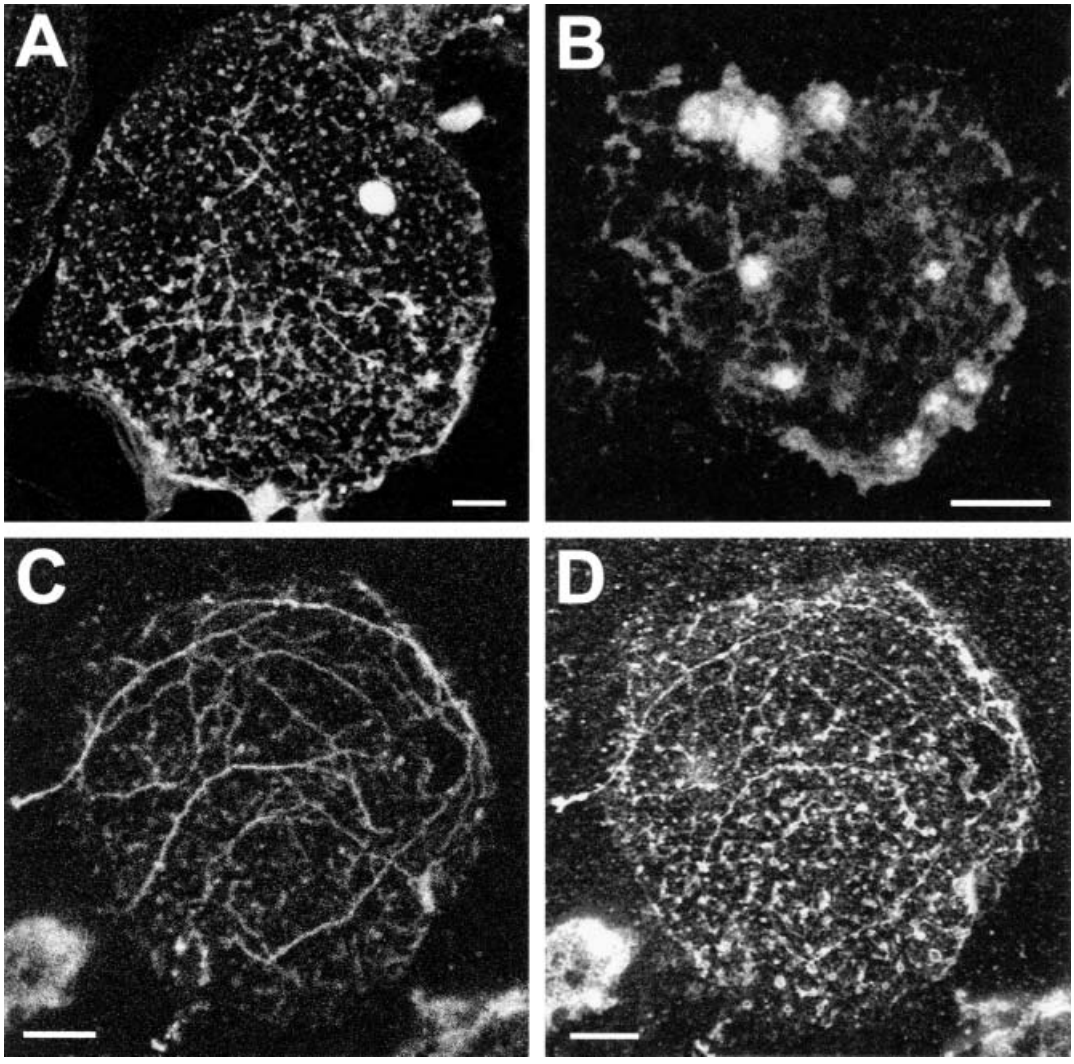

Fig. 3A–D. Immunofluorescence localization by confocal microscopy of an Arp3 homologue on tobacco plasma membrane ghosts. **A** The anti-fission yeast Arp3 antibody labels filamentlike and punctate structures. **B** The negative control of labelling with nonimmune rabbit serum. **C** and **D** Double-labelling of filamentous structures with anti-actin antibody (**C**) and anti-fission yeast Arp3 antibody (**D**) on the same ghost. Bars: 10 μm

essentially the same localization patterns as the antibodies against *D. discoideum* Arp3 (data not shown).

Immunogold localization of an Arp3 homologue in maize roots

Using the same antibodies as for immunofluorescence labelling, immunogold electron microscopy revealed subcellular labelling patterns consistent with the former results. First, plasmodesmata, both at the sidewalls and at young crosswalls, are decorated with gold particles (Fig. 5A). A sidewall with a gold-labeled pit field (group of plasmodesmata) is shown in Fig. 5B. Second, gold labelling was also found associated with cortical endoplasmic reticulum and a round compartment, probably an early endosome, can be discerned among the labeled membranous structures (Fig. 5C). A multivesicular body with an abundance of gold particles is shown in Fig. 5D. Again, the antibody raised against fission yeast Arp3 gave essentially the same localization patterns as the *D. discoideum* Arp3 antibody (data not shown).

Discussion

Immunoblotting reveals candidates for plant Arp3-like proteins in extracts of monocotyledonous and dicotyledonous plants

With the antibodies raised against *D. discoideum* Arp3, a band at about 47 kDa was recognized in maize root extracts, while the anti-fission yeast Arp3 antibody and the anti-

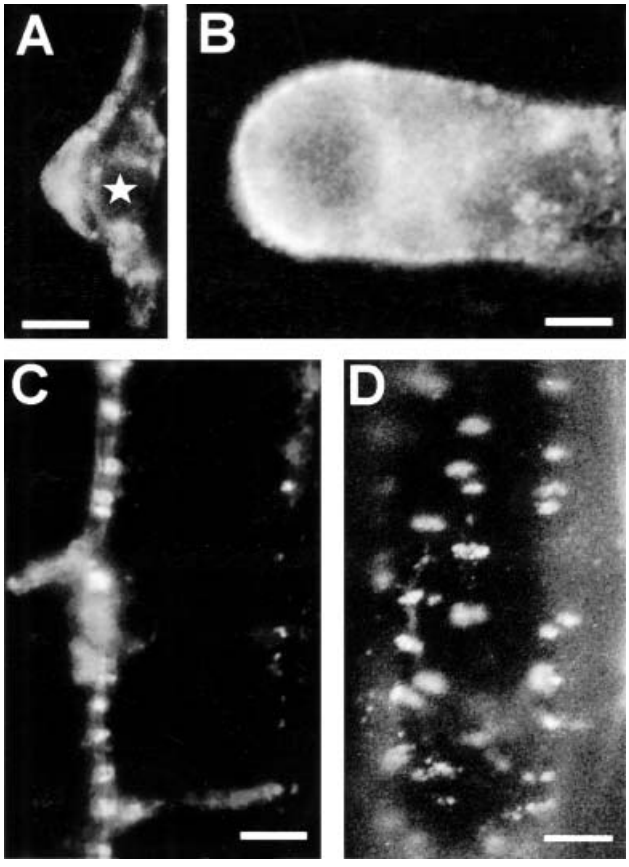

Fig. 4 A–D. Immunofluorescence localization of an Arp3 homologue on sections of maize roots with anti-*D. discoideum* Arp3. **A** In an early-bulging state of a root hair, both a prominent diffuse signal and distinct punctate signals were detected within the cytoplasm. The nucleus is indicated with a star. **B** In an emerging root hair, tip-focused plasma membrane labelling and small spotlike labelling was detected. **C** and **D** In the inner cortical root cells, plasmodesmata and pit fields of sidewalls are prominently labeled as is shown on a radial (**C**) and a tangential (**D**) longitudinal section. Bar: A, 13 μm; B–D, 7 μm

bovine peptide antibody recognized bands at about 50 kDa in tobacco leaf extracts. The theoretical molecular mass of the Arp3 protein in nonplant eukaryotic cells varies from 47 to 48.6 kDa according to the number of amino acids, which ranges from 418 in human cells and *D. discoideum* to 427 in *Acanthamoeba castellanii* and fission yeast (Lees-Miller et al. 1992, Kelleher et al. 1995, Welch et al. 1997b). On Western blot, however, the molecular mass was often found to be higher than the calculated value (up to 50 kDa). A locus on the *A. thaliana* chromosome 1 encodes an Arp3-like protein with 427 amino acids (GenBank accession nr. ADD31071). Therefore, a putative Arp3 homologue in plants can be expected to have a molecular mass between 47 and 50 kDa. In comparison with the soluble protein fraction of the maize root extract, the microsomal protein fraction seems to react somehow stronger and more specifically to the anti-Arp3 antibody. This finding indicates that the detected Arp3-homologue of maize might be closely associated with membranes.

Confocal microscopy reveals immunolocalization of a putative plant Arp3 homologue on tobacco membrane ghosts

In plasma membrane ghosts prepared from protoplasts derived from fully grown tobacco leaves a clear labelling of epitopes closely related with ghost-associated actin filaments was detected. Only actin which is firmly attached to the plasma membrane, also called the cortical actin, will stay on the ghosts. It is unlikely that actin filament bundles originating from the deeper cytoplasm are present on the ghosts. Therefore, it is highly probable that a tobacco Arp3 homologue is associated with arrays of cortical fine actin filaments. Also in nonplant eukaryotic cells, antibodies to Arp3 mainly localized to cortical regions with high actin dynamics (Kelleher et al. 1995, Moreau et al. 1996, Welch et al. 1997b, Winter et al. 1997). In fibroblasts, for example, Arp3 was localized to protruding lamellipodia which contain dynamic cross-linked actin meshworks, whereas actin fibers consisting of bundled filamentous actin only rarely showed labelling for a member of the Arp2/3 complex (Welch et al. 1997b, Machesky and Gould 1999).

Immunolocalization of a putative plant Arp3 homologue in maize roots

Growing maize root apices are ideal organs to use in localization studies. Median sections of root apices allow direct comparison of young diffusely growing cells (inner cortical cells), emerging tip-growing cells (root hairs), and cell-to-cell connections (plasmodesmata).

The putative plant Arp3 was localized to distinct sites in the cytoplasm of inner cortical root cells. Labelling was found mainly associated with small organelles, multivesicular bodies, and endoplasmic reticulum. Possibly, part of the labeled small organelles represent vesicles and/or endosomes. In animal cells, the Arp2/3 complex has recently been shown to be involved in phagocytosis, where the complex was localized to phagocytic cups and phagosomes (Alrutz et al. 2001, May et al. 2001). Moreover, recent studies reported that Arp2/3-mediated actin polymerization powers the movement not only of the plasma membrane but also of intracellular membranes (reviewed by Taunton 2001). Endocytic vesicles and endosomes are among those organelles which are powered via actin cometlike tails (Merrifield et al. 1999, Taunton et al. 2000, Southwick et al. 2003). In *D. discoideum*, the Arp2/3 complex was recruited immediately to

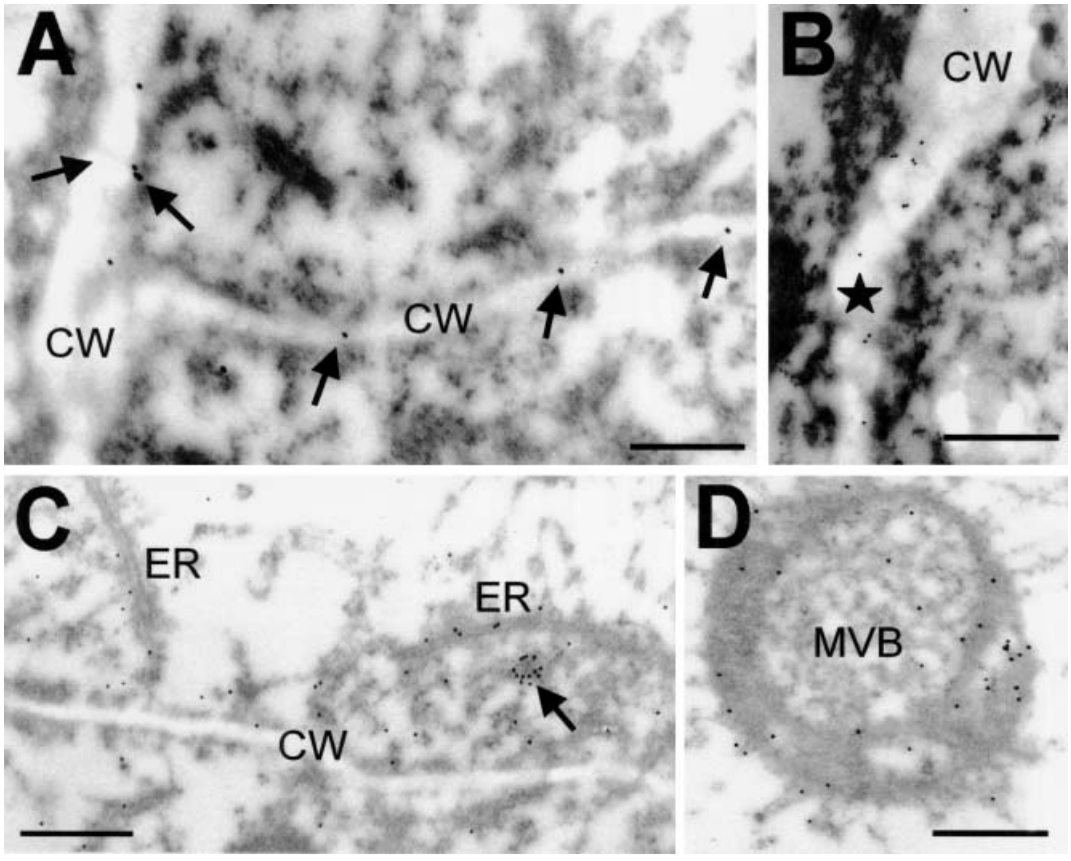

Fig. 5A–D. Immunogold localization of an Arp3 homologue on ultrathin sections of maize root cells with anti-*D. discoideum* Arp3. **A** Plasmodesmata, both at the longitudinal and at young transversal cell walls (*CW*), are decorated with gold particles (arrows). **B** A side cell wall (*CW*) with a gold-labeled pit field (star). **C** Gold labelling was also found associated with cortical endoplasmic reticulum (*ER*) and with a round vesicular compartment (arrow). **D** A multivesicular body (*MVB*) with an abundance of gold particles. Bar: A, 100 nm; B–D, 150 nm

sites where endocytosis was likely to occur and was localized to endosomes not only during engulfment but also later during the postlysosomal neutral-pH phase of the endocytic pathway (Insall et al. 2001). Endocytosis occurs also in plants (Battey et al. 1999, Emans et al. 2002, Baluška et al. 2002), but less is known about its molecular mechanisms (Holstein 2002, Geldner et al. 2003). Like in animal cells, the plant endocytic pathway appears to consist of a series of structurally distinct organelles (Galway et al. 1993), among which multivesicular bodies are prominent (Tanchak and Fowke 1987). These rather large (in the range of 250–500 nm) vesicle-containing bodies are involved in the transport of internalized material to the vacuole. Interestingly, multivesicular endosomes often use actin-based comet tails for their motilities (Taunton et al. 2000).

Our preliminary data suggest that pit fields on the inner cortex in maize root apices are sites accomplishing fluid-phase endocytosis (F. Baluška, J. Šamaj, A. Hlavacka, J. Kendrick-Jones, D. Volkmann unpubl.). Intriguingly in this respect, labelling of root cell plasmodesmata with the anti-Arp3 antibody adds further evidence to the model which depicts plasmodesmata as actin-dependent gateable and contractile structures (Baluška et al. 2001a). The unconventional myosin ATM1 (class VIII) has been localized to plasmodesmata and pit fields in the sidewalls of inner cortex cells (Baluška et al. 2000a). In the latter report, plasmodesmata were speculated to act as actin-filament-organizing centers in root apices. Further evidence that they possess properties of such centers is now provided by the localization of putative Arp3 homologues to these plasmodesmata. Interestingly in this respect, the Arp2/3 complex has been shown to play a role in ring canal expansion during oogenesis in *Drosophila melanogaster* (Hudson and Cooley 2002). These ring canals are actin-lined cytoplasmic connections between egg chamber cells, remotely resembling plant plasmodesmata.

The observed localization of the putative plant Arp3 on endosomelike multivesicular organelles is in agreement with the importance of actin assembly for their formation and/or movement in other organisms. Our preliminary data show

that the green-fluorescent protein fused to tandem FYVE domains, a marker for early endosomes (Gillooly et al. 2000, Stenmark et al. 2002), identifies early endosome of *Medicago sativa* roots which need actin polymerization, but not myosin activities and intact microtubules, for their rapid motilities (F. Baluška, B. Voigt, A. Hlavacka, B. Kost, T. Timmers, H. Stenmark, D. Menzel unpubl.). If this can be confirmed, then it will be a breakthrough observation as all currently published reports describe the moving of plant organelles, such as Golgi stacks, peroxisomes, plastids, and mitochondria, as along F-actin tracks via interactions between actin and myosins (Boevink et al. 1998, Mathur et al. 2002, Van Gestel et al. 2002, Muench and Mullen 2003). Nevertheless, actin-polymerization-driven propulsion remains a possibility for the motility of peroxisomes and mitochondria in plant cells (Mathur et al. 2002).

The localization of a putative plant Arp3 homologue to trichoblast bulges, as well as to the tips of emerging root hairs, fits well into the upcoming concept that a pushing actin machinery could be responsible for the fast polar growth of tip-growing plant cells such as pollen tubes and root hairs (Baluška and Volkmann 2002, Vantard and Blanchoin 2002). Reports on the localization of actin and actin-binding proteins such as profilin and the actin-depolymerizing factor in tip-growing plant cells, as well as studies using various inhibitors, support the presence of a highly dynamic actin array in the tips of pollen tubes and root hairs (Gibbon et al. 1999, Baluška et al. 2000b, Gibbon 2001, Vidali et al. 2001). Signal-mediated actin polymerization just beneath the apical plasma membrane (Machesky and Insall 1999; for plant cells, see Volkmann and Baluška 1999, Staiger 2000) could possibly act as a pushing force on this membrane (Baluška and Volkmann 2002, Vantard and Blanchoin 2002) similar to the protrusive lamellipodial motility reported for animal cells (Borisy and Svitkina 2000). Moreover, the nucleating Arp2/3 complex (Machesky and Gould 1999) has been shown to play a crucial role in the dendritic organization of branching networks of filamentous actin at the leading edge of motile animal cells (Svitkina and Borisy 1999, Blanchoin et al. 2000).

Conclusions

A putative Arp3 homologue was found in tobacco as well as in maize and was localized to subcellular sites known to be enriched with filamentous actin. It was found to be localized to the apical plasma membrane of outgrowing root hairs, to plasmodesmata and pit fields of the maize root inner cortex, as well as to multivesicular endosomelike structures in the cytoplasm. Within the framework of the still limited, but nevertheless increasing, knowledge on plant actin dynamics (Staiger et al. 2000, Vantard and Blanchoin 2002), these data represent circumstantial evidence for the presence of a plant homologue of Arp3. In support of this notion, nucleation and branching of actin filaments was found in plant cell extracts (Blanchoin 2002).

Acknowledgments

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References

Alrutz MA, Srivastava A, Wong K-W, D'Souza-Schorey C, Tang M, Ch'ng L-E, Snapper SB, Isberg RR (2001) Efficient uptake of *Yersinia pseudotuberculosis* via integrin receptors involves a Rac1-Arp2/3 pathway that bypasses N-WASP function. Mol Microbiol 42: 689–703

Baluška F, Volkmann D (2002) Actin-driven polar growth of plant cells. Trends Cell Biol 12: 14

– Barlow PW, Volkmann D (2000a) Actin and myosin VIII in developing root apex cells. In: Staiger CJ, Baluška F, Volkmann D, Barlow PW (eds) Actin: a dynamic framework for multiple plant cell functions. Kluwer, Dordrecht, pp 457–477
– Salaj J, Mathur J, Braun M, Jasper F, Šamaj J, Chua N-H, Barlow PW, Volkmann D (2000b) Root hair formation: F-actin-dependent tip growth is initiated by local assembly of profilin-supported F-actin meshworks accumulated within expansin-enriched bulges. Dev Biol 227: 618–632
– Cvrčková F, Kendrick-Jones J, Volkmannn D (2001a) Sink plasmodesmata as gateways for phloem unloading: myosin VIII and calreticulin as molecular determinants of sink strength? Plant Physiol 126: 39–46
– von Witsch M, Peters M, Hlavačka A, Volkmann D (2001b) Mastoparan alters subcellular distribution of profilin and remodels F-actin cytoskeleton in cells of maize root apices. Plant Cell Physiol 42: 912–922
– Hlavacka A, Šamaj J, Palme K, Robinson DG, Matoh T, McCurdy DW, Menzel D, Volkmann D (2002) F-actin-dependent endocytosis of cell wall pectins in meristematic root cells: insights from brefeldin A-induced compartments. Plant Physiol 130: 422–431

Battey N, James N, Greenland A, Brownlee C (1999) Exocytosis and endocytosis. Plant Cell 11: 643–659

Blanchoin L (2002) Actin polymerization in plant cell extracts. In: Botanikertagung Freiburg, Freiburg, Federal Republic of Germany, book of abstracts, p 84

– Amann KJ, Higgs HN, Marchand J-B, Kaiser DA, Pollard TD (2000) Direct observation of dendritic actin filament networks nucleated by Arp2/3 complex and WASP/Scar proteins. Nature 404: 1007–1011

Boevink P, Oparka K, Santa Cruz S, Martin B, Betteridge A, Hawes C (1998) Stacks on tracks: the plant Golgi apparatus traffics on an actin/ER network. Plant J 15: 441–447

Boldogh IR, Yang H-C, Dan Nowakowski W, Karmon SL, Hays LG, Yates JR III, Pon LA (2001) Arp2/3 complex and actin dynamics are required for actin-based mitochondrial motility in yeast. Proc Natl Acad Sci USA 98: 3162–3167

Borisy GG, Svitkina TM (2000) Actin machinery: pushing the envelope. Curr Opin Cell Biol 12: 104–112

Collings DA, Asada T, Allen NS, Shibaoka H (1998) Plasma membrane-associated actin in Bright Yellow 2 tobacco cells: evidence for interaction with microtubules. Plant Physiol 118: 917–928

Condeelis J (2001) How is actin polymerized in vivo? Trends Cell Biol 11: 288–293

David V, Gouin E, Van Troys M, Grogan A, Segal AW, Ampe C, Cossart P (1998) Identification of cofilin, coronin, Rac and capZ in actin tails using a *Listeria* affinity approach. J Cell Sci 111: 2877–2884

Emans N, Zimmerman S, Fischer R (2002) Uptake of a fluorescent marker in plant cells is sensitive to brefeldin A and wortmannin. Plant Cell 14: 71–86

Galway M, Rennie P, Fowke L (1993) Ultrastructure of the endocytic pathway in glutaraldehyde fixed and high-pressure frozen/freeze-substituted protoplasts of white spruce (*Picea glauca*). J Cell Sci 106: 847–858

Geldner N, Anders N, Wolters H, Keicher J, Kornberger W, Muller P, Delbarre A, Ueda T, Nakano A, Jürgens G (2003) The *Arabidopsis* GNOM ARF-GEF mediates endosomal recycling, auxin transport, and auxin-dependent plant growth. Cell 112: 219–230

Gibbon BC (2001) Actin monomer-binding proteins and the regulation of actin dynamics in plants. J Plant Growth Regul 20: 103–112

– Kovar DR, Staiger CJ (1999) Latrunculin B has different effects on pollen germination and tube growth. Plant Cell 11: 2349–2363

Gillooly DJ, Morrow IC, Lindsay M, Gould R, Bryant NJ, Gaullier J-M, Parton RG, Stenmark H (2000) Localization of phospatidylinositol 3-phosphate in yeast and mammalian cells. EMBO J 19: 4577–4588

Holstein SHE (2002) Clathrin and plant endocytosis. Traffic 3: 614–620

Hudson AM, Cooley L (2002) A subset of dynamic actin rearrangements in *Drosophila* requires the Arp2/3 complex. J Cell Biol 156: 677–687

Insall R, Müller-Taubenberger A, Machesky L, Köhler J, Simmeth E, Atkinson SJ, Weber I, Gerisch G (2001) Dynamics of the *Dictyostelium* Arp2/3 complex in endocytosis, cytokinesis and chemotaxis. Cell Motil Cytoskeleton 50: 115–128

Kelleher JF, Atkinson SJ, Pollard TD (1995) Sequences, structural models, and cellular localization of the actin-related proteins Arp2 and Arp3 from *Acanthamoeba*. J Cell Biol 131: 385–397

Kermode AR, Fisher SA, Polishchuk E, Wandelt C, Spencer D, Higgins TJV (1995) Proteolytic processing and localization of vicilin deletion mutant proteins in the leaf and seed of transgenic tobacco. Planta 197: 501–513

Klahre U, Chua N-H (1999) The Arabidopsis *ACTIN-RELATED PROTEIN 2* (*AtARP2*) promoter directs expression in xylem precursor cells and pollen. Plant Mol Biol 41: 65–73

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680–685

Lees-Miller JP, Henry G, Helfman DM (1992) Identification of act2, an essential gene in the fission yeast *Schizosaccharomyces pombe* that encodes a protein related to actin. Proc Natl Acad Sci USA 89: 80–83

Machesky LM, Gould KL (1999) The Arp2/3 complex: a multifunctional actin organizer. Curr Opin Cell Biol 11: 117–121

– Insall RH (1999) Signaling to actin dynamics. J Cell Biol 146: 267–272

– Atkinson SJ, Ampe C, Vandekerckhove J, Pollard TD (1994) Purification of a cortical complex containing two unconventional actins from *Acanthamoeba* by affinity chromatography on profilin agarose. J Cell Biol 127: 107–115

Mathur J, Mathur N, Hülskamp M (2002) Simultaneous visualization of peroxisomes and cytoskeletal elements reveals actin not microtubule-based peroxisome motility in plants. Plant Physiol 128: 1031–1045

May RC, Caron E, Hall A, Machesky LM (2001) Involvement of the Arp2/3 complex in phagocytosis mediated by FcγR or CR3. Nat Cell Biol 2: 246–248

McCollum D, Feoktistova A, Morphew M, Balasubramanian M, Gould KL (1996) The *Schizosaccharomyces pombe* actin related protein, Arp3, is a component of the cortical actin cytoskeleton and interacts with profilin. EMBO J 15: 6438–6446

McKinney EC, Kandasamy MK, Meagher RB (2002) Arabidopsis contains ancient classes of differentially expressed actin-related protein genes. Plant Physiol 127: 997–1007

Merrifield CJ, Moss SE, Ballestrem C, Imhof BA, Giese G, Wunderlich I, Almers W (1999) Endocytic vesicles move at tips of actin tails in cultured mast cells. Nat Cell Biol 1: 72–74

Miller KG (2002) Extending the Arp2/3 complex and its regulation beyond the leading edge. J Cell Biol 156: 591–593

Moreau V, Madania A, Martin RP, Winsor B (1996) The *Saccharomyces cerevisiae* actin-related protein Arp2 is involved in the actin cytoskeleton. J Cell Biol 134: 117–132

Muench DG, Mullen RT (2003) Peroxisome dynamics in plant cells: a role for the cytoskeleton. Plant Sci 164: 307–315

Potrykus I, Shillito RD (1986) Protoplasts: isolation, culture, plant regeneration. Methods Enzymol 118: 549–678

Reichelt S, Knight AE, Hodge TP, Baluška F, Šamaj J, Volkmann D, Kendrick-Jones J (1999) Characterization of the unconventional myosin VIII in plant cells and its localization at the post-cytokinetic cell wall. Plant J 19: 555–567

Svitkina TM, Borisy GG (1999) Arp2/3 complex and actin depolymerizing factor/cofilin in dendritic organization and treadmilling of actin filament array in lamellipodia. J Cell Biol 145: 1009–1026

Staiger CJ (2000) Signaling to the actin cytoskeleton in plants. Annu Rev Plant Physiol Plant Mol Biol 51: 257–288

– Baluška F, Volkmann D, Barlow PW (eds) (2000) Actin: a dynamic framework for multiple plant cell functions. Kluwer, Dordrecht

Stenmark H, Aasland R, Driscoll PC (2002) The phospatidylinositol 3-phosphate-binding FYVE finger. FEBS Lett 513: 77–84

Southwick FS, Li W, Zhang F, Zeile WL, Purich DL (2003) Actin-based endosome and phagosome rocketing in macrophages: activation by the secretagogue antagonists lanthanum and zinc. Cell Motil Cytoskeleton 54: 41–55

Tanchak MA, Fowke LC (1987) The morphology of multivesicular bodies in soybean protoplasts and their role in endocytosis. Protoplasma 138: 173–182

Taunton J (2001) Actin filament nucleation by endosomes, lysosomes and secretory vesicles. Curr Opin Cell Biol 13: 85–91

– Rowning BA, Coughlin ML, Wu M, Moon RT, Mitchison TJ, Larabell CA (2000) Actin-dependent propulsion of endosomes and lysosomes by recruitment of N-WASP. J Cell Biol 148: 519–530

Van Gestel K, Köhler RH, Verbelen J-P (2002) Plant mitochondria move on F-actin but their positioning in the cortical cytoplasm depends on both F-actin and microtubules. J Exp Bot 53: 659–667

Vantard M, Blanchoin L (2002) Actin polymerization processes in plant cells. Curr Opin Plant Biol 5: 502–506

Verbelen J-P, Lambrechts D, Stickens D, Tao W (1992) Controlling cellular development in a single cell system of *Nicotiana*. Int J Dev Biol 36: 67–72

Vidali L, McKenna ST, Hepler PK (2001) Actin polymerization is essential for pollen tube growth. Mol Biol Cell 12: 2534–2545

Volkmann D, Baluška F (1999) The actin cytoskeleton in plants: from transport networks to signaling networks. Microsc Res Tech 47: 135–154

Welch MD, Iwamatsu A, Mitchison TJ (1997a) Actin polymerization is induced by Arp2/3 protein complex at the surface of *Listeria monocytogenes*. Nature 385: 265–268

– DePace AH, Verma SV, Iwamatsu A, Mitchison TJ (1997b) The human Arp2/3 complex is composed of evolutionary conserved subunits and is localized to cellular regions of dynamic actin filament assembly. J Cell Biol 138: 375–384

Winter D, Podtelejnikov AV, Mann M, Li R (1997) The complex containing actin-related proteins Arp2 and Arp3 is required for the motility and integrity of yeast actin patches. Curr Biol 7: 519–529