Interaction of SPIN90 with the Arp2/3 Complex Mediates Lamellipodia and Actin Comet Tail Formation

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The appropriate regulation of the actin cytoskeleton is essential for cell movement, changes in cell shape, and formation of membrane protrusions like lamellipodia and filopodia. Moreover, several regulatory proteins affecting actin dynamics have been identified in the motile regions of cells. Here, we provide evidence for the involvement of SPIN90 in the regulation of actin cytoskeleton and actin comet tail formation. SPIN90 was distributed throughout the cytoplasm in COS-7 cells, but exposing the cells to platelet-derived growth factor (PDGF) caused a redistribution of SPIN90 to the cell cortex and the formation of lamellipodia (or membrane ruffles), both of which were dramatically inhibited in SPIN90-knockdown cells. In addition, the binding of the C terminus of SPIN90 with both the Arp2/3 complex (actin-related proteins Arp 2 and Arp 3) and G-actin activates the former, leading to actin polymerization in vitro. And when coexpressed with phosphatidylinositol 4-phosphate 5 kinase, SPIN90 was observed within actin comet tails. Taken these findings suggest that SPIN90 participates in reorganization of the actin cytoskeleton and in actin-based cell motility.

The actin cytoskeleton plays key roles in cell motility and morphology, intracellular organization, membrane trafficking, and the intracellular movement of a variety of pathogens (1, 2). Many actin-based structures, especially those involved in membrane protrusion, are assembled through the coordinated polymerization and cross-linking of actin monomers into actin filaments that in turn form orthogonal or parallel filament networks (3). In that regard, the dynamics of actin stress fibers, filopodia, and lamellipodia (or membrane ruffles) are tightly regulated by various nucleation-promoting factors (WASP family proteins) and actin nucleation proteins (e.g. the Arp2/3 complex) (4, 5). Among these components, the Arp2/3 complex localizes at the leading edge of cells, where the actin cytoskeleton is nucleated and reorganized (6, 7). The major activators of the complex include the WASP family proteins, which contain a conserved VCA domain composed of one or two VPH domains, which bind actin monomers, a central region, and an A domain, which binds to the Arp2/3 complex (8). In addition, cortactin, a filamentous actin-associated protein, binds to the Arp2/3 complex via an A domain at its N terminus and stimulates nucleation of actin filaments, ultimately promoting formation and stabilization of actin filament networks (9). In similar fashion, Abp1, an F-actin binding protein, also associates with the Arp2/3 complex and stimulates actin nucleation (10).

The Arp2/3 complex also plays a role in the intracellular motility of pathogens and vesicles. For instance, the pathogenic bacterium Listeria monocytogenes utilizes actin polymerization mediated by the Arp2/3 complex to move within the cytoplasm of infected host cells. Likewise, movement of intracellular vesicles (i.e. endosomes) is dependent upon actin polymerization mediated by the Arp2/3 complex, which localizes along the length of actin comet tails where it nucleates and reorganizes the actin cytoskeleton (6, 11).

SPIN90 was originally identified as an Nck-binding protein through yeast two-hybrid screening (12). Showing high sequence similarity to the AF3p21 gene product, DIP, WISP, and VIP54 (13–17), SPIN90 contains an SH3 domain, three proline-rich regions, a serine/threonine-rich region, and a long C terminus containing a leucine-rich region of unknown function. SPIN90 is also known to act on the actin cytoskeleton, playing a key role during myofibril and sarcomere assembly, and to regulate protein-protein interactions involving WASP, BPIX, and Nck (12, 17). Moreover, the SPIN90-like protein DIP interacts with Grb2 and Sre to regulate Rho GTase activity (Rho and Rac) and cell adhesion and might be involved in regulating the initial steps of cell movement following stimulation of integrin or growth factor receptors (14, 18). Taken together, these findings strongly support the idea that SPIN90 plays a pivotal role in regulating the organization of the actin cytoskeleton; however, the precise way in which it functions during these processes is still unclear. Here we show that SPIN90 interacts directly with the Arp2/3 complex and participates in the regulation of actin-based motility by promoting the complexes activity.

MATERIALS AND METHODS

Plasmid Construction and Antibodies—cDNAs encoding full-length SPIN90 (amino acids 1–722) and variant deletion mutants (N-term, amino acids 1–279; C-term, amino acids 280–722; SH3, amino acids 1–145; PRD, amino acids 1–279; Arp2/3 complex, actin related proteins Arp 2 and Arp 3; HEK 293T, human embryonic kidney 293-T; BHK cell, baby hamster kidney; siRNA, small interfering RNA; VCA, verpinol homology, cofillin homology, and acidic region; VP, verpinol homology; A domain, acidic domain; GFP, green fluorescent protein; Erk, extracellular signal-related kinase; PI(4)P kinase, phosphatidylinositol 4-phosphate 5 kinase; PI(4,5)P2, phosphatidylinositol 4,5-bisphosphate.

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The abbreviations used are: WASP, Wiskott-Aldrich Syndrome protein; GST, glutathione S-transferase; PDGF, platelet-derived growth factor; SH3, src homology 3; PRD, proline-rich domain; Arp2/3 complex, actin related proteins Arp 2 and Arp 3; Hek 293T, human embryonic kidney 293-T; BHK cell, baby hamster kidney; siRNA, small interfering RNA; VCA, verpinol homology, cofillin homology, and acidic region; VP, verpinol homology; A domain, acidic domain; GFP, green fluorescent protein; Erk, extracellular signal-related kinase; PI(4)P kinase, phosphatidylinositol 4-phosphate 5 kinase; PI(4,5)P2, phosphatidylinositol 4,5-bisphosphate.
RNA Interference—The following SPIN90-specific siRNAs were designed on the basis of the human SPIN90 cDNA sequence and targeted four regions: si-824 (nucleotides 824–844, 5'-GGAAA-3'), si-1293 (nucleotides 1293–1303, 5'-GTACCTCGAGCAAACACGCCA-3'), si-1666 (nucleotides 1666–1686, 5'-GTACCTCAATGTGCAAGAGAAACGAGTTTCAAGAGAACTCG-3'), and si-1731 (nucleotides 1731–1751, 5'-GTACCTCGAGCAAACACGCCA-ATGCTCAATGTGCAAGAGAAACGAGTTTCAAGAGAACTCG-3'); the underlined letters denote the SPIN90 siRNA sequence. A pair of 66-nucleotide complementary oligonucleotides with an Acc65I site and a HindIII site or BamHI site and EcoRI site added, respectively, to their 5'-ends was also synthesized separately. SPIN90 siRNAs with the annealed 66-bp cDNA fragment were then cloned into the Acc65I site and HindIII site of the pSIREN-DNR-DsRed-Express vector (BD Biosciences). siRNA expression vectors contained a GFP or DsRed gene driven by a RNAi-Ready pSIREN-DNR-DsRed-Express vector (BD Biosciences). SPIN90 siRNAs with the annealed 66-bp cDNA fragment were then transfected with siRNA expression vectors into COS-7, BHK, and HEK 293T cells for 48 h using a mixture of Lipofectamine and Plus reagent (Invitrogen) and the subjected Western analysis using anti-SPIN90 antibody.

Cell Culture and Immunofluorescence—COS-7, BHK, and HEK 293T cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum at 37 °C in 5% CO2. For immunofluorescence analysis, cells were washed three times with phosphate-buffered saline containing 1 mM CaCl2 and 1 mM MgCl2, fixed for 10 min in 3.5% paraformaldehyde, and then permeabilized for 10 min in 0.2% Triton X-100. Once permeabilized, the cells were incubated at room temperature first with primary antibodies for 1 h and then with fluorophore-conjugated secondary antibodies for an additional 30 min. To assess the cytoskeleton, filamentous actin was labeled with Texas red-phalloidin (Molecular Probes, Eugene, OR) and visualized using a Leica DMRBE microscope equipped with a 63X (1.4 NA) oil objective and fluorescein isothiocyanate- or Texas Red-optimized filter sets (Omega® Optical Inc., Brattleboro, VT). Images were acquired using a Coolsnap™fx CCD camera driven by MetaMorph imaging software (Universal Imaging Co., Downingtown, PA).

Immunoprecipitation—Immunoprecipitation assays were carried out as described previously (16). Briefly, cells were washed with cold phosphate-buffered saline and extracted with modified radioluminescence precipitation assay buffer (50 mM Tris-Hcl, pH 7.4, 1% Nonidet P-40, 150 mM NaCl, 10 mM NaF, and 1 mM Na3VO4) supplemented with protease inhibitors. Cell extracts were sonicated for 30 s to solubilize the insoluble fractions and then centrifuged at 100,000 x g for 30 min. The resultant lysates were immunoprecipitated using antibodies against Arp3 or SPIN90, after which the immunoprecipitates were incubated for 4 h at 4°C with protein A-Sepharose beads (Amersham Pharmacia Biotech) and then subjected to SDS-PAGE and immunoblotted with rabbit anti-SPIN90 polyclonal antibody.

In Vitro Binding Assays for Arp2/3 Complex and G-actin—Arp2/3 complex binding assay was carried out as described previously (9). Purified GST or GST-SPIN90 variant proteins (5 μg) immobilized on glutathione beads were mixed with 20 pmol of Arp2/3 complex (Cytoskeleton Inc, Denver, CO) in binding buffer (5 mM HEPES, pH 7.6, 1 mM EDTA, 0.5 mM dithiothreitol, 50 mM KCl, 2 mM MgCl2, 0.2 mM ATP, 0.5 mg/ml bovine serum albumin, and 0.1% Tween 20) and incubated for 4 h at 4°C. The bound proteins were subjected to SDS-PAGE and immunoblotted with Arp3 antibodies. G-actin binding assays were carried out as described previously (19). Purified GST-SPIN90 protein (5 μg) was incubated with 0.05 μM G-actin (Cytoskeleton Inc, Denver, CO) in binding buffer (10 mM HEPES, pH 7.6, 1 mM MgCl2, 1 mM dithiothreitol, 0.1% Tween 20, 0.2 mM ATP, and 0.5 mg/ml bovine serum albumin) for 1 h at 4°C. After washing with binding buffer, the bound proteins were subjected to SDS-PAGE and immunoblotted with monoclonal anti-actin antibody.

In addition, a VPH synthetic peptide (FAQFLLNIVEDGLPL) and an inactive mutant (FAQFKKNAVEDAHPK) containing L534K, L535K,
I537A, G541A, L542H, and L544K substitutions were commercially synthesized (Anygen, South Korea), conjugated to CNBr-activated-Sepharose (Amersham Pharmacia Biotech) and reacted with 0.05 \( \mu \text{M} \) G-actin for 1 h at 4°C. Bound actin was subjected to SDS-PAGE. For inhibition assays, purified SPIN90-C-term protein (5 \( \mu \text{g} \)) was reacted with 0.05 \( \mu \text{M} \) G-actin and various concentrations of the synthetic peptides (0–60 \( \mu \text{M} \)) for 1 h at 4°C. After washing with binding buffer, the bound proteins were subjected to SDS-PAGE and immunoblotted with monoclonal anti-actin antibody. A densitometric analysis of developed blots was performed using a densitometric scanner (GS-710, Bio-Rad) and Quantity One software (Bio-Rad).

**Actin Polymerization Assay**—Actin polymerization was measured by monitoring the change in fluorescence intensity of pyrene-labeled actin as described previously (9, 19). Rabbit skeletal muscle monomeric actin-labeled with pyrene with 10% efficiency and unlabeled G-actin (Cytoskeleton Inc, Denver, CO) were mixed at ratio of 1:4 in G-actin buffer (5 mM Tris-HCl, pH 8.0, 0.2 mM CaCl\(_2\), 0.5 mM dithiothreitol, and 0.2 mM ATP) and then centrifuged at 200,000 \( \times g \) for 2 h to remove residual filamentous actin. The Arp2/3 complex and SPIN90-C-term, -SH3 or -PRD were then added to the polymerization buffer (5 mM Tris, pH 7.5, 1 mM EGTA, 0.1 mM CaCl\(_2\), 0.5 mM dithiothreitol, 3 mM NaN\(_3\), 50 mM KCl, 2 mM MgCl\(_2\), and 0.2 mM ATP) after which polymerization was initiated by adding G-actin stock. The final concentration of G-actin in the polymerization reaction mixture was 2.5 \( \mu \text{M} \). Changes in fluorescence were then monitored for 30 min using a spectrofluorometer (SHIMADZU RF-5301 PC, Kyoto, Japan) with excitation at 365 nm and emission at 407 nm.

**Live Cell Imaging and Image Analysis**—Cells were incubated for 24 h after transfection with each constructs GFP-SPIN90 full-length or GFP-actin PI(4)P5 kinase and DsRed si-824 and then placed in a parallel plate chamber or a sealed chamber containing oxygen-depleted (Oxirase, Mansfield, OH) Tyrode solution (119 mM NaCl, 2.5 mM KCl, 2 mM CaCl\(_2\), 2 mM MgCl\(_2\), 25 mM HEPES, pH 7.4, and 30 mM glucose). Fluorescence images were acquired using an Olympus IX71 inverted microscope equipped with a 60X oil objective (1.3 NA) (Olympus, Tokyo, Japan) and a CoolSNAP-ES CCD camera driven by MetaMorph imaging software. Time-lapse sequences were constructed by acquiring 500-ms exposures with 3–5 s intervals in between using a VMM1 Uniblitz shutter (Vincent Associates, Rochester, NY). For live imaging inhibition of membrane ruffle formation in SPIN90 knockdown cell, COS-7 cells were transfected with SPIN90 siRNA for 2–3 days and then serum-starved for 20 h. Images were captured every 5 s for 5 min after treatment of PDGF (50 ng/ml). Phase contrast images were acquired using a Leica DM IRB inverted microscope (Germany) equipped with a 40X (0.55 NA) dry lens. The surface area of the cells was estimated using MetaMorph imaging software. The ratio of the cell surface area before and after PDGF treatment was calculated as a percentage in cells transfected with Mock or si-824.

**RESULTS**

**PDGF-induced Accumulation of SPIN90 in Actin-rich Regions of COS-7 Cells**—SPIN90 is known to play a pivotal role in sarcomere assembly during cardiac myocyte differentiation and to associate with various regulators of actin dynamics, including the Arp2/3 activator WASP and βPIX, a GEF for Rho GTPase (12, 17). This led us to hypothesize that SPIN90 is in some way involved in cytoskeletal reorganization. Consistent with that idea, analysis of immunofluorescence images showed that endogenous SPIN90 is widely distributed in the cytoplasm of COS-7 cells (Fig. 1C) but that treating the cells with PDGF leads to its redistribution to the cell cortex (Fig. 1D) where it colocalizes with phallolidin-stained F-actin. By contrast, the distribution of Ibx, a cytoplasmic marker, was unaffected by PDGF (Fig. 1, A and B, left panel).

**Inhibition of Lamellipodia Formation by SPIN90 siRNAs in COS-7 Cells**—To gain further insight into the function of SPIN90, we tried to knock the molecule down using SPIN90 siRNA (Fig. 2A). That the SPIN90 protein was specifically depleted by the knockdown procedure was confirmed by Western blot analysis (Fig. 2, B and C). We found that expression of SPIN90 was reduced 80–90% in cells transfected with
si-824, si-1293, or si-1731, but was unaffected in cells transfected with Mock (empty vector) or si-1666 (ineffective siRNA). Notably, actin staining with phalloidin revealed that PDGF-induced lamellipodia formation was greatly diminished in SPIN90 knockdown cells transfected with si-824, si-1293, or si-1731, whereas lamellipodia were readily observed in cells transfected with mock (vector only) or si-1666 (ineffective siRNA) (Fig. 3, A and B). SPIN90 knockdown cells also showed diminished cell spreading and motility (Fig. 3, C and D). Analysis of phase contrast images showed that exposing mock transfectants to PDGF for 5 min elicited a 10% increase of cell surface area, but only a 1% increase was seen in si-824 transfectants (Fig. 3, C and D and also see supplemental movie 1).

Binding of the C Terminus of SPIN90 to the Arp2/3 Complex—The Arp2/3 complex is known to be a key regulator of actin-filament nucleation and to be concentrated within dynamic actin structures, such as the leading edges of motile cells. By using coimmunoprecipitation and GST pull-down assays, we confirmed that SPIN90-C-term also associates with the Arp2/3 complex \textit{in vivo} and \textit{in vitro} (Fig. 4, A–D). Furthermore, sequence alignment showed that SPIN90 contains an Arp2/3 binding region (A-like domain) in its C terminus (Fig. 4E) and that this A-like domain contains conserved arginine, tryptophan, and hydrophobic residues essential for strong activation of the Arp2/3 complex (20).

**Figure 3. Lamellipodia formation is inhibited by SPIN90 siRNAs in COS-7 cells.** A, COS-7 cells were transfected with SPIN90 siRNAs and then stimulated with PDGF. Arrows indicate GFP-positive siRNA-transfectants, whereas actin is shown in red (Texas Red-phalloidin). Scale bar represents 10 μm. B, histogram showing the ratios of ruffle length to cell circumference on each group of cells. Measurements were made on more than 20 transfected cells in each experiment; error bars indicate the S.E. This experiment was repeated at least four times, **p < 0.01 versus control (Student’s t test). C, phase contrast images of Mock and si-824 transfectants, with and without PDGF. Black arrows indicate lamellipodia formation. The insets show cells expressing GFP under the indicated conditions. Scale bar represents 5 μm (also see supplemental movie 1). D, the cell surface area before and after PDGF-treatment was calculated as a percentage in cells (n > 10) transfected with Mock or si-824. Error bars indicate the S.E. from three independent experiments.
FIGURE 4. SPIN90 binds to and colocalizes with the Arp2/3 complex. A, COS-7 cell lysates were immunoprecipitated with anti-Arp3 antibody and immunoblotted (IB) with rabbit anti-SPIN90 antibody. B, schematic diagrams of the GST-tagged SPIN90 constructs. C, in vitro binding assays were carried out with purified GST-SPIN90 protein fragments (SH3, PRD, or C-term) and Arp2/3 complex; the bound protein was verified using anti-Arp3 antibody. Purified GST-SPIN90 proteins were confirmed by Coomassie Blue staining. D, in vitro binding assays were carried out with the A-like domain of SPIN90 (amino acids 336–407) or SPIN90-C-term and the Arp2/3 complex. The VCA domain of N-WASP was used as a positive control for Arp2/3 complex binding. E, comparison of the SPIN90 A-like domain with the A domains of Cortactin, N-WASP, WASP, Scar, and MyoA. Black boxes indicate conserved amino acids. F, COS-7 cells with or without PDGF treatment (50 ng/ml) for 5 min were stained with mouse anti-SPIN90 polyclonal and anti-Arp3 antibodies. White boxes were magnified. Scale bar represents 10 μm.
of actin polymerization in vitro (21, 22). Immunofluorescence imaging of untreated COS-7 cells revealed that Arp3 and SPIN90 were scattered in the cytoplasm in punctate structures and appeared to colocalize sometimes (Fig. 4F, upper panels). Treating the cells with PDGF induced colocalization of SPIN90 and the Arp2/3 complex at the cell cortex (Fig. 4F, lower panels).

The C-terminal VPH Domain of SPIN90 Binds Directly to G-actin—We next determined which region of SPIN90 is essential for binding G-actin. Sequence analysis showed that the C terminus of SPIN90 contains a VPH domain (amino acids 527–549) with the conserved LLXX(I)XXGXXL sequence (single-letter amino acid code, where X is any amino acid), which is able to bind monomeric G-actin (Fig. 5D) (23). That the VPH domain of SPIN90 actually binds G-actin was then confirmed in two ways. First, in vitro binding assays showed that SPIN90-C-term (a C-terminal SPIN90 fragment containing the VPH domain) binds monomeric G-actin, whereas SPIN90-SH3 and SPIN90-PRD (SPIN90 fragments respectively containing SH3 and PRD domains) did not bind G-actin (Fig. 5A). Second, a synthetic VPH peptide effectively bound G-actin in a dose-dependent manner (Fig. 5B and C), whereas an inactive VPH substitution mutant did not (Fig. 5C).

Activation of the Arp2/3 Complex by SPIN90 C Terminus—To further confirm the role of SPIN90 in actin polymerization and dynamics, actin polymerization in the presence of SPIN90 protein and pyrene-actin was monitored as a function of the increase in fluorescence that occurred when monomeric actin was assembled into filaments. We found that a GST-N-WASP VCA domain fusion protein (VCA, 50 nM), which served as a positive control, readily activated Arp2/3-induced actin polymerization (Fig. 6A). Likewise, SPIN90-C-term concentration-dependently increased Arp2/3-induced actin polymerization (Fig. 6B), whereas SPIN90-SH3 and SPIN90-PRD had no effect (Fig. 6A). It thus appears that the C terminus of SPIN90 is able to bind both monomeric G-actin and the Arp2/3 complex so that the complex activates the actin polymerization process.

SPIN90 Is Present within Actin Comet Tails in Cells Overexpressing PI(4)P5K—Because actin polymerization participates in the movement of vesicles into the cytosol, examination of actin comet tails in cells overexpressing PI(4)P5K can provide clear evidence of the proteins involved in actin dynamics (24–28). Actin comet tails are regulated by PI(4,5)P2, which is produced by phosphorylation of PI(4)P by PI(4)P5K. Overexpression of PI(4)P5K promotes actin polymerization from endosomal vesicles to form motile actin comets (29, 30). Therefore, to determine whether SPIN90 is recruited to
FIGURE 6. The SPIN90 C terminus induces Arp2/3 complex-mediated actin polymerization. A, time course of the actin polymerization reaction (2.5 μM G-actin (2 μM unlabeled actin and 0.5 μM pyrene-labeled actin) and 100 nM Arp2/3 complex) carried out in the presence of SPIN90-SH3 (250 nM, SH3), SPIN90-PRD (250 nM, PRD), or SPIN90-C-term (250 nM, C-term), GST-N-WASP VCA domain fusion protein (50 nM VCA, positive control) and actin alone served as controls. B, actin polymerization was also monitored following addition of the indicated concentrations of purified GST-SPIN90-C-term.

FIGURE 7. SPIN90 associates with PI(4)P5K-induced actin comet tail formation. A, time lapse images of BHK cells coexpressing GFP-SPIN90 and PI(4)P5K reveal that SPIN90 was incorporated into PI(4)P5K-induced actin comet tails (also see supplemental movie 2). B, BHK cells coexpressing GFP-SPIN90 and PI(4)P5K were fixed with 3.5% paraformaldehyde, after which actin was labeled with Texas Red-phalloidin. The magnified image shows an actin tail in which GFP-SPIN90 and actin were colocalized. Scale bar represents 10 μm. C, HEK 293T cells were transfected with either pSIREN-DsRed (Mock), or pSIREN-DsRed containing SPIN90 siRNA (DsRed si-824). The expression of SPIN90 was detected by anti-SPIN90 antibody and Mock and DsRed si-824 expression was by anti-DsRed antibody. Tubulin was used to indicate equal loading. D, actin comet tail formation was assessed by counting the number of tail in the HEK 293T cells (n > 40) coexpressing GFP-actin and PI(4)P5K with Mock and DsRed si-824 and presented by histograms, and the experiment was repeated three times. Error bars indicate the S.E. (also see supplemental movie 3).
the tails of moving endosomes, we recorded time-lapse sequences of BHK cells cotransfected with GFP-SPIN90 and PI(4)P5K. The recorded sequences showed that GFP-SPIN90 localized within comet tails tagged to moving endosomes (Fig. 7A and also see supplemental movie 2) and completely overlapped actin within those comet tails (Fig. 7B).

To then determine whether SPIN90 is actually required for formation of actin comet tails, we have assayed the formation of actin comet tail in SPIN90-knockdown cells. First of all, DsRed-si-824 activity was tested in HEK 293T cells, and immunoblot analysis confirmed dramatic reduction of endogenous SPIN90 expression in HEK 293T cells transfected with DsRed-si-824 (Fig. 7C). For actin comet tail formation, we transfected HEK 293T cells with GFP-actin, PI(4)P5K with Mock or DsRed-si-824, and then examined the cells using video microscopy (see supplemental movie 3). We observed that Mock-transfected cells showed numerous actin comet tails, whereas the numbers of comet tails were dramatically reduced (~55%) in SPIN90-knockdown cells by DsRed-si-824 (Fig. 7D). Taken together, our findings strongly suggest that SPIN90 participates in the formation of actin comet tail and a variety of cellular processes involved in actin reorganization.

**DISCUSSION**

We previously showed that SPIN90 may participate in cytoskeletal reorganization. Aspects of the signaling pathway involved have been demonstrated in studies of the SPIN90-like protein DIP, which regulates actin polymerization and cell adhesion turnover by interacting with Grb2 and Src downstream of the Rho-mDia pathway. It also plays an important role in EGF-induced cell motility via Src kinase-dependent feedback modulation and in regulating Rho and Rac activity (14, 18). In addition, WISH reportedly induces N-WASP-dependent and -independent activation of the Arp2/3 complex, resulting in rapid actin polymerization for filopodia formation (15). Consistent with those reports, SPIN90 is involved in sarcomere assembly in cardiac myocytes, regulating the interaction of the Arp2/3 complex with WASP, βPIX, and Nck in a manner dependent upon cell adhesion and Erk activation (12, 17). Still, almost nothing is known about the specific functions of SPIN90.

In the present study, we characterized the role played by SPIN90 during actin dynamics. SPIN90 colocalized with actin filaments at the leading edges of cells and was enriched along with the Arp2/3 complex at the lamellipodia of PDGF-treated cells, where there was a high degree of actin turnover. Moreover, such PDGF-induced lamellipodia formation was greatly diminished by siRNAs in SPIN90 knockdown cells. These observations strongly support the idea that SPIN90 regulates actin rearrangement downstream of Rac at the leading edge cells. It is well known that the Arp2/3 complex localizes within lamellipodia and is essential for actin polymerization (31), which means that, together, SPIN90 and the Arp2/3 complex may regulate the spatial and temporal distribution of actin nucleation within lamellipodia. PDGF receptor-triggered signal transduction, which is mediated by Nck, increases the lamellipodial extension rate in concert with a decrease in membrane tension (32). In addition, PDGF treatment induced SPIN90-NCK binding (17). However, it is still unclear how SPIN90 responds to extracellular signals that induce cytoskeletal reorganization and actin-based motility. Having said that, our immunohistochemical and in vitro binding assays indicate the SPIN90 C terminus contains both Arp2/3 and G-actin binding regions (A-like and VPH domains, respectively), which likely mediate activation of the Arp2/3 complex, leading to actin polymerization. Consistent with that idea, both the VPH and A domains are well conserved in all WASP family proteins, and a number of studies have shown that both are required for reconstitution of actin polymerization in vitro and in vivo. SPIN90 has an A-like domain and a VPH domain, which are functionally equivalent to the VCA domain of WASP family proteins, inducing activation of the Arp2/3 complex.

In fibroblasts overexpressing PI(4)P5K, SPIN90 appears to locate within actin comet tails; moreover, actin comet tail formation was inhibited in SPIN90 knockdown cells. It is well known that the actin cytoskeleton provides the force needed to detach newly formed vesicles from the plasma membrane and then propel them through the cytosol (24) and that the movement of intracellular vesicles (endosomes) is mediated by actin polymerization via the Arp2/3 complex (36). Indeed, actin polymerization tightly links endocytosis with endosomal vesicle trafficking. Activators of the Arp2/3 complex, including WASP, Cortactin, Abp1, and Pan1p, have all been implicated in endocytosis and vesicle movement, through their interaction with several endocytic proteins (dynamin, intersectin, syndapin, and Hip1R), which provides a connection to actin dynamics (37–41). The WASP-syndapin-dynam and cortactin-Abp1-dynam pathways highlight the importance of Arp2/3-mediated actin nucleation in endocytosis and vesicle movement. It is noteworthy in that regard that SPIN90 has a reverse domain organization similar to that of cortactin and Abp1, with SH3 and PRD domains in its N terminus and actin- and Arp2/3-binding regions (VPH and A-like domains) in its C terminus (9, 10, 42).

Earlier reports suggested that SPIN90 is an Nck binding molecule that participates in cytoskeletal rearrangement (12). However, we have shown here that the C terminus of SPIN90 mediates actin reorganization via direct interaction with the Arp2/3 complex and G-actin. These findings provide new insight into the way SPIN90 could regulate actin polymerization machinery and thus actin-based motility. A future challenge will be to characterize the molecular composition of SPIN90 complexes and their mode of control of actin-based motility.

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