Cdc42-interacting Protein 4 Mediates Binding of the Wiskott-Aldrich Syndrome Protein to Microtubules*

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The Wiskott-Aldrich syndrome is an inherited X-linked immunodeficiency characterized by thrombocytopenia, eczema, and a tendency toward lymphoid malignancy. Lymphocytes from affected individuals have cytoskeletal abnormalities, and monocyes show impaired motility. The Wiskott-Aldrich syndrome protein (WASP) is a multi-domain protein involved in cytoskeletal organization. In a two-hybrid screen, we identified the protein Cdc42-interacting protein 4 (CIP4) as a WASP interactor. CIP4, like WASP, is a Cdc42 effector protein involved in cytoskeletal organization. We found that the WASP-CIP4 interaction is mediated by the binding of the Src homology 3 domain of CIP4 to the proline-rich segment of WASP. Cdc42 was not required for this interaction. Co-expression of CIP4 and green fluorescent protein-WASP in COS-7 cells led to the association of WASP with microtubules. In vitro experiments showed that CIP4 binds to microtubules via its NH₂ terminus. The region of CIP4 responsible for binding to active Cdc42 was localized to amino acids 383–417, and the mutation I398S abrogated binding. Deletion of the Cdc42-binding domain of CIP4 did not affect the colocalization of WASP with microtubules in vivo. We conclude that CIP4 can mediate the association of WASP with microtubules. This may facilitate transport of WASP to sites of substrate adhesion in hematopoietic cells.

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The abbreviations used are: WASP, Wiskott-Aldrich syndrome protein; CIP4, Cdc42-interacting protein 4; GST, glutathione S-transferase; PCR, polymerase chain reaction; PBS, phosphate-buffered saline; SH, Src homology; PH, pleckstrin homology; GFP, green fluorescent protein; WIP, WASP-interacting protein; PIPES, 1,4-piperazinediethanesulfonic acid; PAGE, polyacrylamide gel electrophoresis; TRITC, tetramethylrhodamine B isothiocyanate; GBD, GTPase binding domain; CIP4, Cdc42/Rac interacting and binding; GDPβS, guanyl-5′-yli thiophosphate; GTP·S, guanosine 5′-O-(thiotriphosphate); PACSIN, protein kinase C and casein kinase 2 substrate in neurons; PSTPIP,
EXPERIMENTAL PROCEDURES

Two-hybrid Screen—We used the Interaction Trap two-hybrid screen (35). Yeast and vectors were obtained from Roger Brent (University of Massachusetts, Boston, MA). An human T-cell lymphotrophic virus type I-transformed T-cell line cDNA library for this system was pur-
chased from CLONTECH (Palo Alto, CA). The screen was performed as
described (16, 36) using full-length WASP as bait in the LexA system.
Additional methods were obtained from material supplied with the
library. The specificity of interaction of clones expressing the interac-
tion phenotype was tested with a bait plasmid containing an open
reading frame of Drosophila bicoid protein that was included with the
materials obtained from Dr. R. Brent, and with a bait plasmid contain-
ing an open reading frame of human IGF1-R6 cytoplasmic domain that
was a gift from Bhakti Dey (NCI Metabolism Branch, Bethesda, MD).

Expression Constructs—A cDNA encoding full-length WASP was
cloned into pQBI-25 (Quantum Biotechnologies Inc., Laval, Quebec,
Canada) in order to produce GFP-WASP, and into pCR2 (Invitrogen,
Carlsbad, CA) for in vitro translation. GST-Cdc42 expression constructs
were generously provided by Dr. Anne Ridley (Ludwig Institute for
Cancer Research, University College, London, United Kingdom). A
full-length CIP4 cDNA PCR product from a B-cell cDNA library (gift
from Dr. Colin Buckett, National Institutes of Health, Bethesda, MD) was
cloned into pCR2.1 vector (Invitrogen). This CIP4 cDNA was used
as a template to clone CIP4 and CIP4 fragments into pCR2.1 for
in vitro translation and into pGEX4-T-2 for expression of GST-tagged
proteins. CIP4 cDNA was cloned into a derivative of the mammalian expression
template pRK5 (PharMingen, San Diego, CA) that was modified to include
an NH2-terminal myc epitope tag (pRK5-myc) in order to express myc-
tagged CIP4 in mammalian cells. Point mutations were produced by
PCR using the TnT® coupled transcription-translation system (Promega,
Madison WI) in the presence of [35S]methionine. GST fusion proteins
were expressed using the protease-deficient Escherichia coli bacte-
rial strain BL21. Cells from induced cultures were collected, resuspended
in 50 μl of ice-cold PBS/ml of culture and disrupted by sonication. Triton
X-100 was added to a final concentration of 1% and the lysate was
mixed for 30 min. The lysate was centrifuged at 12,000g for 10 min,
diluted 5-fold with 1% Triton X-100/PBS, and reduced glutathione-
agarose (Sigma) added. After binding of GST fusion proteins, the gluta-
tathione-agarose was washed and stored at 4 °C. The quantity and
purity of the GST fusion proteins was assessed by SDS-PAGE, followed
by Coomassie Blue staining. GST-Cdc42 proteins were loaded with the
non-hydrolyzable GTP analog GTPγS immediately before use by adding
equal volumes of the purified fusion protein bound to glutathione-
agarose in PBS and 100 mM Tris-HCl, pH 7.5, 15 mM EDTA, 1 mg/ml
bovine serum albumin, 2 mM dithiothreitol, 1 mM GTP-γS or GTPγS at
37 °C for 30 min and fixed with 12.5 mM MgCl2.

Antibodies—Affinity-purified anti-CIP4 antibody was prepared as
follows. A PCR product encoding CIP4 residues 118–481 was cloned
into pFLAG-ATS vector (Kodak-IBI) and recombinant FLAG-CIP4 ex-
pressed in E. coli BL-21. The FLAG-CIP4 was purified by affinity
chromatography on anti-FLAG M2 agarose according to manufacturer’s
directions. Rabbits were immunized with 100 μg of purified FLAG-CIP4
initially, followed by 10 μg/week for 10 weeks. Preimmune and immune
sera were collected and assayed by enzyme-linked immunosorbent assay
using GST or GST-CIP4 as antigens. Anti-CIP4 antibody was pu-
filled from the immune sera by affinity chromatography on GST-CIP4
bound to cyanogen bromide-activated Sepharose (Amersham Pharma-
bia Biotech).

Rabbit anti-tubulin antibody, mouse anti-tubulin monoclonal antibi-
dy MA1, and phallolidin-TRITC were purchased from Sigma. Goat
anti-rabbit IgG AlexaFluor594 and goat anti-mouse IgG AlexaFluor488 were
purchased from Molecular Probes. Rabbit anti-myc antibody was pur-
chased from Upstate Biotechnology, and mouse monoclonal anti-myc
was purchased from Invitrogen. Monoclonal anti-WASP was obtained
as described (37).

Cell Transfection—Plasmid DNA was prepared using the Qiagen
Maxi Prep kit. COS-7 cells were maintained in RPMI 1640 medium
(Life Technologies, Inc.) supplemented with 10% fetal bovine serum and
2 mM l-glutamine. Cells were transfected by electroporation.

Co-immunoprecipitation—COS-7 cells transfected with GFP-WASP
Triton X-100, 80 mM K-PIPES, 1 mM EGTA, 1 mM MgSO₄, 30% glycerol.

Cells were fixed and permeabilized by 4% formaldehyde, 0.1% Triton X-100 and cultured for 7 days as described (39). To visualize microtubules, cells were permeabilized with anti-CIP4. Immunoprecipitated proteins were analyzed by SDS-PAGE followed by immunoblotting with either anti-myc (upper panel) or anti-WASP (lower panel).

and myc-CIP4 were lysed at 1.2 × 10⁶ cells/ml in 1% digitonin, 50 mM Tris-Cl, pH 7.4, 150 mM NaCl supplemented with Complete® proteinase inhibitor mixture (Sigma) at 4 °C for 20 min and centrifuged at 16,000 × g for 10 min. The clarified lysate was diluted 1:3 with washing buffer (50 mM Tris-Cl, pH 7.4, 150 mM NaCl with proteinase inhibitor), and affinity-purified rabbit anti-CIP4 or preimmune serum was added to a final concentration of 10 µg/ml or 5 µg/ml, respectively. After incubation at 4 °C for 2 h, protein A-Sepharose (50 µl of a 50% slurry in wash buffer) was added, and the suspension rocked at 4 °C for 1 h. The pellet was washed three times with washing buffer and bound proteins analyzed by SDS-PAGE, followed by Western blot using either monoclonal anti-myc or monoclonal anti-WASP.

In Vitro Microtubule Binding Assays—Microtubules were obtained by incubating 5 mg/ml tubulin (Molecular Probes, Eugene, OR) in G-PEM buffer (100 mM K-PIPES, 1 mM EGTA, 1 mM MgSO₄, 1 mM GTP, pH 6.8) with 30% glycerol at 35 °C for 10 min. Microtubules were stabilized by 10 µM paclitaxel (Molecular Probes) as instructed by the manufacturer. The microtubule binding experiments were performed as described (38). Briefly, 50 µl of in vitro translated, [³⁵S]methionine-labeled CIP4 or CIP4 mutants was diluted in 200 µl of PB (80 mM K-PIPES, pH 6.9, 1 mM EGTA, 1 mM CaCl₂) containing Complete® proteinase inhibitor mixture (Sigma). The mixture was spun at 50,000 × g for 1 h at 4 °C in a 70TI rotor (Beckman). 100 µl of supernatant was incubated with or without 30 µg of microtubules for 30 min at 37 °C in the presence of 10 µM paclitaxel. The microtubule and CIP4 mixtures were layered over 1 ml of 15% sucrose in PB, and spun for 1 h at 40,000 × g (30,100 rpm) in a SW55 rotor (Beckman). Both supernatants (above the sucrose) and pellets were analyzed for the presence of CIP4 and its mutants by 4–20% SDS-PAGE, followed by autoradiography. The gels were then stained with Coomassie Blue to confirm that an equal mass of microtubules was loaded on the gel in each sample.

Immunofluorescence—COS-7 cells were transfected with pQB125-GFP-WASP in the presence or absence of pRK5-myc-CIP4 by electroporation. Cells were plated on coverslips in 24-well culture plates. 24–48 h after transfection, cells were fixed and permeabilized by Cytoperm/Fix kit (PharMingen). Primary human macrophages were prepared and cultured for 7 days as described (39). To visualize microtubules, cells were fixed and permeabilized by 4% formaldehyde, 0.1% Triton X-100, 80 mM K-PIPES, 1 mM EGTA, 1 mM MgSO₄, 30% glycerol at room temperature for 30 min (40) or by 4% paraformaldehyde, 0.3% Triton X-100, 0.05% glutaraldehyde in cytoskeleton buffer (10 mM PIPES, pH 7.0, 150 mM NaCl, 5 mM EGTA, 5 mM MgCl₂, 100 µg/ml streptomycin) at room temperature for 15 min. Both methods gave similar results. After staining, coverslips were mounted on glass slides by FluorSave® reagent (Calbiochem). Confocal laser scanning microscopy was performed using an Olympus confocal laser scanning microscope equipped with argon and krypton ion lasers. Series of optical sections through the cells were collected. Images were assembled using Adobe PhotoShop software.

RESULTS

WASP Interacts with CIP4—Several unique WASP-interacting clones were identified in the two-hybrid screen. One clone, our number 4–2, encoded a portion of the protein CIP4, a 545 amino acid protein that previously had been identified as an SH3-domain containing protein with partial homology to the non-receptor kinase Fer that interacts with the active form of Cdc42 (33). The nucleic acid sequence of clone 4–2 was identical to bases 390–1499 of the published CIP4 sequence (GenBank accession number AF000414) with the exception of a single C insertion at 1911 in the 3′-untranslated region. The cDNA encoded CIP4 amino acids 118–545. The interaction was specific to the WASP-LexA bait protein; no interaction of CIP4 with either Drosophila bicoid protein or the cytoplasmic domain of IgF1-Rβ was detected in the two-hybrid system (data not shown). The region of WASP responsible for interaction with CIP4 was mapped by deletion mutation analysis in the two-hybrid system. This showed that CIP4 interacted with a WASP construct retaining amino acids 1–442, but failed to interact with a WASP construct retaining amino acids 1–201 (data not shown). Since the WASP 1–442 has both the GBD and the proline-rich region (310–420), two mechanisms of interaction were thought possible. First, the CIP4 SH3 domain might bind directly to WASP in the proline-rich region. Alternatively, since the yeast homolog of Cdc42 is similar to the human protein, the binding of endogenous yeast Cdc42 to WASP and CIP4 simultaneously could contribute to the interaction. We therefore performed an in vitro binding assay to explore the mechanisms of CIP4/WASP interaction.

The interaction between radiolabeled, in vitro translated WASP of different lengths and GST-CIP4 full-length and deletion mutants is shown in Fig. 1. Full-length CIP4 (GST-CIP4 wild type (WT)) bound strongly to WASP mutants retaining amino acids 1–442 and 1–502, which contain both the proline-rich region and the GBD. WASP mutants lacking the proline-rich region (1–302 and 1–201) bound CIP4 weakly, whether or not the WASP GBD was present (1–302) or absent (1–201).
This indicates that the proline-rich region of WASP is necessary for effective CIP4 binding. However, full-length WASP bound weakly to GST-CIP4 SH3, which contains the isolated CIP4 SH3 domain. WASP mutant 1–442, which retains the polyproline region but lacks the COOH-terminal actin binding region, bound GST-CIP4 SH3 strongly. This difference may be due to a WASP intramolecular interaction between COOH-terminal acidic residues and basic residues just NH₂-terminal of the GBD, which may render the proline-rich region less available for binding to the CIP4 SH3 domain (41, 42). WASP mutants lacking the polyproline region (1–302 and 1–201) failed to bind the CIP4 SH3 domain. A CIP4 mutant lacking the SH3 domain (GST-CIP4 118–481) bound all WASP mutants weakly, if at all. These results demonstrate that the WASP-CIP4 interaction is mediated chiefly by the binding of the CIP4 SH3 domain to the polyproline region of WASP, and that the presence or absence of the WASP GBD has no influence on this binding.

To further establish that the CIP4-WASP interaction can occur independently of Cdc42 binding, the binding of in vitro translated radiolabeled WASP to both CIP4 and the constitutively active Cdc42 mutant V12 was examined. As shown in Fig. 2, a deletion mutant of WASP specifically lacking the GBD retains the ability to bind CIP4, but Cdc42 binding is lost. This shows that CIP4 can bind to WASP in the absence of Cdc42 binding.

To further investigate the CIP4-WASP interaction, COS-7 cells were co-transfected with GFP-WASP and myc-tagged CIP4. GFP-WASP co-immunoprecipitated with CIP4 as shown in Fig. 3. The CIP4 118–481 mutant did not show binding to WASP, again showing the necessity of the CIP4 SH3 domain. When expressed alone, the CIP4 SH3 domain localized entirely in the cell nucleus as detected by immunofluorescence (data not shown), and was not tested for interaction with WASP by co-immunoprecipitation.

Identification of CIP4 Amino Acids Critical for Cdc42 Binding—The primary sequence of CIP4 does not contain the GBD/CRIB motif characteristic of the binding site of Cdc42. We therefore tested a series of CIP4 deletion mutants for the ability to interact with the active form of Cdc42. Full-length in vitro translated CIP4 interacted specifically with GST-Cdc42-GTP-S or with the constitutively active mutant Cdc42-V12-GTP-S but not to the GDP-S-loaded forms (not shown). The dominant negative Cdc42 mutant N17 failed to bind CIP4 regardless of GTP or GDP loading (not shown). As shown in Fig. 4, full-length CIP4 (1–545) or CIP4 constructs retaining amino acids 1–417 and 1–423 reacted with Cdc42, but CIP4 1–383 or 1–407 did not. A series of substitution mutations in this region of the CIP4 construct 1–417 revealed that the mutation I398S abrogated binding of CIP4 to active Cdc42 (Fig.

**FIG. 5.** Effect of CIP4 on WASP colocalization with F-actin. COS-7 cells were transfected with GFP-WASP in the absence (a–c) or presence (d–f) of CIP4. 24 to 48 h after transfection, cells were fixed and permeabilized. WASP distribution was visualized by GFP fluorescence (a and d). F-actin distribution was detected by phalloloidin-TRITC staining (b and e). The GFP-WASP and F-actin images were superimposed for assessment of colocalization (c and f).

**FIG. 6.** Effect of CIP4 on WASP colocalization with microtubules. COS-7 cells were transfected with GFP-WASP and myc-tagged CIP4. GFP-WASP co-immunoprecipitated with CIP4 as shown in Fig. 3. The CIP4 118–481 mutant did not show binding to WASP, again showing the necessity of the CIP4 SH3 domain. When expressed alone, the CIP4 SH3 domain localized entirely in the cell nucleus as detected by immunofluorescence (data not shown), and was not tested for interaction with WASP by co-immunoprecipitation.
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4B). We conclude that the CIP4 amino acids 383–417 and the amino acid Ile-398 are critical for Cdc42 binding to CIP4.

CIP4 Changes WASP Colocalization from F-actin to Microtubules—Wiskott-Aldrich syndrome patients show structural abnormalities of platelets and peripheral lymphocytes, and decreased density of surface microvilli (3–11). Recent studies suggest a role for WASP in actin polymerization since overexpression of WASP induces the formation of actin-containing clusters (18). In order to test the role of CIP4 on WASP-induced cytoskeleton changes, WASP with GFP at the COOH terminus was transfected into COS-7 cells in the absence or presence of myc-tagged CIP4. In the absence of CIP4, the colocalization of GFP-WASP with F-actin was observed in fixed cells stained with phalloidin-TRITC. As reported by others (18), 40–60% of transfected cells exhibited dot or cluster distribution of GFP-WASP in the perinuclear region, accompanied by the disappearance of stress fibers (Fig. 5, a–c). The overexpressed GFP-WASP protein colocalized in clusters with F-actin (Fig. 5, a–c). In cells transfected with myc-CIP4 alone, a general decrease in cellular F-actin was observed with a few stress fibers and subcortical F-actin remaining (data not shown). This has been reported by others (33). When GFP-WASP was cotransfected with myc-CIP4, no dot or cluster structures of GFP-WASP and F-actin were seen (Fig. 5d). F-actin in co-transfected cells did not co-localize with GFP-WASP (Fig. 5, d–f), and assumed a pattern similar to that seen in cells transfected with myc-CIP4 alone. About 40% of the cells showed a diffuse cytoplasmic distribution of GFP-WASP, and the other 60% showed GFP-WASP in cytoplasmic linear structures reminiscent of a microtubular pattern. This linear pattern was not observed in cells transfected with GFP-WASP alone. We then analyzed the relationship between the linearly distributed GFP-WASP and microtubules by staining the cells with anti-tubulin antibody. Without cotransfection with CIP4, GFP-WASP-expressing cells had a normal microtubule distribution, with microtubules radiating from a perinuclear microtubule organizing center to the periphery (Fig. 6b). No distortion of microtubule structure was seen in cells overexpressing GFP-WASP alone, and there was no correlation between GFP-WASP distribution and microtubules (Fig. 6, a–c). In cells co-expressing GFP-WASP and CIP4, however, a linear distribution of GFP-WASP is seen that stained with anti-tubulin antibody (Fig. 6, d–f). Microtubules in WASP+CIP4 cotransfected cells appeared either thicker than normal or in networks of shorter stretches of microtubules. Staining for CIP4 in cells with or without WASP revealed a cytoplasmic distribution of CIP4 with some membrane association; strong localization of CIP4 to microtubules was not observed in cells overexpressing CIP4.

CIP4 NH2 Terminus Binds Microtubules in Vitro—We tested the ability of CIP4 and CIP4 deletion constructs to bind to microtubules by an in vitro co-sedimentation experiment. In vitro translated, [35S]methionine-labeled CIP4 or CIP4 deletion mutants were incubated with or without paclitaxel-polymerized microtubules. Microtubules were then pelleted by ultracentrifugation. COOH-terminal deletion mutants of CIP4 retaining amino acids 1–118 and 1–290, as well as full-length CIP4, cosedimented with microtubules as shown in Fig. 7. NH2-terminal deletion mutants CIP4 291–481 and CIP4 393–481 did not cosediment with microtubules (Fig. 7). These results indicate that the NH2 terminus of CIP4 mediates microtubule binding. CIP4 1–118 and CIP4 1–290 were more completely removed from the supernatant by microtubules than full-length CIP4, suggesting that the NH2 terminus of CIP4 alone binds to microtubules more strongly than full-length CIP4. Additional experiments showed that CIP4 1–417 and Δ383–481 (lacking the binding site for Cdc42) also bound to microtubules (data not shown).

CIP4 Co-localizes with Microtubules in Vivo—Cells overexpressing CIP4 showed a cytoplasmic distribution of the protein. However, the in vitro studies above and the in vivo effect of
CIP4 expression on WASP distribution suggest that CIP4 should co-localize with microtubules. We therefore examined the distribution of native CIP4 in untransfected COS-7 cells and in primary human macrophages. Fig. 8 shows a COS-7 cell and a differentiated human macrophage after 7 days in culture stained with anti-tubulin and anti-CIP4. There is strong association of CIP4 with microtubules in both cell types. We conclude that CIP4 does indeed associate with microtubules in vivo, and that the cytoplasmic distribution seen in the cells overexpressing CIP4 is the result of the large excess of protein present.

NH2 Terminus of CIP4 Mediates WASP Localization with Microtubules—It has been reported that the F-actin and WASP cluster formation in WASP-overexpressing cells could be inhibited by the actin polymerization inhibitor cytochalasin D (18). A decrease of overall F-actin content in cells overexpressing CIP4 alone has also been observed by us (data not shown) and others (33). It is possible that in the presence of CIP4, actin polymerization is generally inhibited or actin depolymerization is enhanced. This may leave WASP in an F-actin-free status, allowing it to move to microtubules. The PH domain of Dbl family member Lfc has been reported to associate with tubulin (43). Although the function of this conserved domain is not known, we present evidence here that the FCH domain in CIP4 mediates binding of WASP to microtubules. We also report that CIP4 amino acids be-
in vitro binding studies that CIP4 amino acids between 383–417 were necessary for binding active Cdc42 (see Fig. 4). CIP4(1–417) preserved both microtubule and WASP binding ability in vitro (data not shown) and caused WASP to assume the linear distribution characteristic of microtubules (Fig. 9d). These results strongly suggest that both the WASP-binding and microtubule-binding portions of CIP4 are required to cause the association of WASP with microtubules in co-transfected cells. Cdc42 binding by CIP4 did not seem to be necessary for CIP4-mediated localization of WASP to microtubules.

**DISCUSSION**

CIP4 was originally identified in a two-hybrid screen as a protein interacting with active Cdc42 and was found to have partial homology in the NH2-terminal region to the proto-oncogene non-receptor cytoplasmic kinase Fes/Tps and to the related kinase Fer (33). This region of homology was termed the FCH domain. The FCH domain is found in the NH2 termini of CIP4 and CIP4 mutants (panels a–d). Subcellular distribution of GFP-WASP was detected by GFP fluorescence. Coexpression and subcellular distribution of myc-tagged CIP4 and mutants was detected by rabbit anti-Myc antibody (Upstate Biotechnology) followed by goat anti-rabbit Alexa™594 as shown in the insets at the lower right corner of each picture.
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binds to the Wiskott-Aldrich syndrome protein, a hematopoietic restricted protein critical for development of normal immunity and platelet function. Co-expression of WASP and CIP4 in COS-7 cells led to WASP localization on microtubules. CIP4 appears to act as an adaptor that mediates the association of WASP with microtubules, by binding microtubules via its NH$_2$-terminal FCH domain, and WASP via its COOH-terminal SH3 domain. In the overexpression system, Cdc42 binding was not important for this adaptor function, since deletion of the CIP4 region that binds Cdc42 had no effect on CIP4 mediated WASP association with microtubules. It is interesting, however, that both CIP4 and WASP bind active Cdc42, suggesting that they are both regulated by this molecule, which is an important regulator of cytoskeletal structure (46, 47). Further experiments are needed to determine if Cdc42 activation has any influence on CIP4 adaptor function in more physiologic conditions.

Is there evidence that other FCH domain proteins bind microtubules and serve as adaptors similar to CIP4? The mammalian proteins PACSIN (protein kinase C and casein kinase 2 substrate in neurons) and PIP3P (proline, serine, threonine phosphatase-interacting protein) are similar to CIP4 in that they have an NH$_2$-terminal FCH domain and a COOH-terminal SH3 domain (44, 48). PIP3P was shown to bind WASP via its SH3 domain, and, when co-expressed in Chinese hamster ovary cells, caused redistribution of WASP from perinuclear F-actin clusters to a cytoplasmic filamentous network (49). The closely related protein PIP3P2, which is very similar to PIP3P, also associates with microtubules, and that these proteins might serve as adaptors for other proteins linking microtubules with proline-rich proteins via the SH3 domain.

Microtubules are dynamic cytoskeletal structures that contribute to cell shape and polarity, motility, intracellular transport, and signal transduction (for reviews, see Refs. 53 and 54). What cell function might be served by the association of WASP with microtubules, mediated by CIP4? WASP function is known to be required for normal monocyte (macrophage) motility (9–11). Recently, WASP was shown to be an essential component of the macrophage podosome, which is the principal adhesive structure of this cell type (39). The osteoclast, derived from the same progenitor as the macrophage, also adheres to the substrate by podosomes. In the osteoclast, podosomes form at the cell periphery adjacent to the ends of microtubules, and if the microtubules are disrupted, the podosomes form in random patterns (55). This suggests that the microtubules may deliver podosome assembly components or signals to the sites where podosomes form. In fibroblasts, the principal adhesive structure is the focal adhesion. In these cells, the microtubules appear to deliver signals regulating the size and distribution of the focal adhesions (56, 57). It may be that CIP4 is a component of a pathway directing WASP and possibly other proteins onto the microtubules for delivery to sites of adhesive structure assembly. Further experiments aimed at disrupting the activity of CIP4 in cells undergoing formation of adhesive structures will investigate this possibility.

In summary, we have shown that the two Cdc42 effector molecules CIP4 and WASP interact, and that CIP4 mediates the association of WASP with microtubules. This reinforces the emerging understanding of the cytoplasmic microtubule network as a dynamic surface which plays a role in facilitation of cell adhesion, signaling, and macromolecular assembly.

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