# The WASP–WAVE protein network: connecting the membrane to the cytoskeleton

*Tadaomi Takenawa* and Shiro Suetsugu*‡*

Abstract | Wiskott–Aldrich syndrome protein (WASP) and WASP-family verprolin-homologous protein (WAVE) family proteins are scaffolds that link upstream signals to the activation of the ARP2/3 complex, leading to a burst of actin polymerization. ARP2/3-complex-mediated actin polymerization is crucial for the reorganization of the actin cytoskeleton at the cell cortex for processes such as cell movement, vesicular trafficking and pathogen infection. Large families of membrane-binding proteins were recently found to interact with WASP and WAVE family proteins, therefore providing a new layer of membrane-dependent regulation of actin polymerization.

Thrombocytopenia
The presence of fewer than usual platelets.

Eczema
A state of inflammation of the skin that is characterized by redness, skin oedema, itching and dryness.

ARP2/3 complex
(Actin-related protein-2/3 complex). A complex that consists of seven subunits. ARP2 and ARP3 are thought to function as two of the three actin monomers that are required for the nucleation of actin polymerization.

**Department of Biochemistry, Institute of Medical Science, University of Tokyo, 4-6-1, Shirokane-dai, Minato-ku, Tokyo 108-8639, Japan.*
*‡PRESTO, JST, 4-1-8, Honcho, Kawaguchi City, Saitama 332-0012, Japan.*
*Correspondence to T.T. e-mail: takenawa@ims.u-tokyo.ac.jp*
doi:10.1038/nrm2069

Wiskott–Aldrich syndrome (WAS) is an X-linked recessive disease that was described as a clinical triad of immunodeficiency, thrombocytopenia and eczema. Without aggressive treatment, such as bone-marrow transplantation, most patients die by 10 years of age due to recurrent infections, haemorrhage or autoimmune diseases[1–4]. *WASP* was initially identified as the causative gene of WAS[5]. Expression of *WASP* is restricted to haematopoietic cells, and haematopoietic cells from patients with WAS have abnormally smooth cell surfaces. This finding indicated that WASP is associated with the cytoskeleton, because the cell membrane is thought to be supported by the intracellular cytoskeleton. Neural (N-)WASP was identified later through its interaction with growth-factor receptor-bound protein-2 (GRB2; also known as Ash), an adaptor protein that functions downstream of receptor-tyrosine kinases[6]. Although N-WASP was named neural WASP because it was abundant in neural tissue, it is localized in several other tissue types.

WASP and N-WASP proteins possess common domains, such as the C-terminal verprolin-homology domain (V; also known as WASP-homology-2 domain (WH2)), the cofilin-homology domain (also known as central domain (C)) and the acidic domain (A). Collectively, these three domains form the VCA region. The VCA region binds to an actin monomer and to the ARP2/3 complex, leading to a burst of actin polymerization through the activation of the ARP2/3 complex, which mediates nucleation of actin polymerization[7,8]. WASP-family verprolin-homologous protein-1 (WAVE1) was identified in a screen for proteins with sequence homology to the VCA region[9]. A WAVE protein was also identified as a suppressor of the cyclic AMP receptor (cAR) mutant in *Dictyostelium* and was named SCAR[10]. Screening of an expression sequence tag (EST) library led to the identification of WAVE2 and WAVE3 (REF. 11). In mammals, WAVE2 is expressed ubiquitously. By contrast, WAVE1 and WAVE3 are enriched in the brain, but are also localized throughout the body in mammals. The mammalian WASP and WAVE family contains five members: WASP, N-WASP, WAVE1, WAVE2 and WAVE3 (REF. 8).

Budding yeast has Las17, a homologue of WASP and N-WASP[12]. Yeast seem to lack WAVE proteins; however, WAVE proteins are present in *Dictyostelium*, indicating that WAVE is necessary for multicellular organisms, in which cells must migrate for morphogenesis and alter their shapes to adapt to changing environments.

Recently, several new factors that interact with WASP and WAVE family proteins have been identified (Supplementary information S1 (table)). These factors bind to WASP and WAVE proteins and regulate their interactions with the ARP2/3 complex. Here, we will discuss how WASP and WAVE proteins are regulated through these binding partners, which modulate the intermolecular or intramolecular interactions of WASP and WAVE proteins. We will also focus on the members of a family of proteins that can deform membranes into narrow tubules *in vitro* and *in vivo*. These membrane-deforming proteins contain the Bin, amphiphysin, Rvs167 (BAR) domain, the extended

Box 1 | **Actin cytoskeleton and cell movement**

Reorganization of actin filaments provides the force required for multiple biological processes. Most of these processes are coupled with the deformation of the cell membrane. Such membrane-cytoskeleton-coupled processes include: the formation of filopodia, lamellipodia and podosomes for cell movement or cancer-cell invasion; endocytosis, phagocytosis, exocytosis and various membrane-trafficking events; cytokinesis and intracellular movement of pathogenic bacteria.

Two-dimensional cell movement is induced by four ordered steps: front-membrane protrusion, adhesion of the protrusion to substrate, movement of the cell body, and retraction of the rear part of the cell[46]. These processes are highly coordinated and governed by the Rho family of GTPases. During membrane protrusion, rapid actin polymerization is induced at the leading edge, resulting in formation of filopodia and lamellipodia; filopodium formation is mediated by the RhoGTPase CDC42 and lamellipodium formation by Rac. Retraction of the rear of the cell is mediated by RhoA.

The above mentioned adhesion-dependent movement is powered by actin polymerization to protrude the leading edge[131]. However, the mechanism by which motive force is generated was unclear until the discovery of the ARP2/3 complex and Wiskott–Aldrich syndrome proteins (WASP) and WASP-family verprolin-homologous (WAVE) family proteins.

WASP and neural (N-)WASP contain proline-rich sequences that can bind to Src-homology-3 (SH3) domains and profilin. Profilin is a small protein that binds to an actin monomer and then supplies the actin monomer to the barbed end (fast-growing end) of an actin filament[132]. Based on the function of profilin, we postulated that binding of WASP or N-WASP to profilin-bound actin through the WASP and N-WASP proline-rich region and to the actin monomer through the WASP and N-WASP verprolin-homology domain is important for WASP and N-WASP-induced actin-filament reorganization[133,134]. Actin-cytoskeleton reorganization was impaired after expression of N-WASP that lacked the verprolin-homology domain or the profilin-binding region[14,134]. WAVE proteins also bind to profilin[9,119]. However, profilin was not essential for the induction of actin polymerization by WASP, N-WASP and WAVE proteins; actin polymerization can be induced by the association of the ARP2/3 complex to the VCA (verprolin-homology domain, cofilin-homology domain and acidic domain) region in WASP, N-WASP and WAVE proteins. Profilin has a role in supplying actin monomers to a VCA region that is bound to the ARP2/3 complex to accelerate nucleation[22,133,135], and profilin is an important factor for reconstitution of N-WASP-driven actin-based motility[136] (BOX 2).

Fer-CIP4 homology (EFC) domain (also known as the FCH-BAR (F-BAR) domain), or the Rac-binding (RCB) domain (also known as the IRSp53-Mim-homology domain (IMD)). Most of these proteins have Src-homology-3 (SH3) domains that interact with WASP and WAVE proteins. We propose that WASP and WAVE proteins and these membrane-deforming proteins regulate cell shape through effects on both actin polymerization and the cell membrane in several biological processes.

WASP and WAVE proteins are now recognized as scaffold proteins that convert signals from protein–protein and protein–membrane interactions to actin polymerization. WASP and WAVE proteins induce actin polymerization during several biological functions, such as the formation of filopodia and lamellipodia in cell migration (BOX 1), membrane trafficking, podosome and invadopodium formation, cell adhesion, pathogen infection (BOX 2), neurite extension and spine formation (BOX 3).

## WASPs activate the ARP2/3 complex

In mammals, the five WASP and WAVE proteins each comprise approximately 500 amino acids and have similar domain architecture[8] (FIG. 1).

***The VCA region.*** The C-terminal VCA region forms an amphipathic helix[13] and interacts with two proteins. The V domain binds to an actin monomer, and the CA domain binds to the ARP2/3 complex. Actin polymerization is initiated by the assembly of three actin monomers; the ARP2/3 complex has two actin-related molecules, therefore the binding of another actin monomer mimics the assembly of three actin monomers. The VCA region functions as the platform on which an actin monomer binds to the ARP2/3 complex to initiate actin polymerization[14–21] (FIG. 1).

Although the VCA region alone can activate the ARP2/3 complex, full-length WASP and N-WASP with a partial deletion of the acidic or the basic region show higher ARP2/3 activation than does the VCA alone. This indicates that other regions of these proteins also contribute to ARP2/3 activation[22–25].

***The WH1 domain binds to the WIP family.*** The N-terminal regions of WASPs are different from those of WAVEs. WASP and N-WASP contain a WH1 domain (also known as the Ena-VASP-homology-1 (EVH1) domain) that is followed by a basic region and a GTPase-binding domain (GBD; also known as the CDC42/Rac-interactive binding (CRIB) region)[6,15,26]. The WH1 domain binds to a specific proline-rich sequence of the WASP-interacting protein (WIP) family of proteins, which includes WIP, corticosteroids and regional expression-16 (CR16) as well as WIP- and CR16-homologous protein (WICH; also known as WIP-related (WIRE))[27–31].

WIP, CR16 and WICH/WIRE form heterocomplexes with WASP and N-WASP[29–32]. The interaction between WASP or N-WASP and proteins of the WIP family is stable, which indicates that WIP proteins might help to maintain the stability of WASP proteins[33,34]. The interaction with WIP is thought to suppress the activity of WASP or N-WASP[35–37]; however, WIP also functions as a scaffold that links WASP to adaptor proteins such as CrkL and Nck, and is recruited to places of vigorous actin polymerization[38–40].

The WH1 domain is a hot spot for mutations in patients with WAS[4], and WIP-deficient mice have defects in T-cell and B-cell activation. T cells from WIP-knockout mice have defects in their actin cytoskeletons that are similar to the defects of the T cells from WASP-knockout mice. However, the defects of WIP-knockout B cells are different from the defects of WASP-knockout B cells[41,42]. Taken together, these findings indicate that WIP family proteins are important for WASP function, but that WIP can also function independently of WASP in certain cell types. The physiological roles of WIP family proteins remain unclear.

***Binding of small GTPases.*** WASP proteins also interact with phosphoinositides and small GTPases. Phosphoinositides interact with the basic region in WASP and N-WASP[16,43,44]. Negatively charged phosphoinositides are thought to associate with the basic region through electrostatic interactions.

BAR domain
A dimeric coiled-coil domain found in Bin1, amphiphysin, Rvs167, endophilin and related molecules. The BAR domain is curved with positive charges on its concave surface. The curved surface of the BAR domain is thought to correspond to the curvature of the cell membrane or the membrane tubules.

EFC domain
(Extended Fer-CIP4 homology domain; also known as the FCH-BAR (F-BAR) domain). A dimeric coiled-coil domain that has weak similarity to the BAR domain. This domain is found in the pombe-Cdc15-homology (PCH) family of proteins.

Box 2 | **Insights into WASP function through pathogen infection**

Several pathogens move inside infected cells by forming actin comets on their surfaces. Pathogens such as *Listeria*, *Shigella*, vaccinia virus and enteropathogenic *Escherichia coli* (EPEC) use the host-cell actin-regulatory machinery to spread the infection. Insights into how the ARP2/3 complex is activated, and how the resulting actin filaments are organized and maintained, were provided through studies of these pathogens.

*Listeria* have a protein (ActA) that directly activates the ARP2/3 complex[137]. By contrast, *Shigella* have the IcsA (also known as VirG) protein, which binds to and activates neural Wiskott–Aldrich syndrome protein (N-WASP) for the activation of the ARP2/3 complex[138,139]. Vaccinia virus expresses the A36R protein, which is phosphorylated by host-cell Src family kinases, and the Src-homology-2 (SH2) domain of the adaptor protein Nck binds to the phosphorylated tyrosine of A36R[40]. The SH3 domain of Nck then recruits and activates N-WASP or the N-WASP–WIP (WASP-interacting protein) complex.

Actin-comet-based movement can be reconstituted *in vitro*. *Listeria* and N-WASP-bound *Shigella* can move in a solution that contains purified actin, the ARP2/3 complex, the capping protein, cofilin and profilin[136] — these five components have been found in lamellipodia. This movement occurs with plastic beads coated with ActA or N-WASP. Organization of actin filaments from actin comets was branched in a similar manner to that observed in lamellipodia[140]. This complete reconstitution of actin-comet formation is thought to provide the minimum proteins that are required for lamellipodium formation.

N-WASP also has a crucial role in pedestal formation. The pedestal is a protrusive structure that is formed upon attachment of pathogens to the surface of host cells at the site where pathogens interact with the host cell. Pedestal formation is regulated by N-WASP downstream of Nck[79,95,141]. EPEC injects Tir protein into host cells. Tir is phosphorylated by a Src kinase and phosphorylated Tir interacts with the SH2 domain of Nck, which activates N-WASP. Similar Nck-based N-WASP activation seems to be used in cell–cell adhesion[94], indicating that pathogens mimic the host cell-adhesion system.

The role of WAVE proteins in pathogen infection is not as clear. In apical invasion of polarized epithelia by *Salmonella*, activation of Rac is followed by actin rearrangement. WAVE2 is important for this invasion[142].

Next to the basic region, there is a CRIB region. The CRIB region of WASP and N-WASP has been shown to bind to CDC42, a small GTPase that is involved in filopodium formation and cell polarity[15,45,46]. Overexpression of WASP or N-WASP in cultured cells resulted in increased actin filaments at sites of WASP or N-WASP localization[6,15], whereas expression of a dominant-negative form of CDC42 reduced the number of WASP- or N-WASP-induced actin filaments[15,45]. Based on these findings, WASP proteins are thought to function downstream of CDC42 (REF. 46). Other small GTPases related to CDC42, such as Tc10, RhoT and Chp, have also been shown to bind to and to activate N-WASP[47–49].

***Autoinhibition and activation of WASPs.*** Under resting conditions, WASP and N-WASP are folded by an intramolecular interaction between the C-terminal VCA region and the N-terminal region (including the CRIB region and its surrounding regions)[16,45,50,51]. Folded WASP and N-WASP are inactive because the VCA region is masked, thereby inhibiting access of the ARP2/3 complex to the VCA region.

Autoinhibition is released by the competitive binding of other molecules to the CRIB or surrounding regions. CDC42 binds to the CRIB region, releasing the interaction between the CRIB and the VCA. Mouse DAB1, a molecule that regulates cortical layer formation in the brain, also binds to a region close to CRIB and releases autoinhibition of N-WASP *in vitro*[52]. The basic region of N-WASP also contributes to autoinhibition, as an N-WASP-mutant protein that lacks the basic region has higher activity for ARP2/3 activation than full-length N-WASP[24]. Phosphoinositides bind to the basic region and synergize with CDC42 to induce WASP and N-WASP activation[16,45,50,51].

The binding of SH3-domain-containing proteins to the proline-rich region of WASP and N-WASP activates the ARP2/3 complex, but the precise mechanism of this is not clear[53–56]. Various degrees of N-WASP activation were observed to be dependent on the SH3 domains from various proteins[53]. The SH3 domains of adaptor proteins such as Nck, GRB2 and WISH (also known as DIP and SPIN90) activate WASP or N-WASP. CDC42 functions with GRB2, but not with Nck, in the activation of N-WASP[43,54]. The SH3 domain of TOCA1 was shown to activate the N-WASP–WIP complex or the N-WASP–CR16 complex in the presence of active CDC42 (REF. 36).

Phosphorylation of WASP and N-WASP by the Src family of tyrosine kinases occurs close to the CRIB region and releases the intramolecular interaction[57–59]. This phosphorylation seems to be enhanced by the activation of CDC42 (REFS 38,59). Importantly, WASP phosphorylation and binding of CDC42 have a synergistic effect on the activation of the ARP2/3 complex. Therefore, activation of the ARP2/3 complex by WASP and N-WASP is locally optimized by the additive effects of various types of signalling molecule.

Phosphorylated N-WASP is degraded through proteasome-mediated proteolysis[58,60]. The degradation of N-WASP influences its activity. The molecular chaperone heat-shock protein-90 (HSP90) was found to elongate the half-life of N-WASP through the inhibition of the degradation of N-WASP[60]. The WH1 domain of WASP binds to the kinase domain in Src family kinases to negatively regulate Src-kinase activity[61]. Therefore, WIP binding to WASP might regulate the phosphorylation of WASP by suppressing the binding of WASP to Src family kinases.

## Possible regulation by membrane curvature

***Association with curvature-sensing proteins.*** In endocytosis, actin polymerization seems to have important roles in vesicle fission and in subsequent vesicle trafficking inside the cell. The endocytosis machinery includes many membrane-binding proteins, and most of these bind to N-WASP as well as to the GTPase dynamin[56,62–65]. The discovery of the syndapin (also known as pacsin) family of proteins in mammals showed that N-WASP is involved in vesicular trafficking, particularly in endocytosis[66–68]. These membrane-binding proteins include proteins with BAR domains and EFC domains.

A large number of proteins that contain BAR or EFC domains bind to N-WASP through their SH3 domains. These domains are frequently found in proteins that are involved in endocytosis[56,62–65,69,70] (FIGS 2a,3 and Supplementary information S1 (table)). BAR and EFC domains have been characterized as membrane-binding domains[56,62–65] (FIG. 3). These domains bind

IRSp53
An adaptor protein that contains an N-terminal Rac-binding (RCB) domain, which binds to Rac, actin filaments and the cell membrane. Its C-terminal Src-homology-3 (SH3) domain binds to WAVE2, Ena (also known as VASP) and other proteins, and its CDC42/Rac-interactive binding (CRIB) region is responsible for binding to CDC42.

Filopodium
A spiky structure that protrudes from the cell. Bundled actin filaments fill the inside of a filopodium.

Lamellipodium
A flat cellular structure that protrudes in the direction of cell movement. Branched actin filaments fill the inside of a lamellipodium.

**Box 3 | N-WASP and WAVE1 in the nervous system**

Among Wiskott–Aldrich syndrome protein (WASP) and WASP-family verprolin-homologous (WAVE) family proteins, neural (N-)WASP, WAVE1 and WAVE3 are enriched in the brain[6,11]. N-WASP is involved in neurite extension as expression of a dominant-negative N-WASP blocks neurite extension[58,60,143], whereas the ARP2/3 complex is possibly a negative regulator of growth-cone translocation[144]. Knockdown of N-WASP by small interfering RNA enhances neurite extension[145], which further complicates the role of N-WASP in growth-cone extension.

Some molecules that bind to N-WASP are also enriched in the brain. These binding molecules include slit-robo (sr)GAP[146] and formin-binding protein-17 (FBP17; also known as rapostlin)[147], which both contain EFC domains. The yeast homologue of FBP17, Toca-1, seems to be a negative regulator of neurite extension[145]. Although all three WAVE proteins localize at growth cones, their role in neurite and growth-cone extension is unclear[111]. The WAVE1 complex seems to be transported by kinesin-1, and this transport has been suggested to be important for axon elongation[148].

Several reports have also indicated the involvement of N-WASP in spine formation — spine is an actin-rich protrusion on a dendrite[149,150]. Phosphorylation and dephosphorylation of WAVE1 are also involved in spine formation. Kim *et al.* recently reported that the WAVE1 complex was active during spine formation, and that cyclin-dependent kinase-5 (CDK5) phosphorylates the proline-rich region of WAVE1 in the WAVE1 complex, resulting in the inhibition of the capability of WAVE1 to activate the ARP2/3 complex. The phosphorylation sites in the proline-rich region of WAVE1 are not conserved in WAVE2 and WAVE3. Activation of protein kinase A reduces the phosphorylation of WAVE1, leading to actin polymerization for spine formation[114].

to phosphatidylserine and phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)$P_2$), and deform artificial liposomes and cell membranes into tubules. The deformation of the membrane by the BAR and EFC domains might occur during the formation of endocytic vesicles. Alternatively, these domains might simply sense the curvature of the membrane and recruit WASP and N-WASP. Blocking actin polymerization with latrunculin treatment resulted in localization of EFC-domain-containing proteins to tube-like structures, which indicated that N-WASP-mediated actin polymerization is important for fission and subsequent movement of vesicles[56,63].

BAR- and EFC-domain-containing proteins form homodimers. The SH3 domains of the BAR and EFC proteins bind to dynamin, which is involved in mechanical fission of membrane tubules to form vesicles[56,63,64,69,71]. Therefore, N-WASP-induced actin polymerization might function cooperatively with dynamin in vesicle fission in endocytosis mediated by BAR- and EFC-domain proteins (FIG. 4).

Las17, the yeast WASP homologue, was identified in a screen for mutants that were defective in endocytosis[66–68]. Las17 and verprolin-1 (Vrp1) — a yeast homologue of WIP — are recruited to clathrin-coated pits in the early stage of endocytosis with Bzz1 and Rvs167, which are EFC- and BAR-domain-containing proteins[66,68,72]. Recruitment of N-WASP or Las17 to clathrin-coated pits and the involvement of the actin cytoskeleton in endocytosis have also been shown in mammalian cells[68,73]. The role of WASP and N-WASP in endocytosis is well conserved from yeast to mammals.

***Direction of actin polymerization.*** Although actin polymerization is essential for endocytosis, the direction of actin polymerization — whether the barbed end of the actin is facing towards the endocytosis vesicles or towards the plasma membrane — is unclear (FIG. 4a,b). In lamellipodia and filopodia, the barbed ends are directed towards the plasma membrane. Therefore, actin polymerization occurs centrifugally or outwardly in lamellipodia and filopodia[7,74,75] (FIG. 4c).

The formation of actin comets on vesicles with endocytic properties is observed in cultured cells and in *Xenopus laevis* eggs under several conditions[76,77]. N-WASP is localized at the vesicles of these actin comets. Therefore, actin polymerization seems to occur inwardly from the plasma membrane; the barbed ends are directed towards the vesicles that move away from the plasma membrane[77] (FIG. 4a). In this model, actin polymerization directly generates force for vesicle movement.

There is a mutant strain of yeast in which actin filaments accumulate at sites of endocytosis. Photobleach analysis of actin in this yeast mutant indicated that actin polymerization occurs towards the plasma membrane (that is, the barbed ends are facing towards the plasma membrane, not to the endocytosis vesicles). Therefore, the direction of actin polymerization for endocytosis seems to be the same as the direction of actin polymerization during protrusive lamellipodium formation (FIG. 4b,c). In this case, the force generated by actin polymerization might function for vesicle movement or for vesicle fission. For vesicle movement, polymerizing pointed ends of actin filaments might possibly generate the direct force for comet-like vesicle movement[68,75] (FIG. 4b). For vesicle fission, the pushing of the plasma membrane by actin polymerization might generate the force for fission of the vesicles from the plasma membrane (FIG. 4b). Other unexpected functions of actin polymerization remain to be considered.

## Other biological functions of WASPs

WASP and N-WASP are involved in several biological functions that are accompanied by the activation of the ARP2/3 complex. These functions include filopodium formation, podosome formation, pathogen infection (BOX 2) and neurite extension (BOX 3).

***Filopodium formation.*** Filopodia are thought to be generated by the activation of CDC42 or other small GTPases. Because N-WASP is activated by CDC42 *in vitro*[16], N-WASP was initially thought to induce filopodium formation[45]. N-WASP has been shown to be localized to certain types of filopodium[78]; however, filopodia still form in N-WASP-deficient cells[79,80].

Filopodia contain straight bundles of actin filaments, but N-WASP-induced actin filaments are branched because they are induced by the activation of the ARP2/3 complex[19,20,81], indicating that N-WASP alone does not induce the formation of filopodia. N-WASP-binding proteins that induce bundling of actin filaments have not yet been discovered, and it is still unclear how N-WASP generates bundled actin filaments.

Filopodia can be generated independently of the ARP2/3 complex and WASP family proteins; for example, the formation of certain types of filopodium occurs independently of ARP2/3 and WAVE2 (REF. 82). Formins, which also mediate actin nucleation, induce the formation

**Podosome**
A structure that protrudes into the extracellular matrix and that is enriched in actin filaments, matrix-degrading enzymes, focal adhesion molecules and molecules involved in vesicle trafficking.

**Invadopodium**
A structure that is similar to a podosome, but larger. Sometimes, podosomes in transformed cells are called invadopodia.

**Phosphoinositides**
A phospholipid species, members of which function as signalling molecules and contain an inositol ring. There are seven poly-phosphoinositides, PtdIns(3)P, PtdIns(4)P, PtdIns(5)P, PtdIns(3,4)$P_2$, PtdIns(3,5)$P_2$, PtdIns(4,5)$P_2$ and PtdIns(3,4,5)$P_3$, corresponding to phosphorylation at the hydroxyl moiety in the inositol ring.

**Latrunculin**
A natural toxin produced by sponges of the *Latrunculia* genus. It binds to actin monomers and prevents them from polymerizing.

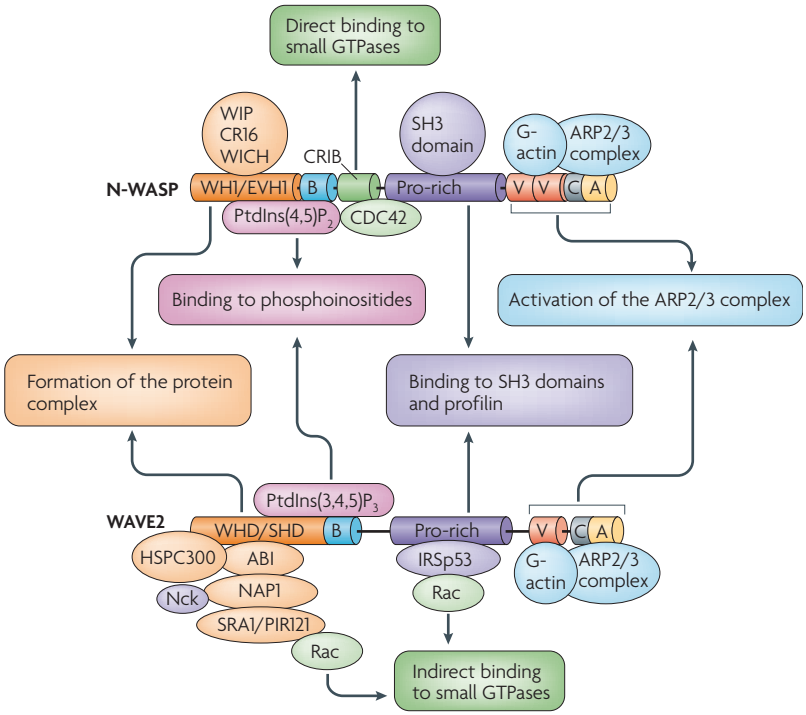


Figure 1 | **Domains and basic binding partners of N-WASP and WAVE2.** Neural Wiskott–Aldrich syndrome protein (N-WASP) and WASP-family verprolin-homologous protein-2 (WAVE2) are the ubiquitous isoforms of the WASP and WAVE families, respectively. The function of each domain is shown. N-WASP has an N-terminal WASP-homology-1 (WH1; also known as Ena-VASP-homology-1 (EVH1)) domain that WASP-interacting protein (WIP), corticosteroid and regional expression-16 (CR16) or WIP- and CR16-homologous protein (WICH; also known as WIP-related (WIRE)) can bind to. WAVE2 has an N-terminal WAVE (also known SCAR)-homology domain (WHD/SHD) that mediates the protein complex formation with HSPC300, Abelson-interacting protein (ABI), NAP1, and SRA1 (or the closely related PIR121). The basic region (B) is common for N-WASP and WAVE2, and phosphoinositides (specifically, PtdIns(4,5)$P_2$ or PtdIns(3,4,5)$P_3$) bind to the basic region. This interaction is important for protein localization or activation of the ARP2/3 complex. N-WASP contains a CDC42/Rac-interactive binding (CRIB) region for CDC42 binding. WAVE2 binds to Rac through SRA1/PIR121 in the WAVE2 complex and through IRSp53 that binds to the proline-rich (Pro-rich) region of WAVE2. N-WASP, WAVE2 and other WASP and WAVE family proteins have a proline-rich region for binding to Src-homology-3 (SH3)-domain-containing proteins and profilin. The binding of SH3-domain-containing proteins to N-WASP or WAVE2 contributes to the optimization of ARP2/3 activation. The adaptor protein Nck also binds to NAP1. The C-terminal region is known as the verprolin-homology domain (V), the cofilin-homology domain (C) and the acidic domain (A). Actin monomer (G-actin) binds to the V domain, whereas the ARP2/3 complex binds to the CA domain. The simultaneous binding of G-actin and the ARP2/3 complex to the VCA region contributes to the activation of ARP2/3-complex-mediated actin polymerization.

of straight actin filaments and are thought to be involved in filopodium formation[83]. Therefore, ARP2/3 activation and other actin-nucleation mechanisms, such as formin-mediated actin nucleation, can function synergistically to induce filopodium formation.

***Podosome and invadopodium formation.*** In macrophages and certain types of invasive cancer cell, a structure that is known as a podosome, or an invadopodium, protrudes into the extracellular matrix (ECM) during invasion. Podosome and invadopodium formation is thought to be essential for invasion and metastasis. Invasion of cells into the ECM is mediated through actin reorganization and the activation of matrix metalloproteinases (MMPs), which degrade the ECM. Src family tyrosine kinases[84,85] and N-WASP are required for podosome and invadopodium formation. Expression of a dominant-negative mutant of N-WASP in Src-transformed cells inhibited invadopodium formation and ECM degradation[86–88]. The binding of the Src family substrate cortactin to N-WASP is essential for this process[86]. Cortactin also binds to the ARP2/3 complex and weakly activates actin polymerization[89,90]. It is thought that N-WASP-induced activation of the ARP2/3 complex is essential for actin-cytoskeleton reorganization that is associated with the protrusion of podosomes and invadopodia.

Although it is not known whether endocytosis occurs in podosomes[84], molecules that are involved in endocytosis, such as dynamin, are also localized to podosomes[84,85,91]. Cortactin also binds to dynamin[92]. Therefore, N-WASP might be involved in podosome formation, not only through regulation of actin polymerization for protrusion, but also through interactions with molecules that are involved in endocytosis[84,85].

N-WASP and WASP are also involved in cell-substratum adhesions through their interactions with the focal adhesion kinase (FAK)[93]. FAK and other focal adhesion molecules localize in podosomes; therefore N-WASP and WASP might function with these cell-adhesion molecules in podosomes.

***N-WASP-binding adaptor proteins.*** WASP and N-WASP bind to several adaptor proteins including Nck, GRB2/Ash, WISH/DIP/SPIN90 and Crk[38,53–55]. For example, Crk seems to be involved in the activation of WASP at immunological synapses, which are contact sites between cells of the immune system (such as between T cells and antigen-presenting cells)[38]. In the kidney, phosphorylation of nephrin by Src family kinases mediates the activation of N-WASP-induced actin polymerization by the adaptor Nck[94]. Similar molecules are used by some pathogens, including vaccinia virus and enteropathogenic *Escherichia coli* (EPEC), to induce actin comet or pedestal formation for prevalence of infection[40,95] (BOX 2). Clustering of Nck at the cell surface induces N-WASP-induced actin polymerization, indicating that Nck-mediated actin polymerization might regulate the reorganization of the actin cytoskeleton at cell adhesions upon activation of Src family kinases and recruitment of adaptor proteins[96].

## The WAVE complex

The N-terminal region of each member of the WAVE family contains a WAVE-homology domain (WHD) and a basic region[10,11] (FIG. 1). The basic region of WAVE2 binds to PtdIns(3,4,5)$P_3$; PtdIns(3,4,5)$P_3$ binding is important for the localization of WAVE2 (REF. 97).

The WHD of all three WAVEs is predicted to be a coiled-coil region and to contribute to the heterocomplex formation. The WAVE1 complex was identified first[98], and the same protein complex for WAVE2 and WAVE3 was later identified[99–102]. Each WAVE complex exists as a pentameric heterocomplex that consists of WAVE,

Nephrin
Nephrin is a cell–cell adhesion molecule that belongs to the immunoglobulin superfamily and is localized at the slit diaphragm of kidney glomerulus. Mutations in the gene that encodes nephrin have been associated with the congenital nephrotic syndrome.

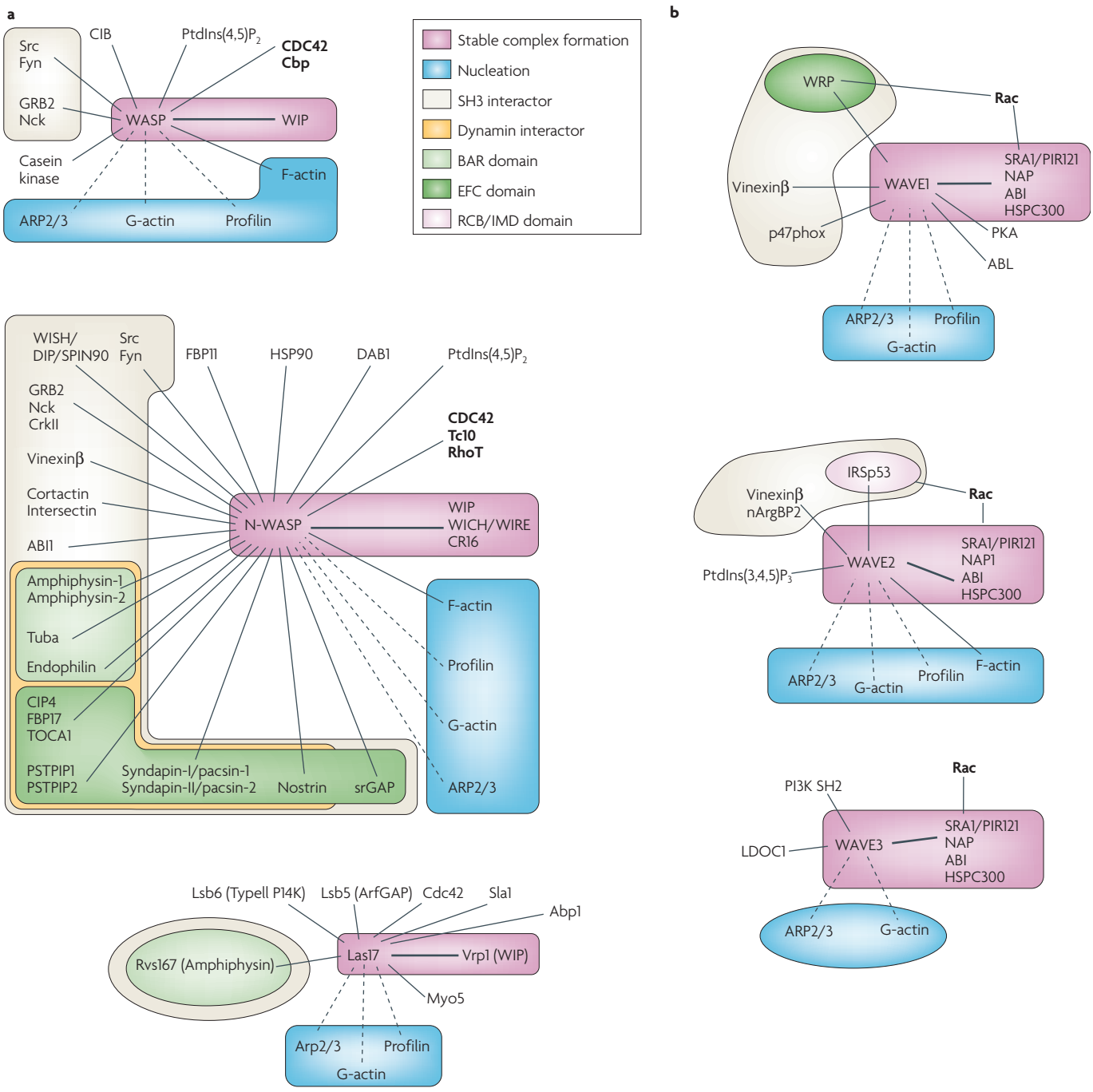


Figure 2 | **WASP and WAVE interactors.** Proteins that interact with mammalian Wiskott–Aldrich syndrome protein (WASP), neural (N-)WASP and yeast Las17 (**a**), or mammalian WASP-family verprolin-homologous protein-1 (WAVE1), WAVE2 and WAVE3 (**b**), are shown by connecting lines according to their characteristics. Pink areas include proteins that form stable complexes with WASP family proteins and WASP-interacting protein (WIP) family proteins (**a**) or WAVE proteins, Abelson-interacting protein (ABI), NAP1, SRA1 (or the closely related PIR121) and HSPC300 (**b**). Blue areas include proteins that are involved in nucleation of actin polymerization. Beige areas include proteins that have Src-homology-3 (SH3) domains for interactions. Dark green and light green areas include EFC- and BAR-domain-containing proteins, respectively. The yellow area includes proteins that interact with dynamin. The light pink circle indicates the protein that has a Rac-binding (RCB) domain (also known as a IRSp53-Mim-homology (IMD)). BAR, EFC and RCB/IMD domains have homology to each other and are a large protein family. Bold font indicates small GTPases. Proteins that are not classified in the categories listed above are shown in roman font. ABL, Abelson; CIB, calcium- and integrin-binding protein; CR16, corticosteroid and regional expression-16; FBP, formin-binding protein; GRB2, growth-factor receptor-bound protein-2; PI3K, phosphatidylinositol-3 kinase; PSTPIP, proline, serine, threonine phosphatase-interacting protein; PKA, protein kinase A; PtdIns, phosphatidylinositol; srGAP, slit-roboGAP; WICH, WIP- and CR16-homologous protein (also known as WIP-related (WIRE)) ; WRP, WAVE-associated RacGAP protein; Vrp1, verprolin-1.

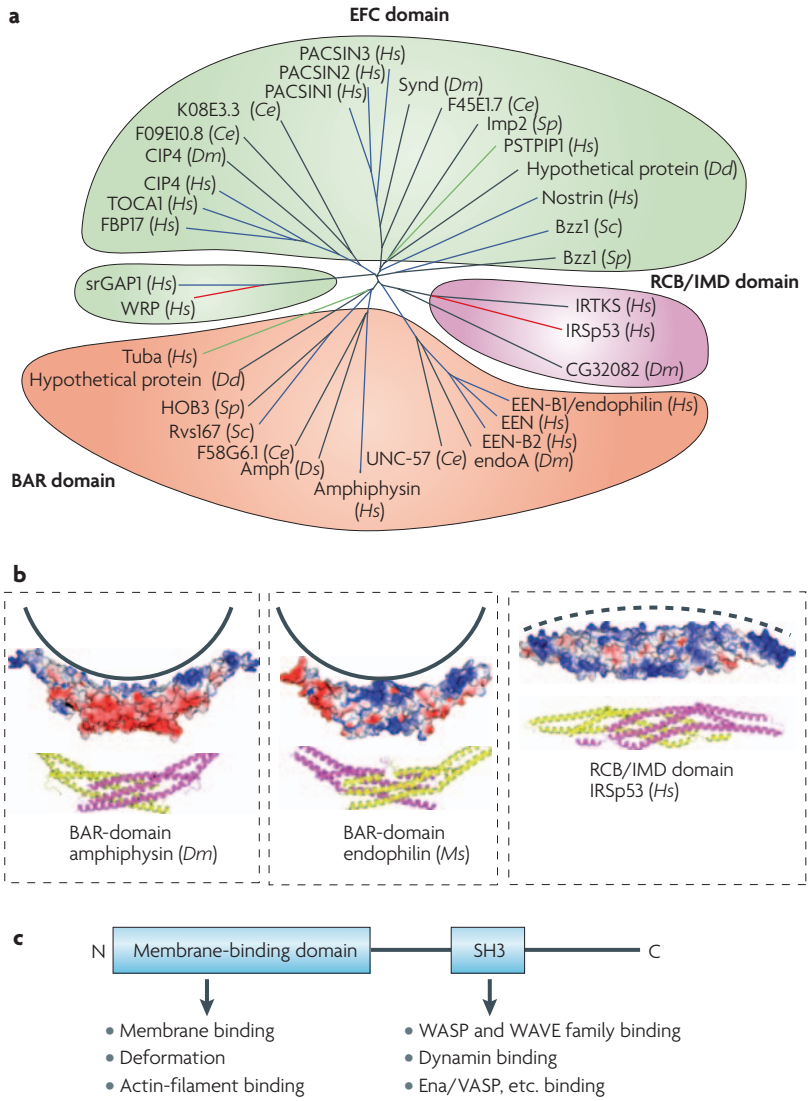


Figure 3 | **Phylogenetic analysis of WASP- and WAVE-binding proteins that have BAR, EFC or RCB/IMD domains. a** | Phylogenetic analysis of BAR-, EFC - or Rac-binding (RCB; also known as a IRSp53-Mim-homology (IMD))-domain-containing proteins that are reported to interact with Wiskott–Aldrich syndrome protein (WASP) and WASP-family verprolin-homologous protein (WAVE). The homologues in *Saccharomyces cerevisiae* (*Sc*), *Schizosaccharomyces pombe* (*Sp*), *Dictyostelium discoideum* (*Dd*), *Caenorhabditis elegans* (*Ce*), *Drosophila melanogaster* (*Dm*), and *Homo sapiens* (*Hs*) are shown. Proteins on blue, red or green lines are reported to bind to WASP or neural (N-)WASP, WAVE proteins, or both, respectively. **b** | The structures of BAR-domain amphiphysin, BAR-domain endophilin and RCB/IMD-domain IRSp53 are shown (upper images show surface electrostatics and lower images show ribbon diagrams of secondary structure). The solid line in the BAR-domain structure indicates where the curved cell membrane is thought to interact with the BAR domain. The dashed line in the RCB/IMD structure indicates binding of the RCB/IMD domain to the membrane. **c** | Typical domain structure of a BAR-, EFC or RCB/IMD-domain-containing protein. The BAR, EFC or RCB/IMD domain is in the N-terminal, and the Src-homolgy-3 (SH3) domain is in the C-terminal of the protein.

ABI (Abelson-interacting protein), NAP1 (also known as p125NAP1), SRA1 (or the closely related PIR121; SRA1 is also known as CYFIP1) and HSPC300 (also known as BRICK). This pentameric heterocomplex is referred to as the WAVE complex[98–101] (FIGS 1,2b).

The WHD domains of all three WAVEs interact with the predicted coiled-coil region of ABI1/2 and HSPC300, which is a small peptide of approximately 75 amino acids[100,103]. ABI1 and ABI2 were originally identified as molecules that interact with the ABL tyrosine kinase, but ABI1/2 forms the 1:1 protein complex with each WAVE. ABI1/2 links NAP1 to WAVEs and NAP1 binds to SRA1/PIR121. SRA1/PIR121 and NAP1 are homologues of *Caenorhabditis elegans* GEX-2 and GEX-3, respectively, which are involved in ventral enclosure (a process of dermal-cell migration during *C. elegans* development)[104]. The ARP2/3 complex is also required for ventral enclosure[105]. Importantly, NAP1 binds to Nck, and SRA1/PIR121 binds to activated Rac[106,107]. Therefore, Nck and Rac might regulate WAVE-mediated activation of the ARP2/3 complex through NAP1 and SRA1/PIR121.

Formation of the WAVE complex contributes to the localization and stability of the various WAVE proteins[103,108–110]. For example, decreased expression of any protein of the complex results in both mislocalization of WAVE2 and decreased amounts of each protein in the WAVE2 complex[108–110]. However, the localizations of WAVE proteins are not determined only by the formation of the complex. WAVE1, WAVE2 and WAVE3 are localized differently in growth cones[111], and WAVE1 and WAVE2 localize differently in fibroblasts[112,113].

The role of the WAVE complex in the activation of the ARP2/3 complex remains controversial. WAVE1 and HSPC300 can dissociate from the rest of the complex (which includes ABI, NAP1 and SRA1/PIR121) in the presence of Rac or Nck *in vitro*. The released WAVE1 can activate ARP2/3 (REF. 98), whereas the rest of the complex, ABI, NAP1, and SRA1/PIR121, seems to trans-inhibit the WAVE1 activity. However, a recent report indicated that the WAVE1 complex purified from brain can also activate ARP2/3 (REF. 114).

The WAVE2 complex was shown to be stable after incubation with active Rac or incubation with stimuli that induce Rac activation, and could be as active as a VCA-region fragment of WAVE2 (constitutively active fragment)[99,101]. It is still unclear whether WAVE2 activity is suppressed by the intramolecular interaction, as is the case for N-WASP. WAVE2 activity might be regulated by the control of its localization alone.

## WAVE2 is crucial for lamellipodium formation

In lamellipodium or filopodium formation, actin polymerization occurs towards the plasma membrane at the cell periphery (FIG. 4c). Studies of WAVE2-knockout fibroblasts showed that WAVE2 is essential for lamellipodium formation downstream of Rac[112,115,116]. However, the molecular link between Rac and WAVE2 was not clear until the discovery of the WAVE protein complex. One of the proteins in the WAVE complex, SRA1/PIR121, binds to activated Rac[107]. In lamellipodia, actin filaments are branched at 70° (REF. 74), which is the angle at which the ARP2/3 complex and WAVE2 generate branched filaments *in vitro*[19,20,81], indicating that the branched actin filaments in lamellipodia are the direct result of WAVE2 function.

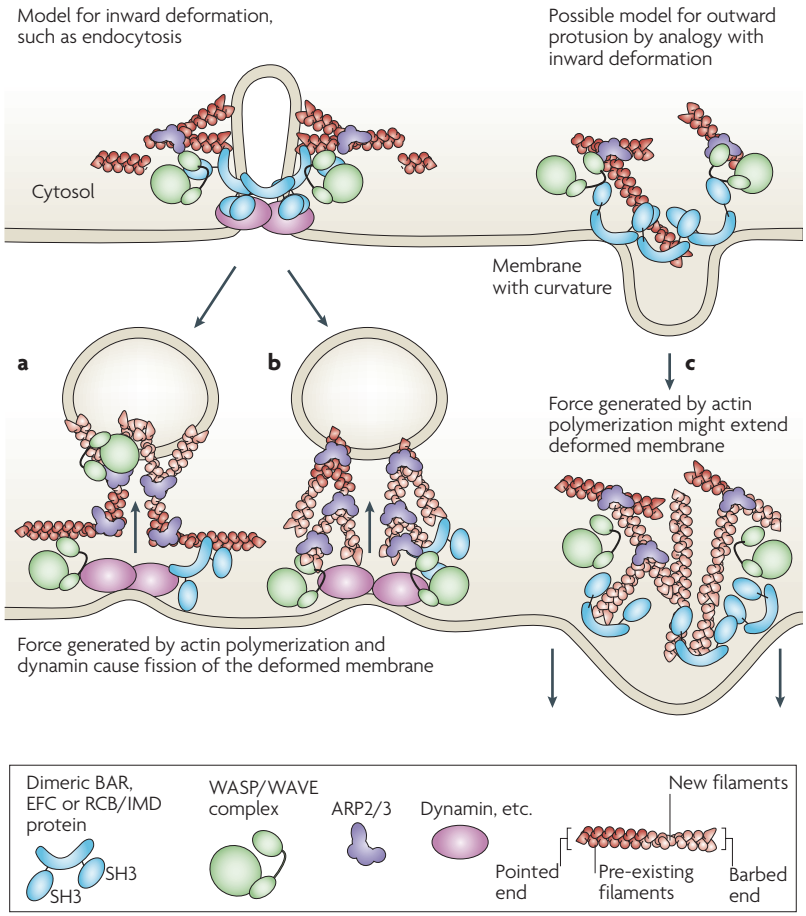


Figure 4 | **Functional models of WASP and WAVE proteins in inward or outward deformation of the membrane.** Wiskott–Aldrich syndrome protein (WASP) and WASP-family verprolin-homologous protein (WAVE) proteins and the BAR, EFC or Rac-binding (RCB; also known as a IRSp53-Mim-homology (IMD))-domain-containing proteins function as functional units that organize the curvature of both the membrane and the cytoskeleton. In endocytosis, the direction of actin polymerization (that is, the direction of the barbed end) can be facing towards the endocytosis vesicles (**a**), or towards the plasma membrane (**b**). In both cases, the BAR- or EFC-domain-containing proteins recruit both neural (N-)WASP and dynamin to induce membrane fission. In model **a**, the elongating barbed end pushes the vesicles for vesicle movement, whereas in model **b**, the elongating pointed end pushes the vesicles or the elongating barbed end towards the plasma membrane and thereby generates force for vesicle fission. For outward protrusions, such as lamellipodia and filopodia, actin polymerization occurs outwardly: the barbed end faces towards the plasma membrane (**c**), and polymerization might possibly be initiated by the RCB/IMD-domain-containing proteins that can recruit WAVE, VASP and other proteins. If model **b** occurs, it is still unclear how endocytosis (**b**) and outward protrusion (**c**) are differentially regulated during actin polymerization. Membrane-binding domains such as BAR, EFC and RCB/IMD might sense the curvature of the membrane to determine the direction of reorganization of the actin cytoskeleton. Small GTPases and protein kinases regulate the activity of the WASP and WAVE family to induce actin polymerization through the ARP2/3 complex. Src-homology-3, SH3.

***IRSp53 optimizes WAVE2 at the membrane.*** WAVE2 has a specific linker molecule, IRSp53. IRSp53 was originally identified as an insulin-receptor substrate, but it was subsequently shown to bind to both Rac and WAVE2 (REFS 117,118). IRSp53 also binds to WAVE1 and WAVE3, but its affinities for WAVE1 and WAVE3 are much weaker than its affinity for WAVE2 (REFS 102,118,119). WAVE2 and IRSp53 have also been shown to be involved in spine formation[120]. IRSp53 is colocalized with WAVE2 (REF. 121), but is not essential for the localization of WAVE2 at the leading edge of lamellipodia[101]. Furthermore, IRSp53 enhances the activity of the WAVE2 complex in the presence of Rac and PtdIns(3,4,5)$P_3$ *in vitro*, indicating that IRSp53 optimizes WAVE2 activity when Rac is active during lamellipodium formation[101]. Therefore, the WAVE2 complex can bind to Rac through IRSp53 and through SRA1/PIR121 of the WAVE2 complex.

IRSp53 has a CDC42-binding site that does not overlap with its RCB/IMD domain, and CDC42-binding to IRSp53 decreases the affinity of IRSp53 for WAVE2 (REFS 101,122). The RCB/IMD domain binds to activated Rac and actin filaments[118,123], whereas the SH3 domain of IRSp53 binds transiently to the proline-rich region of WAVE2 (REF. 101).

The RCB/IMD domain contains α-helical bundles that are similar to the BAR domain[62,124,125]. Accordingly, the RCB/IMD domain of IRSp53 binds to the cell membrane[101]. The BAR domain has a gradually curved structure, and its concave surface is positively charged, which favours binding to the negatively charged cell membrane to induce tubulated membrane or to sense membrane curvature[65] (FIG. 3). The surface of the RCB/IMD domain is also positively charged. However, the RCB/IMD domain is straight, and the RCB/IMD domain deforms the membrane in a different direction to that induced by the BAR and EFC domains in a Rac-dependent manner[125] (FIG. 3). Membrane protrusions that seem to lack actin filaments have been observed in IRSp53-overexpressing cells[121,125,126]. Although it is possible that a trace amount of actin filaments is present, this observation strongly indicates that the protrusion is composed of membrane without actin filaments. Therefore, actin polymerization is probably not the driving force for the membrane protrusion. IRSp53 might affect membrane organization independently of actin filaments. This possible actin-independent protrusion by IRSp53 might function with actin-polymerization-dependent protrusion during lamellipodium or filopodium formations.

As discussed above, the direction of actin polymerization at the plasma membrane for endocytosis could be the same as the direction of actin polymerization for protrusions such as lamellipodia and filopodia (FIG. 4b,c). Curvature sensing by the RCB/IMD domain, the BAR domain and EFC domain might possibly determine whether the plasma membrane is put forward for protrusion or invaginated and pinched off for endocytosis vesicles.

## WAVE1 regulates dorsal ruffle formation

WAVE1 is not essential for protrusion of the leading edge of lamellipodia[112,113], but it is involved in the formation of dorsal ruffles, circular assemblies of actin filaments formed upwardly in the dorsal surface of cells[112]. In WAVE1-knockout cells, extension of the leading edge occurs faster than in wild-type cells[113]; however, the leading edges in WAVE1-knockout cells are unstable and have shorter half-lives than those of wild-type cells.

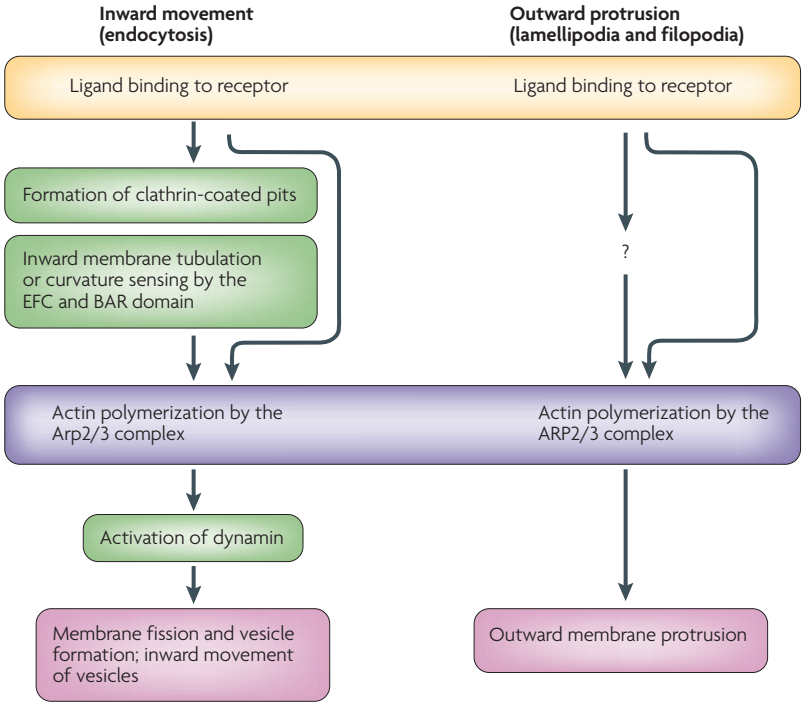


Figure 5 | **Regulation of inward or outward protrusions of the cell membrane.** Many processes are involved in endocytosis. Assembly of clathrin-coated pits followed by the binding of EFC- and BAR-domain-containing proteins to the membrane occurs in endocytosis. Then, actin polymerization is induced by the ARP2/3 complex and by neural Wiskott–Aldrich syndrome protein (N-WASP). Simultaneously, dynamin seems to cause fission of the invaginated membrane to make vesicles. In contrast to the invagination of the membrane for vesicular trafficking, outward protrusive structures involve fewer molecules that bind to the membrane. Novel molecules of membrane-binding capability might participate in the protrusion of the membrane if protrusions have evolved from the same processes, as did endocytosis and intracellular trafficking.

Consistent with this finding, WAVE1 is localized slightly behind the leading edge[112,113], and therefore, WAVE1 might be important for the accumulation of actin filaments behind the leading edge to increase the mechanical force necessary for protrusion.

Several proteins bind to WAVE1. WRP (WAVE-associated RacGAP protein) binds to WAVE1 through its SH3 domain[127] and has an EFC domain and a RacGAP domain[56,63]. WRP inactivates Rac and might recruit WAVE1 to the membrane. Therefore, WAVE1 and WRP might function cooperatively to stabilize the actin cytoskeleton and to complete lamellipodium formation. The RII subunit of cAMP-dependent kinase (PKA) and p47phox (NADPH-oxidase adapter) are also reported to bind specifically to WAVE1 (REFS 128,129), but the role of these molecules for WAVE1 function is not clear.

***Phosphorylation of WAVE1.*** The activity of WAVE1 in ARP2/3-complex activation is regulated by phosphorylation. Phosphorylation of WAVE1 suppresses ARP2/3-complex activation by WAVE1 without affecting the stability of the WAVE1 complex[114]. WAVE2 is phosphorylated by mitogen-activated protein kinase (MAPK), but the significance of this phosphorylation remains to be determined[130].

## WASP and WAVE and cell-shape changes

Cell shape had long been thought to be determined by the cytoskeleton beneath the cellular membrane. However, this idea could be revised by the discovery of several membrane-deforming proteins that bind to WASP and WAVE proteins.

The proteins of the largest population among WASP- and WAVE-binding proteins consist of an N-terminal membrane-binding domain (BAR, EFC or RCB/IMD domain) and a C-terminal SH3 domain (FIGS 2,3). These N-terminal domains bind to the cell membrane and the SH3 domains bind to and activate WASP and WAVE proteins. The proteins containing a BAR- or an EFC-domain are conserved from yeast to mammals (FIG. 3). WASP is found in yeast, but WAVE is not, and WAVE-binding proteins, including IRSp53 and WRP, are present only in multicellular organisms (FIG. 3).

Conservation of WASP and WAVE proteins, as well as their binding proteins, indicates that SH3-mediated interaction of BAR- or EFC-domain-containing proteins with the WASP–WIP complex is the original functional unit for changes in cell shapes. For Las17, the yeast WASP homologue, the binding of Las17 to Cdc42 has not been detected, but the binding of Las17 to Vrp1, a yeast homologue of WIP, and the binding of Las17 to Rvs167, a BAR-domain-containing protein, and Bzz1, an EFC-domain-containing protein, is conserved during endocytosis[12,66,72]. The functions of WASP or WAVE downstream of small GTPases seem to be a later evolutionary development.

In single-cell organisms, changes of cell shape occur under limited conditions, such as endocytosis, exocytosis and cytokinesis. WASP and EFC-domain-containing proteins probably have been developed for endocytosis or inward vesicle movement. BAR and the EFC domains bind to phosphatidylserine and PtdIns(4,5)$P_2$ for sensing or for the deformation of the membrane, whereas SH3 domains recruit WASP or N-WASP as well as dynamin, leading to the generation of endocytosis vesicles (FIG. 5).

On the other hand, drastic changes in the shapes of outward protrusions occur during cell movement or cell adhesions in multicellular organisms. Although the machinery for sensing or for generating membrane curvature in outward protrusions is not well characterized, the machinery for endocytosis might have evolved to fill the cell's need to form protrusive structures for cell movement and cell adhesions. The BAR-related RCB/IMD domain of IRSp53 is a strong candidate for membrane-deformation capability and for curvature-sensing capability for outward protrusion[125], if membrane-deformation and sensing occurs in protrusion.

The endocytosis machinery is known to require many membrane-binding proteins. These molecules include clathrin for coated-pit formation and dynamin for membrane fission. No such proteins have been identified for membrane protrusions (FIG. 5). If similar molecules, including WASPs, WAVEs and the proteins with BAR, EFC and RCB/IMD domains, are involved in both membrane protrusion and endocytosis, then the identification of membrane-binding proteins associated with cell protrusion will be a subject of future studies.

## Conclusions

Molecules that regulate the actin cytoskeleton have been studied extensively in the past decade. The identification of the WASP and WAVE family proteins and of the ARP2/3 complex in the 1990s greatly enriched our understanding of how extracellular stimuli trigger the rearrangement of the actin cytoskeleton. The exponential growth of actin filaments that are linked to each other by branching seems to generate the force for cell-shape alterations.

However, cell shape is determined by the shape of the cell membrane, because the most outer layer of the cell is the membrane. Discovery of the membrane-deforming capability of BAR-domain-related membrane-binding proteins that include BAR, EFC and RCB/IMD domains indicate another mechanism in the shape changes of cellular membranes. BAR-domain-related proteins seem to couple to WASP and WAVE family proteins. The protein complexes of WASP and WAVE and BAR-domain-related proteins seem to synergistically regulate the cytoskeleton and membrane shape. Possible functional units of WASP and WAVE proteins and BAR-domain-related proteins are involved in both outward protrusion and inward vesicle trafficking. Therefore, these two types of cell-shape change might have an identical origin.

Currently, most BAR-domain-related proteins are involved in endocytosis and associate with WASP proteins. Fewer molecules associate with WAVE proteins (FIGS 2,3 and Supplementary information S1 (table)). Therefore, it is unclear how WAVE proteins are predominantly adapted to protrusion formation rather than to endocytosis. The answer of how WASP and WAVE proteins function differently for various morphological changes of several cells has not yet been obtained. Furthermore, it is still unclear how each WAVE protein is regulated and functions differently. These questions remain to be solved to clarify the mechanisms of cell-shape and body formation.


1. Wiskott, A. Familiarer, angeborener Morbus Welhofii? *Monatsschr. Kinderheilkd.* **68**, 212–216 (1937).
2. Aldrich, R. A., Steinberg, A. G. & Campbell, D. C. Pedigree demonstrating a sex-linked recessive condition characterized by draining ears, eczematoid dermatitis and bloody diarrhea. *Pediatrics* **13**, 133–139 (1954).
3. Thrasher, A. J. WASp in immune-system organization and function. *Nature Rev. Immunol.* **2**, 635–646 (2002).
4. Ochs, H. D. & Notarangelo, L. D. Structure and function of the Wiskott–Aldrich syndrome protein. *Curr. Opin. Hematol.* **12**, 284–291 (2005).
5. Derry, J. M., Ochs, H. D. & Francke, U. Isolation of a novel gene mutated in Wiskott–Aldrich syndrome. *Cell* **78**, 635–644 (1994).
**Discovery of WASP.**
6. Miki, H., Miura, K. & Takenawa, T. N-WASP, a novel actin-depolymerizing protein, regulates the cortical cytoskeletal rearrangement in a PIP2-dependent manner downstream of tyrosine kinases. *EMBO J.* **15**, 5326–5335 (1996).
**Discovery of N-WASP.**
7. Pollard, T. D. & Borisy, G. G. Cellular motility driven by assembly and disassembly of actin filaments. *Cell* **112**, 453–465 (2003).
8. Takenawa, T. & Miki, H. WASP and WAVE family proteins: key molecules for rapid rearrangement of cortical actin filaments and cell movement. *J. Cell Sci.* **114**, 1801–1809 (2001).
9. Miki, H., Suetsugu, S. & Takenawa, T. WAVE, a novel WASP-family protein involved in actin reorganization induced by Rac. *EMBO J.* **17**, 6932–6941 (1998).
**Discovery of WAVE1.**
10. Bear, J. E., Rawls, J. F. & Saxe III, C. L. SCAR, a WASP-related protein, isolated as a suppressor of receptor defects in late *Dictyostelium* development. *J. Cell Biol.* **142**, 1325–1335 (1998).
11. Suetsugu, S., Miki, H. & Takenawa, T. Identification of two human WAVE/SCAR homologues as general actin regulatory molecules which associate with Arp2/3 complex. *Biochem. Biophys. Res. Commun.* **260**, 296–302 (1999).
**Discovery of WAVE2 and WAVE3.**
12. Li, R. Bee1, a yeast protein with homology to Wiscott–Aldrich syndrome protein, is critical for the assembly of cortical actin cytoskeleton. *J. Cell Biol.* **136**, 649–658 (1997).
13. Panchal, S. C., Kaiser, D. A., Torres, E., Pollard, T. D. & Rosen, M. K. A conserved amphipathic helix in WASP/Scar proteins is essential for activation of Arp2/3 complex. *Nature Struct. Biol.* **10**, 591–598 (2003).
14. Miki, H. & Takenawa, T. Direct binding of the verprolin-homology domain in N-WASP to actin is essential for cytoskeletal reorganization. *Biochem. Biophys. Res. Commun.* **243**, 73–78 (1998).
15. Symons, M. *et al.* Wiskott–Aldrich syndrome protein, a novel effector for the GTPase CDC42Hs, is implicated in actin polymerization. *Cell* **84**, 723–734 (1996).
16. Rohatgi, R. *et al.* The interaction between N-WASP and the Arp2/3 complex links Cdc42-dependent signals to actin assembly. *Cell* **97**, 221–231 (1999).
**Demonstration of N-WASP autoinhibition.**
17. Machesky, L. M. & Insall, R. H. Scar1 and the related Wiskott–Aldrich syndrome protein, WASP, regulate the actin cytoskeleton through the Arp2/3 complex. *Curr. Biol.* **8**, 1347–1356 (1998).
18. Machesky, L. M. *et al.* Scar, a WASp-related protein, activates nucleation of actin filaments by the Arp2/3 complex. *Proc. Natl Acad. Sci. USA* **96**, 3739–3744 (1999).
**References 17 and 18 reported the identification of the ARP2/3 complex as a WASP/WAVE binding partner and showed that ARP2/3 is activated by WAVE.**
19. Pantaloni, D., Boujemaa, R., Didry, D., Gounon, P. & Carlier, M.-F. The Arp2/3 complex branches filament barbed ends: functional antagonism with capping proteins. *Nature Cell. Biol.* **2**, 385–391 (2000).
20. Blanchoin, L. *et al.* Direct observation of dendritic actin filament networks nucleated by Arp2/3 complex and WASP/Scar proteins. *Nature* **404**, 1007–1011 (2000).
21. Fujiwara, I., Suetsugu, S., Uemura, S., Takenawa, T. & Ishiwata, S. Visualization and force measurement of branching by Arp2/3 complex and N-WASP in actin filament. *Biochem. Biophys. Res. Commun.* **293**, 1550–1555 (2002).
22. Yarar, D., D'Alessio, J. A., Jeng, R. L. & Welch, M. D. Motility determinants in WASP family proteins. *Mol. Biol. Cell* **13**, 4045–4059 (2002).
23. Suetsugu, S., Miki, H. & Takenawa, T. Identification of another Actin-related protein (Arp) 2/3 complex binding site in neural Wiskott–Aldrich syndrome protein (N-WASP), that complements actin polymerization induced by the Arp2/3 complex activating (VCA) domain of N-WASP. *J. Biol. Chem.* **276**, 33175–33180 (2001).
24. Suetsugu, S., Miki, H., Yamaguchi, H. & Takenawa, T. Requirement of the basic region of N-WASP/WAVE2 for actin-based motility. *Biochem. Biophys. Res. Commun.* **282**, 739–744 (2001).
25. Rodal, A. A., Manning, A. L., Goode, B. L. & Drubin, D. G. Negative regulation of yeast WASp by two SH3 domain-containing proteins. *Curr. Biol.* **13**, 1000–1008 (2003).
26. Aspenstrom, P., Lindberg, U. & Hall, A. Two GTPases, Cdc42 and Rac, bind directly to a protein implicated in the immunodeficiency disorder Wiskott–Aldrich syndrome. *Curr. Biol.* **6**, 70–75 (1996).
27. Volkman, B. F., Prehoda, K. E., Scott, J. A., Peterson, F. C. & Lim, W. A. Structure of the N-WASP EVH1 domain-WIP complex: insight into the molecular basis of Wiskott–Aldrich syndrome. *Cell* **111**, 565–576 (2002).
28. Ramesh, N., Anton, I. M., Hartwig, J. H. & Geha, R. S. WIP, a protein associated with Wiskott–Aldrich syndrome protein, induces actin polymerization and redistribution in lymphoid cells. *Proc. Natl Acad. Sci. USA* **94**, 14671–14676 (1997).
**Identification of WIP.**
29. Aspenstrom, P. The WASP-binding protein WIRE has a role in the regulation of the actin filament system downstream of the platelet-derived growth factor receptor. *Exp. Cell Res.* **279**, 21–33 (2002).
30. Kato, M. *et al.* WICH, a novel verprolin homology domain-containing protein that functions cooperatively with N-WASP in actin-microspike formation. *Biochem. Biophys. Res. Commun.* **291**, 41–47 (2002).
31. Ho, H. Y., Rohatgi, R., Ma, L. & Kirschner, M. W. CR16 forms a complex with N-WASP in brain and is a novel member of a conserved proline-rich actin-binding protein family. *Proc. Natl Acad. Sci. USA* **98**, 11306–11311 (2001).
32. Aspenstrom, P. The mammalian verprolin homologue WIRE participates in receptor-mediated endocytosis and regulation of the actin filament system by distinct mechanisms. *Exp. Cell Res.* **298**, 485–498 (2004).
33. Krzewski, K., Chen, X., Orange, J. S. & Strominger, J. L. Formation of a WIP-, WASp-, actin-, and myosin IIA-containing multiprotein complex in activated NK cells and its alteration by KIR inhibitory signaling. *J. Cell Biol.* **173**, 121–132 (2006).
34. Sawa, M. & Takenawa, T. *Caenorhabditis elegans* WASP-interacting protein homologue WIP-1 is involved in morphogenesis through maintenance of WSP-1 protein levels. *Biochem. Biophys. Res. Commun.* **340**, 709–717 (2006).
35. Martinez-Quiles, N. *et al.* WIP regulates N-WASP-mediated actin polymerization and filopodium formation. *Nature Cell Biol.* **3**, 484–491 (2001).
36. Ho, H. Y. *et al.* Toca-1 mediates Cdc42-dependent actin nucleation by activating the N-WASP–WIP complex. *Cell* **118**, 203–216 (2004).
37. Hertzog, M. *et al.* The β-thymosin/WH2 domain; structural basis for the switch from inhibition to promotion of actin assembly. *Cell* **117**, 611–623 (2004).
38. Sasahara, Y. *et al.* Mechanism of recruitment of WASP to the immunological synapse and of its activation following TCR ligation. *Mol. Cell* **10**, 1269–1281 (2002).
39. Moreau, V. *et al.* A complex of N-WASP and WIP integrates signalling cascades that lead to actin polymerization. *Nature Cell Biol.* **2**, 441–448 (2000).
40. Frischknecht, F. *et al.* Actin-based motility of vaccinia virus mimics receptor tyrosine kinase signalling. *Nature* **401**, 926–929 (1999).
41. Anton, I. M. *et al.* WIP deficiency reveals a differential role for WIP and the actin cytoskeleton in T and B cell activation. *Immunity* **16**, 193–204 (2002).
42. Snapper, S. B. *et al.* Wiskott–Aldrich syndrome protein-deficient mice reveal a role for WASP in T but not B cell activation. *Immunity* **9**, 81–91 (1998).

43. Rohatgi, R., Ho, H. Y. & Kirschner, M. W. Mechanism of N-WASP activation by CDC42 and phosphatidylinositol 4,5-bisphosphate. *J. Cell Biol.* **150**, 1299–1310 (2000).
44. Higgs, H. N. & Pollard, T. D. Activation by Cdc42 and PIP(2) of Wiskott–Aldrich syndrome protein (WASp) stimulates actin nucleation by Arp2/3 complex. *J. Cell Biol.* **150**, 1311–1320 (2000).
45. Miki, H., Sasaki, T., Takai, Y. & Takenawa, T. Induction of filopodium formation by WASP-related actin-depolymerizing protein N-WASP. *Nature* **391**, 93–96 (1998).
46. Hall, A. Rho GTPase and the actin cytoskeleton. *Science* **279**, 509–514 (1998).
47. Abe, T., Kato, M., Miki, H., Takenawa, T. & Endo, T. Small GTPase Tc10 and its homologue RhoT induce N-WASP-mediated long process formation and neurite outgrowth. *J. Cell Sci.* **116**, 155–168 (2003).
48. Hemsath, L., Dvorsky, R., Fiegen, D., Carlier, M. F. & Ahmadian, M. R. An electrostatic steering mechanism of Cdc42 recognition by Wiskott–Aldrich syndrome proteins. *Mol. Cell* **20**, 313–324 (2005).
49. Aronheim, A. *et al.* Chp, a homologue of the GTPase Cdc42Hs, activates the JNK pathway and is implicated in reorganizing the actin cytoskeleton. *Curr. Biol.* **8**, 1125–1128 (1998).
50. Kim, A. S., Kakalis, L. T., Abdul-Manan, N., Liu, G. A. & Rosen, M. K. Autoinhibition and activation mechanisms of the Wiskott–Aldrich syndrome protein. *Nature* **404**, 151–158 (2000).
51. Prehoda, K. E., Scott, J. A., Mullins, D. R. & Lim, W. A. Integration of multiple signals through cooperative regulation of the N-WASP–Arp2/3 complex. *Science* **290**, 801–806 (2000).
52. Suetsugu, S. *et al.* Regulation of actin cytoskeleton by mDab1 through N-WASP and ubiquitination of mDab1. *Biochem. J.* **384**, 1–8 (2004).
53. Fukuoka, M. *et al.* A novel neural Wiskott–Aldrich syndrome protein (N-WASP) binding protein, WISH, induces Arp2/3 complex activation independent of Cdc42. *J. Cell Biol.* **152**, 471–482 (2001).
54. Carlier, M.-F. *et al.* Grb2 links signaling to actin assembly by enhancing interaction of neural Wiskott–Aldrich syndrome protein (N-WASP) with actin-related protein (Arp2/3) complex. *J. Biol. Chem.* **275**, 21946–21952 (2000).
55. Rohatgi, R., Nollau, P., Ho, H. Y., Kirschner, M. W. & Mayer, B. J. Nck and phosphatidylinositol 4,5-bisphosphate synergistically activate actin polymerization through the N-WASP–Arp2/3 pathway. *J. Biol. Chem.* **276**, 26448–26452 (2001).
56. Tsujita, K. *et al.* Coordination between the actin cytoskeleton and membrane deformation by a novel membrane tubulation domain of PCH proteins is involved in endocytosis. *J. Cell Biol.* **172**, 269–279 (2006).
57. Cory, G. O., Garg, R., Cramer, R. & Ridley, A. J. Phosphorylation of tyrosine 291 enhances the ability of WASp to stimulate actin polymerization and filopodium formation. Wiskott–Aldrich syndrome protein. *J. Biol. Chem.* **277**, 45115–45121 (2002).
58. Suetsugu, S. *et al.* Sustained activation of N-WASP through phosphorylation is essential for neurite extension. *Dev. Cell* **3**, 645–658 (2002).
**The first report that WASP and WAVE proteins are degraded by proteasomes.**
59. Torres, E. & Rosen, M. K. Contingent phosphorylation/dephosphorylation provides a mechanism of molecular memory in WASP. *Mol. Cell* **11**, 1215–1227 (2003).
60. Park, S. J., Suetsugu, S. & Takenawa, T. Interaction of HSP90 to N-WASP leads to activation and protection from proteasome-dependent degradation. *EMBO J.* **24**,1557–1570 (2005).
61. Schulte, R. J. & Sefton, B. M. Inhibition of the activity of SRC and Abl tyrosine protein kinases by the binding of the Wiskott–Aldrich syndrome protein. *Biochemistry* **42**, 9424–9430 (2003).
62. Habermann, B. The BAR-domain family of proteins: a case of bending and binding? *EMBO Rep.* **5**, 250–255 (2004).
63. Itoh, T. *et al.* Dynamin and the actin cytoskeleton cooperatively regulate plasma membrane invagination by BAR and F-BAR proteins. *Dev. Cell* **9**, 791–804 (2005).
**References 56 and 63 report the identification of the EFC domain as a membrane-deforming domain. The role of PCH family proteins in linking membrane deformation with the cytoskeleton was proposed in this paper.**
64. Gundelfinger, E. D., Kessels, M. M. & Qualmann, B. Temporal and spatial coordination of exocytosis and endocytosis. *Nature Rev. Mol. Cell Biol.* **4**, 127–139 (2003).
65. Peter, B. J. *et al.* BAR domains as sensors of membrane curvature: the amphiphysin BAR structure. *Science* **303**, 495–499 (2004).
**The structure of BAR domain indicates that the shape of the protein dictates the shape of the membrane.**
66. Naqvi, S. N., Zahn, R., Mitchell, D. A., Stevenson, B. J. & Munn, A. L. The WASp homologue Las17p functions with the WIP homologue End5p/verprolin and is essential for endocytosis in yeast. *Curr. Biol.* **8**, 959–962 (1998).
**Proposes a role for WASP family proteins in association with WIP in endocytosis.**
67. Qualmann, B. & Kelly, R. B. Syndapin isoforms participate in receptor-mediated endocytosis and actin organization. *J. Cell Biol.* **148**, 1047–1062 (2000).
**N-WASP is shown to be involved in endocytosis through binding to an EFC-domain-containing protein.**
68. Kaksonen, M., Toret, C. P. & Drubin, D. G. A modular design for the clathrin- and actin-mediated endocytosis machinery. *Cell* **123**, 305–320 (2005).
69. Otsuki, M., Itoh, T. & Takenawa, T. T. N-WASP is recruited to rafts and associates with endophilin A in response to EGF. *J. Biol. Chem.* **278**, 6461–6469 (2002).
70. Madania, A. *et al.* The *Saccharomyces cerevisiae* homologue of human Wiskott–Aldrich syndrome protein Las17p interacts with the Arp2/3 complex. *Mol. Biol. Cell* **10**, 3521–3538 (1999).
71. Kamioka, Y. *et al.* A novel dynamin-associating molecule, formin-binding protein 17, induces tubular membrane invaginations and participates in endocytosis. *J. Biol. Chem.* **279**, 40091–40099 (2004).
72. Soulard, A. *et al. Saccharomyces cerevisiae* Bzz1p is implicated with type I myosins in actin patch polarization and is able to recruit actin-polymerizing machinery *in vitro*. *Mol. Cell. Biol.* **22**, 7889–7906 (2002).
73. Merrifield, C. J., Perrais, D. & Zenisek, D. Coupling between clathrin-coated-pit invagination, cortactin recruitment, and membrane scission observed in live cells. *Cell* **121**, 593–606 (2005).
74. Svitkina, T. M. & Borisy, G. G. 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 (1999).
75. Kaksonen, M., Toret, C. P. & Drubin, D. G. Harnessing actin dynamics for clathrin-mediated endocytosis. *Nature Rev. Mol. Cell Biol.* **7**, 404–414 (2006).
76. Rozelle, A. L. *et al.* Phosphatidylinositol 4,5-bisphosphate induces actin-based movement of raft-enriched vesicles through WASP–Arp2/3. *Curr. Biol.* **10**, 311–320 (2000).
77. Taunton, J. *et al.* Actin-dependent propulsion of endosomes and lysosomes by recruitment of N-WASP. *J. Cell Biol.* **148**, 519–530 (2000).
78. Nakagawa, H. *et al.* N-WASP, WAVE and Mena play different roles in the organization of actin cytoskeleton in lamellipodia. *J. Cell Sci.* **114**, 1555–1565 (2001).
79. Lommel, S. *et al.* Actin pedestal formation by enteropathogenic *Escherichia coli* and intracellular motility of *Shigella flexneri* are abolished in N-WASP-defective cells. *EMBO Rep.* **2**, 850–857 (2001).
80. Snapper, S. B. *et al.* N-WASP deficiency reveals distinct pathways for cell surface projections and microbial actin-based motility. *Nature Cell Biol.* **3**, 897–904 (2001).
81. Suetsugu, S., Miki, H., Yamaguchi, H., Obinata, T. & Takenawa, T. Enhancement of branching efficiency by the actin filament-binding activity of N-WASP/WAVE2. *J. Cell Sci.* **114**, 4533–4542 (2001).
82. Steffen, A. *et al.* Filopodia formation in the absence of functional WAVE and Arp2/3 complexes. *Mol. Biol. Cell* **17**, 2581–2591 (2006).
83. Schirenbeck, A., Bretschneider, T., Arasada, R., Schleicher, M. & Faix, J. The Diaphanous-related formin dDia2 is required for the formation and maintenance of filopodia. *Nature Cell Biol.* **7**, 619–625 (2005).
84. Buccione, R., Orth, J. D. & McNiven, M. A. Foot and mouth: podosomes, invadopodia and circular dorsal ruffles. *Nature Rev. Mol. Cell Biol.* **5**, 647–657 (2004).
85. Linder, S. & Aepfelbacher, M. Podosomes: adhesion hot-spots of invasive cells. *Trends Cell Biol.* **13**, 376–385 (2003).
86. Mizutani, K., Miki, H., He, H., Maruta, H. & Takenawa, T. Essential role of neural Wiskott–Aldrich syndrome protein in podosome formation and degradation of extracellular matrix in src-transformed fibroblasts. *Cancer Res.* **62**, 669–674 (2002).
87. Weaver, A. *et al.* Interaction of cortactin and N-WASP with Arp2/3 complex. *Curr. Biol.* **12**, 1270 (2002).
88. Yamaguchi, H. *et al.* Molecular mechanisms of invadopodium formation: the role of the N-WASP–Arp2/3 complex pathway and cofilin. *J. Cell Biol.* **168**, 441–452 (2005).
89. Weaver, A. M. *et al.* Cortactin promotes and stabilizes Arp2/3-induced actin filament network formation. *Curr. Biol.* **11**, 370–374 (2001).
90. Uruno, T. *et al.* Activation of Arp2/3 complex-mediated actin polymerization by cortactin. *Nature Cell Biol.* **3**, 259–266 (2001).
91. Krueger, E. W., Orth, J. D., Cao, H. & McNiven, M. A. A dynamin–cortactin–Arp2/3 complex mediates Actin reorganization in growth factor-stimulated cells. *Mol. Biol. Cell* **14**, 1085–1096 (2003).
92. Schafer, D. A. *et al.* Dynamin2 and cortactin regulate actin assembly and filament organization. *Curr. Biol.* **12**, 1852–1857 (2002).
93. Wu, X., Suetsugu, S., Cooper, L. A., Takenawa, T. & Guan, J. L. Focal adhesion kinase regulation of N-WASP subcellular localization and function. *J. Biol. Chem.* **279**, 9565–9576 (2004).
94. Jones, N. *et al.* Nck adaptor proteins link nephrin to the actin cytoskeleton of kidney podocytes. *Nature* **440**, 818–823 (2006).
95. Gruenheid, S. *et al.* Enteropathogenic *E. coli* Tir binds Nck to initiate actin pedestal formation in host cells. *Nature Cell Biol.* **3**, 856–859 (2001).
96. Rivera, G. M., Briceno, C. A., Takeshima, F., Snapper, S. B. & Mayer, B. J. Inducible clustering of membrane-targeted SH3 domains of the adaptor protein Nck triggers localized actin polymerization. *Curr. Biol.* **14**, 11–22 (2004).
97. Oikawa, T. *et al.* PtdIns(3,4,5)P3 binding is necessary for WAVE2-induced formation of lamellipodia. *Nature Cell Biol.* **6**, 420–426 (2004).
98. Eden, S., Rohatgi, R., Podtelejnikov, A. V., Mann, M. & Kirschner, M. W. Mechanism of regulation of WAVE1-induced actin nucleation by Rac1 and Nck. *Nature* **418**, 790–793 (2002).
**Identification of the WAVE complex that consists of WAVE1, ABI1/2, NAP1/p125NAP1, SRA1/PIR121 and HSPC300. Proposes trans-inhibition of WAVE. The presence of the Rac-binding molecule, SRA1/PIR121, in the WAVE complex was also described.**
99. Innocenti, M. *et al.* Abi1 is essential for the formation and activation of a WAVE2 signalling complex. *Nature Cell Biol.* **6**, 319–327 (2004).
**Demonstration of constitutive formation of the WAVE2 complex.**
100. Gautreau, A. *et al.* Purification and architecture of the ubiquitous Wave complex. *Proc. Natl Acad. Sci. USA* **101**, 4379–4383 (2004).
101. Suetsugu, S. *et al.* Optimization of WAVE2-complex-induced actin polymerization by membrane-bound IRSp53, PIP3, and Rac. *J. Cell Biol.* **173**, 571–585 (2006).
**The WAVE2 complex was purified from cells and its activity in the ARP2/3 activation was examined. Reconcilement of two proposals, through SRA1/PIR121 and IRSp53, for Rac association with WAVE2.**
102. Stovold, C. F., Millard, T. H. & Machesky, L. M. Inclusion of Scar/WAVE3 in a similar complex to Scar/WAVE1 and 2. *BMC Cell Biol.* **6**, 11 (2005).
103. Leng, Y. *et al.* Abelson-interactor-1 promotes WAVE2 membrane translocation and Abelson-mediated tyrosine phosphorylation required for WAVE2 activation. *Proc. Natl Acad. Sci. USA* **102**,1098–1103 (2005).
104. Soto, M. C. *et al.* The GEX-2 and GEX-3 proteins are required for tissue morphogenesis and cell migrations in *C. elegans*. *Genes Dev.* **16**, 620–632 (2002).
105. Sawa, M. *et al.* Essential role of the *C. elegans* Arp2/3 complex in cell migration during ventral enclosure. *J. Cell Sci.* **116**, 1505–1518 (2003).
106. Kitamura, T. *et al.* Molecular cloning of p125Nap1, a protein that associates with an SH3 domain of Nck. *Biochem. Biophys. Res. Commun.* **219**, 509–514 (1996).
107. Kobayashi, K. *et al.* p140Sra-1 (specifically Rac1-associated protein) is a novel specific target for Rac1 small GTPase. *J. Biol. Chem.* **273**, 291–295 (1998).
108. Steffen, A. *et al.* Sra-1 and Nap1 link Rac to actin assembly driving lamellipodia formation. *EMBO J.* **23**, 749–759 (2004).

109. Kunda, P., Craig, G., Dominguez, V. & Baum, B. Abi, Sra1, and Kette control the stability and localization of SCAR/WAVE to regulate the formation of actin-based protrusions. *Curr. Biol.* **13**, 1867–1875 (2003).
110. Rogers, S. L., Wiedemann, U., Stuurman, N. & Vale, R. D. Molecular requirements for actin-based lamella formation in *Drosophila* S2 cells. *J. Cell Biol.* **162**, 1079–1088 (2003).
111. Nozumi, M., Nakagawa, H., Miki, H., Takenawa, T. & Miyamoto, S. Differential localization of WAVE isoforms in filopodia and lamellipodia of the neuronal growth cone. *J. Cell Sci.* **116**, 239–246 (2003).
112. Suetsugu, S., Yamazaki, D., Kurisu, S. & Takenawa, T. Differential roles of WAVE1 and WAVE2 in dorsal and peripheral ruffle formation for fibroblast cell migration. *Dev. Cell* **5**, 595–609 (2003).
**Description of differential roles for WAVE1 and WAVE2. The requirement of WAVE2 in lamellipodia formation is established in this paper and in reference 113.**
113. Yamazaki, D., Fujiwara, T., Suetsugu, S. & Takenawa, T. A novel function of WAVE in lamellipodia: WAVE1 is required for stabilization of lamellipodial protrusions during cell spreading. *Genes Cells* **10**, 381–392 (2005).
114. Kim, Y. *et al.* Phosphorylation of WAVE1 regulates actin polymerization and dendritic spine morphology. *Nature* **442**, 814–817 (2006).
**Involvement of WAVE1 in spine formation. CDK5-mediated phosphorylation of WAVE1 is reported to inhibit WAVE1-induced ARP2/3 activation in spine formation.**
115. Yan, C. *et al.* WAVE2 deficiency reveals distinct roles in embryogenesis and Rac-mediated actin-based motility. *EMBO J.* **22**, 3602–3612 (2003).
116. Yamazaki, D. *et al.* WAVE2 is required for directed cell migration and cardiovascular development. *Nature* **424**, 452–456 (2003).
117. Yeh, T. C., Ogawa, W., Danielsen, A. G. & Roth, R. A. Characterization and cloning of a 58/53-kDa substrate of the insulin receptor tyrosine kinase. *J. Biol. Chem.* **271**, 2921–2928 (1996).
118. Miki, H., Yamaguchi, H., Suetsugu, S. & Takenawa, T. IRSp53 is an essential intermediate between Rac and WAVE in the regulation of membrane ruffling. *Nature* **408**, 732–735 (2000).
**Identification of IRSp53 as a linker molecule between Rac and WAVE2.**
119. Oda, A. *et al.* WAVE/Scars in platelets. *Blood* **105**, 3141–3148 (2005).
120. Choi, J. *et al.* Regulation of dendritic spine morphogenesis by insulin receptor substrate 53, a downstream effector of Rac1 and Cdc42 small GTPases. *J. Neurosci.* **25**, 869–879 (2005).
121. Nakagawa, H. *et al.* IRSp53 is colocalised with WAVE2 at the tips of protruding lamellipodia and filopodia independently of Mena. *J. Cell Sci.* **116**, 2577–2583 (2003).
122. Krugmann, S. *et al.* Cdc42 induces filopodia by promoting the formation of an IRSp53:Mena complex. *Curr. Biol.* **11**, 1645–1655 (2001).
123. Yamagishi, A., Masuda, M., Ohki, T., Onishi, H. & Mochizuki, N. A novel actin bundling/filopodium-forming domain conserved in insulin receptor tyrosine kinase substrate p53 and missing in metastasis protein. *J. Biol. Chem.* **279**, 14929–14936 (2004).
124. Millard, T. H. *et al.* Structural basis of filopodia formation induced by the IRSp53/MIM homology domain of human IRSp53. *EMBO J.* **24**, 240–250 (2005).
**First report of the structure of the RCB/IMD domain of IRSp53.**
125. Suetsugu, S. *et al.* The RAC-binding domain/IRSP53-MIM homology domain of IRSP53 induces RAC-dependent membrane deformation. *J. Biol. Chem.* **281**, 35347–35358 (2006).
**Reports Rac-dependent membrane deformation by the RCB/IMD domain.**
126. Govind, S., Kozma, R., Monfries, C., Lim, L. & Ahmed, S. Cdc42Hs facilitates cytoskeletal reorganization and neurite outgrowth by localizing the 58-kD insulin receptor substrate to filamentous actin. *J. Cell Biol.* **152**, 579–594 (2001).
127. Soderling, S. H. *et al.* The WRP component of the WAVE-1 complex attenuates Rac-mediated signalling. *Nature Cell Biol.* **4**, 970–975 (2002).
128. Wu, R. F., Gu, Y., Xu, Y. C., Nwariaku, F. E. & Terada, L. S. Vascular endothelial growth factor causes translocation of p47phox to membrane ruffles through WAVE1. *J. Biol. Chem.* **278**, 36830–36840 (2003).
129. Westphal, R. S., Soderling, S. H., Alto, N. M., Langeberg, L. K. & Scott, J. D. Scar/WAVE-1, a Wiskott–Aldrich syndrome protein, assembles an actin-associated multi-kinase scaffold. *EMBO J.* **19**, 4589–4600 (2000).
130. Miki, H., Fukuda, M., Nishida, E. & Takenawa, T. Phosphorylation of WAVE downstream of mitogen-activated protein kinase signaling. *J. Biol. Chem.* **274**, 27605–27609 (1999).
131. Theriot, J. A. & Mitchison, T. J. Actin microfilament dynamics in locomoting cells. *Nature* **352**, 126–131 (1991).
132. Pantoloni, D. & Carlier, M.-F. How profilin promotes actin filament assembly in the presence of thymosin β4. *Cell* **75**, 1007–1014 (1993).
133. Yang, C. *et al.* Profilin enhances Cdc42-induced nucleation of actin polymerization. *J. Cell Biol.* **150**, 1001–1012 (2000).
134. Suetsugu, S., Miki, H. & Takenawa, T. The essential role of profilin in the assembly of actin for microspike formation. *EMBO J.* **17**, 6516–6526 (1998).
**Demonstrates that profilin is important for actin reorganization induced by WASP and WAVE family proteins.**
135. Mimuro, H. *et al.* Profilin is required for sustaining efficient intra- and intercellular spreading of *Shigella flexneri*. *J. Biol. Chem.* **275**, 28893–28901 (2000).
136. Loisel, T. P., Boujemaa, R., Pantaloni, D. & Carlier, M. F. Reconstitution of actin-based motility of *Listeria* and *Shigella* using pure proteins. *Nature* **401**, 613–616 (1999).
**Reconstitution of actin-based motility from purified proteins without any live organisms.**
137. Welch, M. D., Iwamatsu, A. & Mitchison, T. J. Actin polymerization is induced by Arp2/3 protein complex at the surface of *Listeria monocytogenes*. *Nature* **385**, 265–269 (1997).
138. Suzuki, T., Miki, H., Takenawa, T. & Sasakawa, C. Neural Wiskott–Aldrich syndrome protein is implicated in the actin-based motility of *Shigella flexneri*. *EMBO J.* **17**, 2767–2776 (1998).
139. Egile, C. *et al.* Activation of the CDC42 effector N-WASP by the *Shigella flexneri* IcsA protein promotes actin nucleation by Arp2/3 complex and bacterial actin-based motility. *J. Cell Biol.* **146**, 1319–1332 (1999).
140. Cameron, L. A., Svitkina, T. M., Vignjevic, D., Theriot, J. A. & Borisy, G. G. Dendritic organization of actin comet tails. *Curr. Biol.* **11**, 130–135 (2001).
141. Kalman, D. *et al.* Enteropathogenic *E. coli* acts through WASP and Arp2/3 complex to form actin pedestals. *Nature Cell Biol.* **1**, 389–391 (1999).
142. Shi, J., Scita, G. & Casanova, J. E. WAVE2 signaling mediates invasion of polarized epithelial cells by *Salmonella typhimurium*. *J. Biol. Chem.* **280**, 29849–29855 (2005).
143. Banzai, Y., Miki, H., Yamaguchi, H. & Takenawa, T. Essential role of neural Wiskott–Aldrich syndrome protein in neurite extension in PC12 cells and rat hippocampal primary culture cells. *J. Biol. Chem.* **275**, 11987–11992 (2000).
144. Strasser, G. A., Rahim, N. A., VanderWaal, K. E., Gertler, F. B. & Lanier, L. M. Arp2/3 is a negative regulator of growth cone translocation. *Neuron* **43**, 81–94 (2004).
145. Kakimoto, T., Katoh, H. & Negishi, M. Regulation of neuronal morphology by Toca-1, an F-BAR/EFC protein that induces plasma membrane invagination. *J. Biol. Chem.* **281**, 29042–29053 (2006).
146. Wong, K. *et al.* Signal transduction in neuronal migration: roles of GTPase activating proteins and the small GTPase Cdc42 in the Slit-Robo pathway. *Cell* **107**, 209–221 (2001).
147. Fujita, H., Katoh, H., Ishikawa, Y., Mori, K. & Negishi, M. Rapostlin is a novel effector of Rnd2 GTPase inducing neurite branching. *J. Biol. Chem.* **277**, 45428–45434 (2002).
148. Kawano, Y. *et al.* CRMP-2 is involved in kinesin-1-dependent transport of the Sra-1/WAVE1 complex and axon formation. *Mol. Cell. Biol.* **25**, 9920–9935 (2005).
149. Irie, F. & Yamaguchi, Y. EphB receptors regulate dendritic spine development via intersectin, Cdc42 and N-WASP. *Nature Neurosci.* **5**, 1117–1118 (2002).
150. Udo, H. *et al.* Serotonin-induced regulation of the actin network for learning-related synaptic growth requires Cdc42, N-WASP, and PAK in Aplysia sensory neurons. *Neuron* **45**, 887–901 (2005).



**Competing interests statement**
The authors declare no competing financial interests.


## DATABASES

**The following terms in this article are linked online to:**
**Entrez Gene:** http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
*WASP*
**OMIM:** http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
CYFIP1 | WAS
**UniProtKB:** http://ca.expasy.org/sprot
ABI1 | CDC42 | GRB2 | Las17 | SRA1 | WAVE1 | WAVE2 | WAVE3 | WICH

## FURTHER INFORMATION

**Tadaomi Takenawa's homepage:** http://www.adm.u-tokyo.ac.jp/IRS/IntroPage_E/intro61821481_e.html
**Shiro Suetsugu's homepage:** http://read.jst.go.jp/ddbs/plsql/fs0_knkysh_detail2_e?KEY=shiro&KOU=RECORD&OP=AND&KEY=suetsugu&KOU=RECORD&CODE=5000044740&OP=DUMMY

## SUPPLEMENTARY INFORMATION

**See online article:** S1 (table)
**Access to this links box is available online.**