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Regulation of actin dynamics by WASP and WAVE family proteins☆

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Signal-dependent regulation of actin dynamics is essential for a variety of cellular processes, including formation of the membrane protrusions required for cell locomotion. Wiskott–Aldrich syndrome protein (WASP), neural (N)-WASP and WASP family verprolin-homologous (WAVE, also named Scar) proteins are thought to play a role in these processes by relaying activation signals from small GTPases, such as Cdc42 and Rac, to the actin-nucleating complex Arp2/3. Although much biochemical and structural biological work has defined the paradigms through which WASP and N-WASP regulation is achieved, only recently have the mechanisms that control WAVE proteins begun to be clarified. WAVE proteins assemble into macromolecular complexes, which are essential for the regulation of WAVE nucleation-promoting activity, dynamic localization and stability. In this article, we discuss recent studies that highlight novel modalities through which WAVE proteins are regulated and, in turn, mediate the site-directed actin polymerization required for membrane protrusion, thus enabling cell motility.

The highly dynamic and reversible polymerization of actin is at the foundation of several complex cellular processes such as cell migration, neurite extension, and bud growth in yeast [1]. Rho GTPase family members (e.g. RhoA, Cdc42 and Rac) play a key role in these processes, acting as molecular switches on which signaling inputs converge and are transduced into a coordinated array of output pathways, leading to site-directed actin polymerization [2]. RhoA acts predominantly through the bundling of pre-existing actin filaments, regulating stress-fiber formation, whereas Cdc42 and Rac stimulate *de novo* actin polymerization and are required for filopodia and lamellipodia, respectively [2].

The rate-limiting step of actin polymerization *in vitro* is the assembly of a trimer comprising three actin monomers [1]. This is enhanced *in vivo* by several factors that stimulate such nucleation (for review, see Ref. [3]). Among these factors, a family of regulatory proteins, including Wiskott–Aldrich syndrome protein (WASP), neural (N)-WASP and WASP family verprolin-homologous (WAVE) proteins, function by relaying signaling from Cdc42 and Rac to the actin-nucleation machinery – the Arp2/3 complex [3]. The first member of this protein family, WASP, was identified as the protein expressed by the gene mutated in patients with Wiskott–Aldrich syndrome [4]. This disorder is characterized by variable defects in blood clotting and immune defenses as a result of deficiency in the actin cytoskeleton of white blood cells and platelets [5]. WASP is expressed exclusively in hematopoietic cells. Other cells and tissues express the more ubiquitous WASP-related protein N-WASP, originally isolated from rat brain as an interactor for the adaptor molecule Grb2 [6], and WAVE, a WASP homolog that was identified independently in mammals [7,8] and *Dictyostelium discoideum*, in which it was named Scar [9]. Based on distinct structural features in their N-terminal portion, WAVE proteins define a subfamily of proteins that includes three members: WAVE1, WAVE2 and WAVE3 (Figure 1 and online Supplementary Figure). WASP, N-WASP and WAVE share common biochemical and structural features that enable them to form a tripartite unit with G-actin and the Arp2/3 complex, leading to nucleation and formation of a branched filament meshwork [10]. However, remarkably divergent features among WASP, N-WASP and WAVE proteins underline different modalities through which these proteins are regulated and interconnected with upstream signaling. Although a large body of biochemical and structural biological work has defined the paradigms through which WASP and N-WASP regulation is achieved [3,11], only recently have the mechanisms that control WAVE proteins begun to be clarified. In this article, we will discuss emerging evidence obtained through biochemical, genetic and cell biology approaches that concur to fill this gap in knowledge, providing a molecular scenario that accounts for the role of WAVE proteins in different biological processes and signaling pathways.

Structural homology and divergence of WASP, N-WASP and WAVE proteins

All WASP and WAVE proteins share a similar modular organization with a conserved C-terminal 'output' domain and a much larger and divergent N-terminal region involved in targeting and regulating the activity of the output domain (Figure 1 and online Supplementary Figure). This domain initiates the growth of new actin

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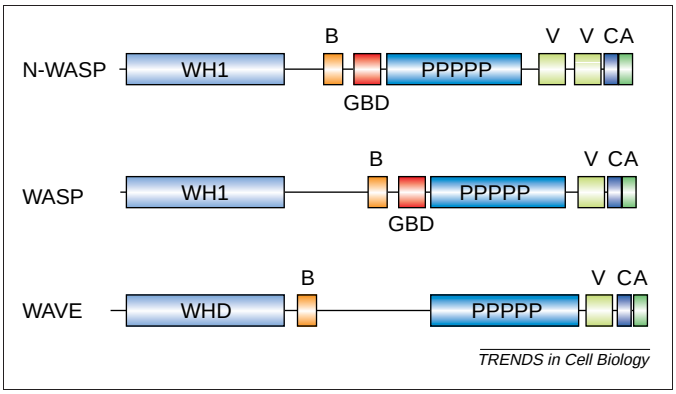

Figure 1. Modular domain organization of Wiskott–Aldrich syndrome protein (WASP), neural (N)-WASP and WASP family verprolin-homologous (WAVE) proteins. The various protein domains and regions are indicated. The WASP homology 1 (WH1) domain is conserved throughout evolution in WASP and N-WASP but is absent in the three WAVE isoforms, which harbor a WAVE homology domain (WHD) instead. B indicates a stretch of basic amino acids that mediate F-actin and (for WASP and N-WASP) phosphatidylinositol (4,5)-bisphosphate binding. The GTPase-binding domain (GBD) interacts directly with activated, GTP-loaded Cdc42. PPPPP indicates a proline-rich region that contains Src-homology-3- and profilin-binding sites. V, C and A form the VCA (verprolin homology, cofilin homology and acidic domains, respectively) modular 'output' domain, which is responsible for initiating the growth of new actin filaments by bringing together actin monomers and the actin-nucleating Arp2/3 complex (see also online Supplementary Figure).

filaments by bringing together actin monomers and the actin-nucleating Arp2/3 complex [1]. This is achieved through a VCA module consisting of a verprolin homology domain [V, also called WASP homology (WH)2 domain] that binds to an actin monomer [12], a C-terminal acidic (A) region that associates with the Arp2/3 complex [13] and an intermediate conserved sequence (C), named cofilin homology domain. The C region acts in concert with the V and A region [14–16], driving the conformational changes of Arp2/3 that are necessary to stimulate nucleation. Comparative analysis of the catalytic properties of the VCA module of WASP, N-WASP and WAVE1 revealed that the isolated domains display unique kinetics of actin assembly. The VCA domain of N-WASP has a distinctly higher rate of nucleation than the VCA of both WASP and WAVE1, which are shorter and have less charge [17–19]. Notably, changing the number of WASP or WAVE1 V domains, a region that is duplicated in N-WASP (Figure 1), was reported to have variable effects on nucleation activity [18,19]. However, the insertion of three amino acids found in the A domain of WASP and N-WASP increased the activity of WAVE1 without affecting its affinity for Arp2/3 [18]. Thus, despite nearly equal affinities of the various VCA domains for binding to Arp2/3, the rate of actin nucleation can differ significantly, indicating that a fine tuning in the catalytic process of Arp2/3 activation by VCA domains takes place, which might contribute to determining the architecture of actin networks produced by the different nucleation-promoting factors.

The N-terminal regulatory features

The overall conservation among all of the WASP and WAVE family members within the catalytic VCA domain is in contrast to the divergence of their N-terminal portion, which includes >85% of the entire amino acid sequence (see online Supplementary Figure). WASP and N-WASP display a common modular organization, which includes the WH1 domain [also called enabled/vasodilator-stimulated phosphoprotein (Ena/VASP) homology 1], a basic region (B), a GTPase-binding domain (GBD) [also called CRIB (Cdc42 and Rac interactive binding domain)] and a proline-rich region (Figure 1 and online Supplementary Figure).

The WH1 domain encompasses the first 150 amino acids [6,20]. Its functional importance is emphasized by the observation that 28 of the 35 identified missense mutations that cause Wiskott–Aldrich syndrome map within this domain [5]. Several putative functions have been ascribed to this domain. However, a biochemical and structural approach recently revealed that N-WASP WH1 does not bind to the acidic phospholipid phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2], as suggested previously [6], but associates tightly with a 25-residue motif of WASP-interacting protein (WIP) [21] – a member of a family of regulators of N-WASP-mediated actin polymerization [22] that includes WIP- and CR16-homologous protein (WICH) and CR16 [23,24]. WIP wraps around two-thirds of the domain [21], generating an extended interaction surface. This might provide a molecular explanation for some manifestations of Wiskott–Aldrich syndrome because amino acid exchanges in the WH1 domain resulting from mutations in the *WASP* gene could lead to disruption of its interaction with WIP family members, resulting in protein misfunction.

The B region, the GBD and the proline-rich region have all been implicated in regulating WASP and N-WASP function, either by mediating autoinhibitory interactions that block the activity of the output VCA domain, as in the case of the B region and the GBD, or by reversing this inhibition [16,25]. The autoinhibitory interactions are relieved by binding to upstream activators [16,25]. For example, activated GTP-loaded Cdc42 and PtdIns(4,5)P_2 interact with the GBD and B domain, respectively, and a plethora of Src-homology (SH)3-containing proteins [3,26] associate with the polyproline motifs in the proline-rich region. Moreover, the B and the proline-rich regions have also been implicated in F-actin [27] and profilin [28–30] binding, respectively.

The N-terminal region of WAVE proteins is much less characterized. In contrast to WASP and N-WASP, WAVE proteins do not contain GBD motifs or WH1 domains [3] (Figure 1 and online Supplementary Figure). The lack of a surface directly linking WAVE proteins to Rho GTPases suggests that WAVE proteins are regulated in a different way from WASP and N-WASP. Consistent with this, WAVE proteins instead possess an N-terminal conserved region, known as the WAVE homology domain (WHD) [3,8,9]. This region displays a significant sequence similarity to WH1 of WASP and N-WASP, implying that it might fold in an analogous fashion (see online Supplementary Figure). However, it exerts distinct functional roles. The WHD domain does not associate with WIP but, instead, mediates direct binding to Abl-interacting protein 1 (Abi1) [31], a scaffolding molecule originally identified as an interactor for the non-receptor tyrosine kinase Abl [32], which is involved in Ras to Rac signaling [33]. This interaction is crucial for the assembly of a WAVE-based macromolecular

complex, which, in turn, mediates a direct association with activated Rac and positively regulates *in vitro* WAVE2–Arp2/3-dependent actin polymerization [31,34]. Furthermore, similar to WASP and N-WASP, WAVE proteins contain a basic motif and a proline-rich region. The latter mediates, for example, the association with insulin receptor substrate (IRS)p53, which has been implicated in physically linking WAVE2 and Rac [35]. Unlike F-actin binding [27], little is known about whether the basic motif mediates any physiologically relevant protein–lipid interactions, as in the case of WASP and N-WASP.

Distinct modes of integrating upstream signaling by WASP/N-WASP and WAVE proteins

The WASP/N-WASP model of signal integration: cooperation among Cdc42, PtdIns(4,5)P_2, SH3-containing proteins and phosphorylation

The structural features of WASP and N-WASP molecules have been instrumental in guiding the search for their regulatory mechanisms. WASP and N-WASP exist as autoinhibited monomers *in vitro* [16,36] and, possibly, *in vivo*, as suggested by the observation that ectopic expression of full-length N-WASP has no effect on actin dynamics [37]. Therefore, external stimuli must control N-WASP activity by promoting its conversion from an inactive to an active conformation. This occurs through several mechanisms that are based on protein–protein and/or protein–lipid interactions. In the N-WASP closed conformation, the N-terminal B region and the GBD are packed over hydrophobic residues of the C domain in the VCA module, which are essential for activation of Arp2/3 [16,25]. Two structural models have been proposed to explain how this intramolecular interaction leads to autoinhibition. In the direct inhibition model, both the B region and the GBD act as a cooperative unit that binds to the VCA–Arp2/3 complex, imposing structural constraints that prevent catalytic activation of Arp2/3 [16,25]. In the sequestration model, the GBD is sufficient to sequester the VCA domain, impeding catalysis and Arp2/3 binding [15].

Whatever the case, the cooperative binding of PtdIns(4,5)P_2 and Cdc42 to the B region and the GBD, respectively, causes a conformational change that results in release of the C terminus, enabling its interaction with, and activation of, Arp2/3. This cooperative activation mechanism demonstrates how combinations of simple binding domains can be used to integrate and amplify coincident signals (Figure 2). Reinforcing this view, WASP and N-WASP regulation can also be exerted through two additional signal-dependent mechanisms: (i) by binding to several SH3-domain-containing signaling proteins [3] and (ii) by phosphorylation [38–40]. For example, the SH2 and SH3 adaptor Grb2, which is a weak activator by itself, cooperates with Cdc42 to elicit full WASP and N-WASP activity, in a similar way to the actions of Nck and PtdIns(4,5)P_2 [3]. By contrast, WASP-interacting SH3 protein (WISH) can elicit maximal activation of N-WASP in a Cdc42-independent manner (for review, see Ref. [3]). The biochemical effects of the association of other SH3-containing signaling molecules, such as Src, Fyn,

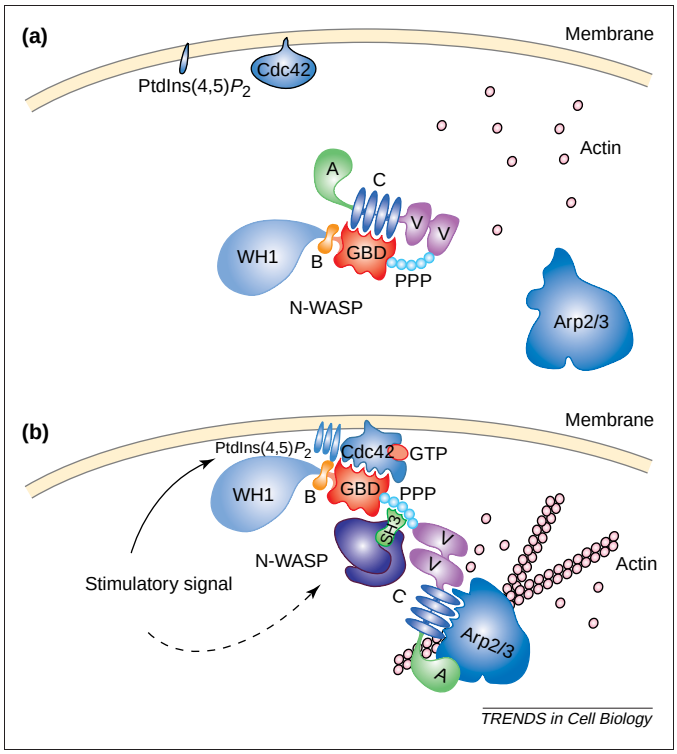

Figure 2. Model of activation of neural Wiskott–Aldrich syndrome protein (N-WASP) by multiple signals. **(a)** In unstimulated cells, Wiskott–Aldrich syndrome protein (WASP) and N-WASP are locked in an autoinhibited state because the N-terminal basic (B) region and the GTPase-binding domain (GBD) are packed over hydrophobic residues of the C domain in the VCA (verprolin homology, cofilin homology and acidic domains, respectively) module, which are essential for activation of Arp2/3. **(b)** Several stimulatory events, such as those emanating from of a variety of activated membrane receptors, might lead to increased and localized phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2] production and Cdc42 activation. Cooperative binding of PtdIns(4,5)P_2 and Cdc42 to the N-WASP B region and GBD, respectively, causes a conformational change that results in release of the C terminus, which enables activation of Arp2/3, leading to formation of branched actin filaments. PtdIns(4,5)P_2 might also uncap filament barbed ends, favoring actin polymerization. Additionally, interaction with several proteins containing Src-homology 3 (SH3) domains and post-translational modifications, such as phosphorylation, assists in modulating the activity of N-WASP. Abbreviations: A, acidic region; C, cofilin homology domain; PPP, proline-rich region; V, verprolin homology domain; WH1, WASP homology domain.

p85 and phospholipase Cγ, with WASP and N-WASP remain to be explored in detail [3]. Several endocytic proteins [endophilin A, intersectin and syndapin (or pacsin1)] might modulate N-WASP activity, suggesting its involvement in trafficking or internalization processes (for review, see Ref. [41]). These studies all support a role for WASP and N-WASP as the convergent 'nodes' of different signaling pathways for the execution of actin-dependent processes. A final level of integration and regulation is exerted by phosphorylation. WASP and N-WASP are phosphorylated on both serine and tyrosine residues [38–40]. Constitutive phosphorylation by casein kinase 2 of a serine located within the VCA domain increases the nucleation-promoting activity of WASP [40]. Src family kinases (but also focal adhesion kinase) [38,39] mediate signal-dependent phosphorylation of both WASP and N-WASP on Y291 and Y253, respectively [38,39]. This post-translational modification synergizes with PtdIns(4,5)P_2, leading to maximal WASP activition. Moreover, the observation that tyrosine phosphorylation, in addition to the subsequent dephosphorylation, occurs only

in the presence of activated Cdc42 suggests a mechanism for regulation of initiation, duration and amplitude of N-WASP activation [42].

Complex assembly leads the way to WAVE regulation

Unlike N-WASP, WAVE proteins do not contain a GBD in their N termini. Consequently, no direct association of WAVE proteins with Rho GTPases has been detected. Nevertheless, WAVE proteins function downstream of Rac in mediating membrane protrusion, as indicated by the fact that WAVE1 dominant negative mutants abolish Rac-dependent ruffling and neurite extension [7]. Additionally, whereas full-length WASP is autoinhibited in *in vitro* actin-polymerization assays, WAVE is active [13,31,43,44], suggesting the existence of specific and distinct modes of regulation. Consistent with this, WAVE assembles into multimolecular complexes to function *in vivo*. These complexes must serve as a link to the incoming signal from Rac and ensure that the actin-nucleation activity of WAVE proteins is regulated and spatially restricted to the cellular leading edge, where actin polymerization for protrusion is initiated and controlled. WAVE complexes have been reported to exert both positive and negative modes of regulation. For example, WAVE2 was found to bind to activated Rac through IRSp53, which, in turn, can stimulate WAVE2 nucleating activity *in vitro*, suggesting a positive mode of regulation [35]. However, it should be noted that IRSp53 was reported to bind only to WAVE2, not to WAVE1 or WAVE3 [35]. Furthermore, the ability of IRSp53 to associate with Rac specifically has been questioned [45]. Thus, a full understanding of the contribution of IRSp53 to WAVE2-mediated actin remodeling awaits further investigation.

An alternative mechanism for controlling the activity of WAVE proteins through Rac was proposed recently [43]. In this study, WAVE1 was found to be kept inactive through its association with four other proteins: Nap1, an Nck-associated protein [46]; PIR121 or Sra-1, identified as Rac effectors [47,48]; and HSPC300, a small 9-KDa protein [43]. This complex could not stimulate actin polymerization in *in vitro* assays, although addition of active Rac relieved this inhibition by inducing the disassembly of the inhibitory Nap1–PIR121 subcomplex from the WAVE1–HSPC300 unit, which was then capable of actin nucleation [43]. This *trans*-inhibitory model is attractive because, similar to the WASP and N-WASP systems, it provides a simple explanation as to how unwanted actin nucleation in the cytoplasm can be prevented. However, it does not account for the mechanisms through which the activity of the complex is restricted to specific sites within cells destined for the formation of membrane extensions. Additional uncertainties concerning the physiological relevance of this mode of WAVE regulation emerged from the fact that Abi proteins were also identified as biochemical and genetic interactors for WAVE-based complexes [49,50]. A detailed analysis of the topological and functional relationships of the various partners of WAVE within these complexes, involving *in vitro* reconstitution and characterization of subcomplexes of WAVE, revealed that Abi1 is an essential component of the PIR121–Nap1-containing WAVE complex [31]. Abi1 was shown to interact directly with the WHD domain of WAVE2, to increase WAVE2 actin-polymerization activity and to mediate the assembly of a WAVE–Abi1–Nap1–PIR121 complex [31].

Unexpectedly, WAVE1–Abi1–Nap1–PIR121 and WAVE2–Abi1–Nap1–PIR121 complexes reconstituted *in vitro* were as active as the WAVE1–Abi1 or WAVE2–Abi1 subcomplexes at stimulating Arp2/3. Also, the addition of activated Rac either *in vitro* or *in vivo* did not disrupt the complex [31,34]. It is difficult to reconcile immediately this latter finding with those reported by Eden *et al.* [43]. Several possibilities could account for the differences. For instance, the exact composition and structure of the complexes, in addition to their state of post-translational modification, might vary among different tissues. However, recent genetic data have provided further support to the notion that, in *Drosophila* and mammalian cell systems, WAVE proteins form stable complexes that are not disrupted following Rac activation or during cell migration. Systematic, individual ablation of the expression of WAVE, Nap1, PIR121/Sra1 and Abi1 by RNA interference (RNAi)-based approaches in *Drosophila* S2 Schneider cells [50,51] and mammalian cells [31,34] led to a complete loss of Rac-induced actin remodeling and lamellipodia, suggesting that PIR121 or Sra-1, Nap1 (or kette, also called HEM, the *Drosophila* homolog of Nap1) and Abi play positive, rather than inhibitory, roles in WAVE regulation. The observation that all components of the WAVE complex are dynamically relocalized to the very edge of membrane protrusions following Rac activation (Figure 3c) supports the idea that proper targeting of a WAVE-containing macromolecular unit defines where *de novo* actin polymerization occurs [31,34,51]. The simple interpretation of these data is consistent with a model in which the WAVE–Abi1–Nap1–PIR121 signaling unit is recruited to lamellipodia following Rac activation, thus leading to site-directed nucleation of actin filaments (Figure 3). An unexpected function of this complex in regulating WAVE expression can be inferred from the observation that individual ablation of Abi1 or any other component of the complex in different cellular model systems leads to degradation of WAVE [31,34,51,52]. This indicates that proper assembly of the complex is also required for WAVE stabilization (Box 1). An additional level of complexity in the regulation of WAVE function is suggested by the identification of WRP [WAVE-associated Rac GTPase-activating protein (GAP)] as a component of a WAVE1 complex [49]. This raises the intriguing possibility that WAVE-based complex(es), in addition to mediating Rac-dependent actin nucleation, might also regulate the duration of signaling by attenuating Rac activation, thus generating a negative feedback loop.

Furthermore, a previously unrecognized role for the WAVE1 isoform as a scaffolding molecule has been proposed, following the observation that this isoform might also act as a kinase-anchoring protein that binds to protein kinase A (PKA) and c-Abl [53]. This supports the notion that the assembly of various WAVE multimolecular complexes is a prerequisite for integration of different signaling pathways.

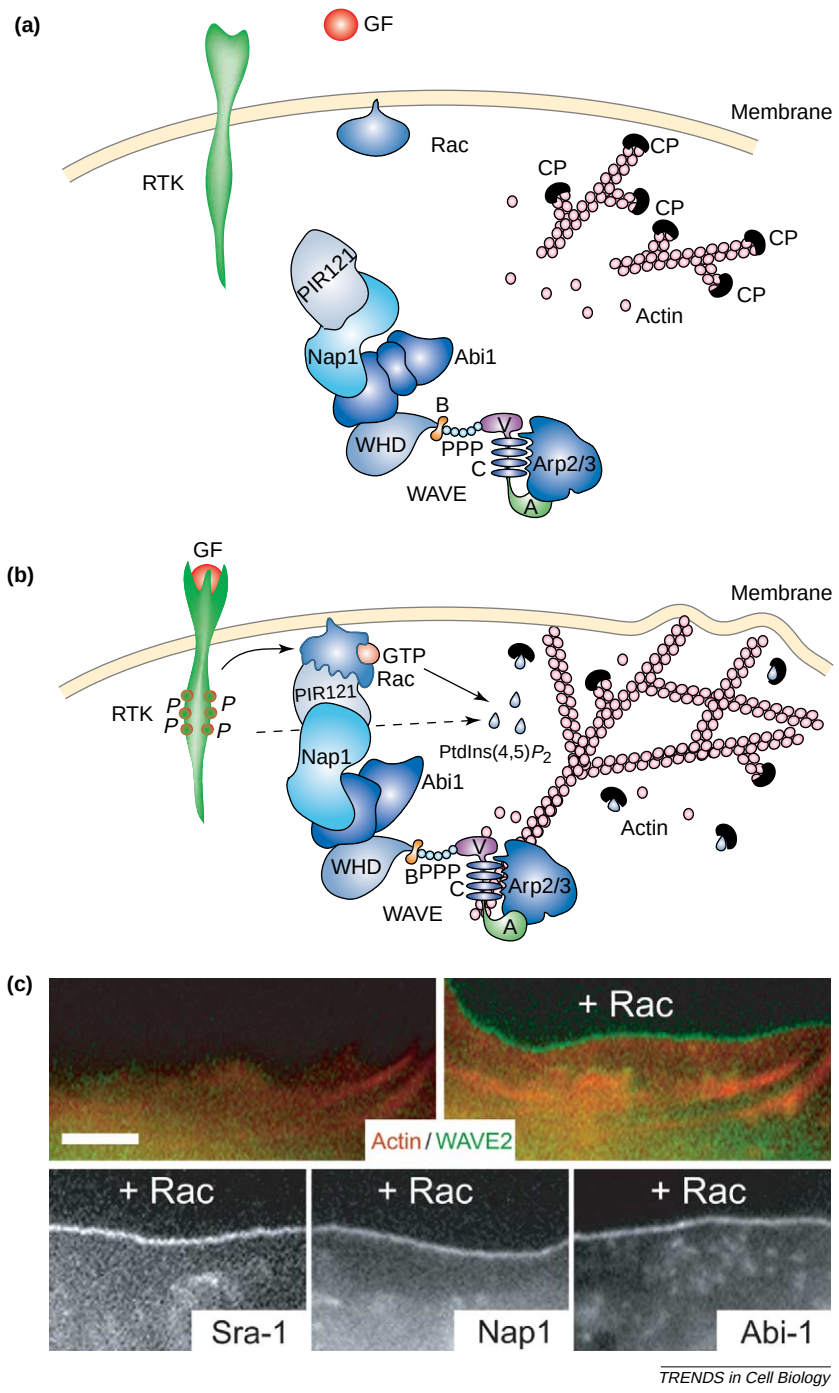

Figure 3. Positive modes of activation of Wiskott–Aldrich syndrome protein (WASP) family verprolin-homologous (WAVE)-containing complexes. In this model of activation of WAVEs, the WAVE–Abi1–Nap1–PIR121 complex is stable and, presumably, can bind to Arp2/3 in the cytoplasm of unstimulated, non-migrating cells, but actin assembly is prevented **(a)**. Under these conditions, filaments exist, although barbed ends are mostly capped by capping proteins (CPs) [78], thereby preventing unwanted actin polymerization. **(b)** Following, for example, Rac activation through receptor tyrosine kinase (RTK) stimulation, coordination between uncapping of filaments and *de novo* actin nucleation occurs. This is achieved through the site-restricted production of localized phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2] [78], which negatively regulates the activity of CPs, and through Rac-dependent relocalization of WAVE-based complexes to the leading edge [31,34,51] to elicit the generation and efficient growth of new filaments necessary to drive cell protrusion and motility. An alternative mode of action (for review, see Refs [79,80]) has also been proposed, in which the assembly of a WAVE1–HSPC300–Nap1–PIR121 complex results in *trans*-inhibition of WAVE1 activity. According to the model, this inhibition is relieved following binding to activated Rac. Abbreviations: A, acidic region; Abi1, Abl-interacting protein 1; B, basic region; C, cofilin homology domain; GF, growth factor; PPP, proline-rich region; SH3, Src homology 3 domain; V, verprolin homology domain; WHD, WAVE homology domain. **(c)** Co-translocation of Sra-1, Nap1, Abi1 and WAVE2 following Rac activation. Upper panels show a fibroblast coexpressing green fluorescent protein (GFP) fused to actin (pseudocolored red) and a red fluorescent protein fused to WAVE2 (pseudocolored green) (see also online Supplementary Video), before (top-left panel) and after (top-right panel) microinjection of constitutively active Rac. Lower panels show cells individually expressing GFP-tagged Sra-1, Nap1 or Abi1 after Rac injections. WAVE2, Sra-1, Nap1 and Abi1 accumulate significantly at the tips of the lamellipodia induced by this treatment (+Rac). Scale bar = 5 μm.

Box 1. Role of WAVE in multicellular organisms: flies and nematodes

Drosophila

Detailed comparison of loss of function for Wiskott–Aldrich syndrome protein (WASP), WASP family verprolin-homologous (WAVE) protein and the Arp2/3 complex during *Drosophila* development revealed a distinct requirement for WAVE and WASP. WAVE/SCAR contributes to Arp2/3-complex-mediated production of bulk actin levels, regulating morphogenesis and cell migration [68]. Notably, those phenotypes caused by ablation of the genes encoding SCAR and Arp2/3 (e.g. during oogenesis and in blastoderm embryos) are not observed in the WASP ortholog *Wsp*-knockout embryos [65,66,68]. Conversely, Wsp function is essential in early cell-fate decisions because loss of function results in a bias for neurons rather than precursor cells of sensory organs [65,66]. The primary role for WAVE in Arp2/3-mediated actin assembly in *Drosophila* is supported further by the findings that mutant kette (the Nap1 ortholog) embryos phenocopy mutants of WAVE and Arp2/3 but not Wsp [75]. Interestingly, genetic interaction between *SCAR* and *Kette* has been suggested recently. Glial cell migration is impaired in *Kette*-null mutants, which is a phenotype that is partially suppressed by the removal of one copy of the *SCAR* gene [81]. However, interpreting these results is made complicated by the observation that the individual removal of any of the WAVE complex (SCAR–Abi–Kette–Sra1) components leads to destabilization and degradation of the complex. This is mediated, at least in part, by the proteasome [51], and is similar to what has been observed in mammalian and *Dictyostelium discoideum* cell systems [31,34,52]. This reveals an unforeseen mode of regulation for the WAVE complex, the integrity of which is required not only for its activity and localization but also for protection from degradation (see also Table 1).

Caenorhabditis elegans

In the nematode *Caenorhabditis elegans*, no direct analysis of the function of the WAVE homolog has yet been reported. However, ablation of expression of Arp2/3-complex subunits by RNA interference (RNAi) leads to embryonic lethality, as a consequence of defective ventral closure, which is probably due to impaired migration of hypodermal cells [64]. Notably, knockdowns of *ced-10* (the ortholog of *Rac1*) [77], or *gex-2* (*sra-1*) and *gex-3* (*nap1*) [76], which are all implicated in WAVE regulation, display a similar phenotype, suggesting that interference with WAVE might phenocopy that of Arp2/3.

Box 2. Functional role of WAVE in unicellular organisms: yeast and *Dictyostelium discoideum*

In yeast, polar budding and receptor-mediated endocytosis depend on the presence and proper positioning of Arp2/3-dependent actin patches. Disruption of the *las17* (also called *bee1p*) gene, the yeast Wiskott–Aldrich syndrome protein (WASP) and WASP family verprolin-homologous (WAVE) homolog, leads to impairment of actin-patch formation and, consequently, of polar budding and endocytosis [61]. Although they have confirmed a genetic and physical interaction between the WASP-like protein and the Arp2/3 complex [62,82], studies in yeast have not aided our understanding of the complex differential contributions of the WASP and WAVE family members in higher organisms.

In this respect, the slime mold *Dictyostelium discoideum* has been a useful model organism, because it displays ameboid actin-based motility and phagocytosis, in a similar way to neutrophils. *D. discoideum WAVE*, named *Scar*, was identified in a screen for genes that alleviate the phenotype of cAMP-receptor loss of function [9]. *Scar*-null cells are unusually small, and display reduced F-actin content and significant deficiencies in cell motility, chemotaxis [9] and uptake of liquids and particles [63]. Blagg *et al.* [52] recently reported the disruption of the gene encoding pirA (the PIR121 or Sra-1 homolog). Cells null for this gene are abnormally large and display decreased motility but excess pseudopod formation. Significantly, the latter phenotype was not observed in a *WAVE/Scar*-null background, suggesting epistasis between *Scar* and *pirA* in *D. discoideum*. Surprisingly, however, *pirA*-null cells express small amounts of Scar protein, which argues against the possibility that elevated WAVE/Scar activity is responsible for the observed phenotype.

Lessons from knockouts

WASP, N-WASP and WAVE family proteins display a remarkable degree of conservation across different species (see online Supplementary Figure), ranging from mammals to yeast. Genetic approaches performed in different model organisms (Boxes 1, 2 and Table 1) have been instrumental in gaining further insight into the physiological role of the various WAVE members and their mode of action. Recently, these studies have been extended to mice, revealing both redundant and specific roles for the different WAVE family members.

Both *Wave1*- and *Wave2*-knockout mice have been described recently [54–57]. The phenotypes of the two *Wave1*-knockout mice described differ significantly in severity. In one case, postnatal lethality, limb weakness, resting tremor and neuroanatomical malformation were reported [54], whereas in the other, mice were characterized mainly by reduced anxiety, and sensorimotor and cognitive deficits [55]. Both mice suffered from brain deficiencies, although no alteration of embryonic development was observed. This was surprising because WAVE1 is widely expressed at this stage and is restricted to the brain only later in development [54,55,58]. More insights into brain-specific WAVE function might be obtained from future *Wave3*-knockout mice and *Wave1–Wave3* double knockouts because WAVE3 expression is restricted to the brain [58].

Knockouts of the ubiquitously expressed *Wave2* isoform yielded drastic phenotypes [56,57]. Embryonic lethality between embryonic day (E)9 and E12 was described, accompanied by developmental retardation, brain malformation [56] and hemorrhaging [56,57]. Hemorrhaging is linked to impaired angiogenesis, resulting from the inability of endothelial cells to migrate in response to vascular endothelial growth factor [57]. A more general role for WAVE2 in cell motility is supported by the finding that W*ave2*-null fibroblasts display impaired cell migration and defective lamellipodia and ruffle formation in response to platelet-derived growth factor [56]. This strengthens the conclusions obtained at the cellular level after small interfering RNA (siRNA)-mediated ablation of both W*ave* expression and the other components of the WAVE complex [31,34]. Interestingly, the time frame of embryonic lethality in W*ave2*-null offspring coincides with the progressive disappearance of WAVE1 in the embryo, suggesting redundancy among WAVE family members at earlier developmental stages. However, a more recent study demonstrated differential roles for WAVE1 and WAVE2 during fibroblast cell migration in dorsal and peripheral ruffles, respectively [59]. It was suggested that WAVE2 is involved in leading-edge extension during directed migration, whereas WAVE1 is required for migration through extracellular matrix in a 3D environment. These observations are in contrast to the finding that both WAVE1 and WAVE2 accumulate at the tips of peripheral

Table 1. Loss-of-function studies on WASP and WAVE families and associated proteins[a]

Gene	Organism	Method	Phenotype	Refs
KO and RNAi studies on *WASP* and *WAVE* family genes				
las17/bee1p	Sc	KO	Defective endocytosis; no polarized cortical actin patches; altered F-actin organization; defects in budding and cytokinesis	[61,62]
scar	Dd	KO	Impaired macropinocytosis and phagocytosis; defects in chemotaxis; reduced cell size; altered shape; reduced F-actin levels	[63]
wsp-1	Ce	RNAi	35% of embryos display embryonic lethality, larval arrest or incomplete ventral enclosure; defective hypodermal cell migration; reduction in F-actin at leading edge of hypodermal cells	[64]
WASp	Dm	KO	Defects in early cell-fate decisions of specific neuronal cell lineages; loss of sensory bristles; adult lethal	[65,66]
WASp	Dm (tc)	RNAi	Reduced phagocytosis	[67]
SCAR	Dm	KO	Lethal during oogenesis; reduced F-actin amounts; aberrant cell and organ morphology; blastoderm- and axon-guidance defects	[68]
SCAR	Dm (tc)	RNAi	Lack of lamellipodia formation; impaired phagocytosis	[50,51,67]
Wasp	Mm	KO	Chronic colitis; defects in T-cell proliferation and differentiation; impaired proliferation after TCR stimulation	[69,70]
WASP	Hs	Multiple point mutations	Wiskott–Aldrich Syndrome (immunodeficiencies and defects in blood cell morphogenesis; lack of podosomes; defective T-cell signaling)	[4,5]
N-WASP	Mm	KO	Embryonic lethal (E11); developmental delay with prominent neural tube and cardiac abnormalities	[71]
N-WASP	Mm	KO	Embryonic lethal (E11.5); lack of axial rotation; abnormalities in intra- and extra-embryonic mesoderm development	[72]
WAVE1	Mm	Genetrap	Postnatal lethality (P20+); resting tremor; neuroanatomical malformations	[54]
WAVE1	Mm	KO	Reduced anxiety; sensorimotor retardation; deficits in learning and memory	[55]
WAVE2	Mm	KO	Embryonic lethal (E12.5); malformations of developing brain; defective response of fibroblasts to PDGF	[56]
WAVE2	Mm	KO	Hemorrhage; embryonic lethal (E10); defective response of endothelial cells to VEGF	[57]
WAVE2	Hs (tc)	RNAi	Almost complete abolishment of lamellipodia and ruffle formation	[31]
KO and RNAi studies on WAVE complex and related genes				
Abi1	Hs (tc)	RNAi	Lack of lamellipodia formation and membrane ruffling	[31]
Sra-1	Hs, Mm (tc)	RNAi	Similar to Abi1 RNAi	[34]
Nap1	Hs, Mm (tc)	RNAi	Similar to Abi1 RNAi	[34]
Nap1	Hs (tc)	RNAi	Rounding; detachment; apoptosis	[73]
PirA	Dd	KO	Altered morphology; defective migration and polarity; increased F-actin levels	[52]
Arp2/3 complex	Dm	KO	Embryonic lethal; defects in oogenesis, cytoplasmic transport and ring-canal formation	[74]
Hem/kette	Dm	KO	Embryonic lethal; fused commissures; defective glial migration; altered F-actin in mesoderm and ectoderm	[75]
Hem/kette	Dm (tc)	RNAi	Similar to SCAR RNAi	[50,51]
Abi	Dm (tc)	RNAi	Similar to SCAR RNAi	[50,51]
Rac	Dm (tc)	RNAi	Similar to SCAR RNAi	[51]
Arp2/3 complex	Dm (tc)	RNAi	Similar to SCAR RNAi	[50,51]
HSPC300	Dm (tc)	RNAi	Partially impaired lamellipodia formation	[51]
gex-2 (Sra-1)	Ce	KO	Embryonic lethal; defective hypodermal cell migration; lack of ventral closure; cell shape changes	[76]
gex-3 (Nap1)	Ce	KO	Identical to *gex-2* KO	[76]
ced-10 (Rac1)	Ce	KO	Similar to *gex-2* and *gex-3* KO but less severe; impaired phagocytosis of apoptotic cells and axon pathfinding	[77]
arp2/3 complex	Ce	RNAi	Defective ventral closure; similar to *gex-2*, *gex-3* and *ced-10* KO	[64]

[a]Abbreviations: Abi, Abl-interacting protein 1; Ce, *Caenorhabditis elegans*; Dd, *Dictyostelium discoideum*; Dm, *Drosophila melanogaster*; E, embryonic day; Hs, *Homo sapiens*; KO, knockout; Mm, *Mus musculus*; P, postnatal day; PDGF, platelet-derived growth factor; RNAi, RNA interference; Sc, *Saccharomyces cerevisiae*; tc, tissue culture; TCR, T-cell receptor; VEGF, vascular endothelial growth factor.

membrane protrusions in other cell types [60]. Further investigations are required to determine whether the function of WAVE proteins might differ in other cells depending on the relative expression levels of the various isoforms, with respect to different developmental stages, cells and organs.

Concluding remarks

Site-directed, dynamic assembly of actin filaments is a crucial event for a wide range of processes through which living cells change shape, extend protrusions or wrap around a particle to form phagocytic cups [10]. A key step within these processes is the regulation of *de novo* actin nucleation by WAVE proteins, which serve as nodes, mediating the propagation of signaling cascades that lead to site-directed actin polymerization. The combination of genetic and biochemical approaches has paved the way towards understanding how this regulation is achieved, revealing that assembly of WAVE-containing macromolecular units is crucial for exerting its function.

In particular, a recent series of studies have revealed how Abi, Nap1 and PIR121 or Sra-1 – the core components of this unit – promote WAVE function by acting at different levels: (i) they modulate Arp2/3-mediated actin polymerization directly [31,43]; (ii) they serve as a physical link to upstream input in the signaling cascade of events centered on Rac activation [31,34,43]; (iii) they are essential for localizing the complex to its proper sites, where *de novo* actin polymerization occurs [31,34,51]; and (iv) they control WAVE degradation, ensuring that its activation is coupled tightly to the appropriate activating signals [31,34,51,52].

Additional facets of WAVE regulation are highlighted by the discovery of other interactors (Abl, PKA, WRP and IRSp53) and possibly distinct WAVE complexes, which have been proposed to exert both positive and negative effects. The definition of their precise functional roles and order of assembly in various processes, tissues and pathways requires further investigation. For example, the role of the signal-dependent post-translational modifications – primarily phosphorylation – of both WAVE and the components of the WAVE complexes has received scant attention so far. Similarly, the use of advanced microscopy techniques, such as fluorescence energy transfer (FRET) or fluorescence recovery after photobleaching (FRAP), to explore dynamic complex formation and turnover will help to clarify further the exact mechanism of the WAVE mode of action. Moreover, it is likely that the combinatorial assembly of different, additional components of the WAVE-based complexes will be vital for regulating its function as a master molecule in dynamic actin reorganization.

Finally, generation of free barbed ends by *de novo* nucleation of actin filaments is only one of the events required for polarized growth of the dendritic actin network. Capping and uncapping of filament free ends, in addition to severing and depolymerization from pointed ends, might contribute significantly to the regulation of actin dynamics that supports membrane protrusion and, ultimately, cell migration. All of these activities must be coordinated and spatially regulated to generate forces properly so that motility can occur. The molecular mechanisms driving these integrated processes are far from understood, and their elucidation for a comprehensive knowledge of how actin dynamics control motility represents one of the major challenges for the future.

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References

1 Pollard, T.D. and Borisy, G.G. (2003) Cellular motility driven by assembly and disassembly of actin filaments. *Cell* 112, 453–465
2 Hall, A. (1998) Rho GTPases and the actin cytoskeleton. *Science* 279, 509–514
3 Takenawa, T. and Miki, H. (2001) WASP and WAVE family proteins: key molecules for rapid rearrangement of cortical actin filaments and cell movement. *J. Cell Sci.* 114, 1801–1809
4 Derry, J.M. *et al.* (1994) Isolation of a novel gene mutated in Wiskott–Aldrich syndrome. *Cell* 78, 635–644
5 Imai, K. *et al.* (2003) WASP (Wiskott–Aldrich syndrome protein) gene mutations and phenotype. *Curr. Opin. Allergy Clin. Immunol.* 3, 427–436
6 Miki, H. *et al.* (1996) 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
7 Miki, H. *et al.* (1998) WAVE, a novel WASP-family protein involved in actin reorganization induced by Rac. *EMBO J.* 17, 6932–6941
8 Suetsugu, S. *et al.* (1999) Identification of two human WAVE/SCAR homologues as general actin regulatory molecules which associate with the Arp2/3 complex. *Biochem. Biophys. Res. Commun.* 260, 296–302
9 Bear, J.E. *et al.* (1998) SCAR, a WASP-related protein, isolated as a suppressor of receptor defects in late *Dictyostelium* development. *J. Cell Biol.* 142, 1325–1335
10 Pantaloni, D. *et al.* (2001) Mechanism of actin-based motility. *Science* 292, 1502–1506
11 Prehoda, K.E. and Lim, W.A. (2002) How signaling proteins integrate multiple inputs: a comparison of N-WASP and Cdk2. *Curr. Opin. Cell Biol.* 14, 149–154
12 Miki, H. and Takenawa, T. (1998) Direct binding of the verprolin-homology domain in N-WASP to actin is essential for cytoskeletal reorganization. *Biochem. Biophys. Res. Commun.* 243, 73–78
13 Machesky, L.M. and Insall, R.H. (1998) Scar1 and the related Wiskott–Aldrich syndrome protein, WASP, regulate the actin cytoskeleton through the Arp2/3 complex. *Curr. Biol.* 8, 1347–1356
14 Marchand, J.B. *et al.* (2001) Interaction of WASP/Scar proteins with actin and vertebrate Arp2/3 complex. *Nat. Cell Biol.* 3, 76–82
15 Panchal, S.C. *et al.* (2003) A conserved amphipathic helix in WASP/Scar proteins is essential for activation of Arp2/3 complex. *Nat. Struct. Biol.* 10, 591–598
16 Kim, A.S. *et al.* (2000) Autoinhibition and activation mechanisms of the Wiskott–Aldrich syndrome protein. *Nature* 404, 151–158
17 Yamaguchi, H. *et al.* (2000) Two tandem verprolin homology domains are necessary for a strong activation of Arp2/3 complex-induced actin polymerization and induction of microspike formation by N-WASP. *Proc. Natl. Acad. Sci. U. S. A.* 97, 12631–12636
18 Zalevsky, J. *et al.* (2001) Different WASP family proteins stimulate different Arp2/3 complex-dependent actin-nucleating activities. *Curr. Biol.* 11, 1903–1913
19 Yamaguchi, H. *et al.* (2002) Two verprolin homology domains increase the Arp2/3 complex-mediated actin polymerization activities of N-WASP and WAVE1 C-terminal regions. *Biochem. Biophys. Res. Commun.* 297, 214–219
20 Symons, M. *et al.* (1996) Wiskott–Aldrich syndrome protein, a novel effector for the GTPase CDC42Hs, is implicated in actin polymerization. *Cell* 84, 723–734
21 Volkman, B.F. *et al.* (2002) Structure of the N-WASP EVH1 domain-WIP complex: insight into the molecular basis of Wiskott–Aldrich Syndrome. *Cell* 111, 565–576
22 Ramesh, N. *et al.* (1997) WIP, a protein associated with Wiskott–Aldrich syndrome protein, induces actin polymerization and redistribution in lymphoid cells. *Proc. Natl. Acad. Sci. U. S. A.* 94, 14671–14676
23 Kato, M. *et al.* (2002) 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
24 Zettl, M. and Way, M. (2002) The WH1 and EVH1 domains of WASP and Ena/VASP family members bind distinct sequence motifs. *Curr. Biol.* 12, 1617–1622
25 Prehoda, K.E. *et al.* (2000) Integration of multiple signals through cooperative regulation of the N-WASP–Arp2/3 complex. *Science* 290, 801–806
26 Rohatgi, R. *et al.* (2000) Mechanism of N-WASP activation by Cdc42 and phosphatidylinositol 4,5- bisphosphate. *J. Cell Biol.* 150, 1299–1310
27 Suetsugu, S. *et al.* (2001) Enhancement of branching efficiency by the actin filament-binding activity of N-WASP/WAVE2. *J. Cell Sci.* 114, 4533–4542

28 Suetsugu, S. *et al.* (1998) The essential role of profilin in the assembly of actin for microspike formation. *EMBO J.* 17, 6516–6526
29 Yang, C. *et al.* (2000) Profilin enhances Cdc42-induced nucleation of actin polymerization. *J. Cell Biol.* 150, 1001–1012
30 Yarar, D. *et al.* (2002) Motility determinants in WASP family proteins. *Mol. Biol. Cell* 13, 4045–4059
31 Innocenti, M. *et al.* (2004) Abi1 is essential for the formation and activation of a WAVE2 signaling. *Nat. Cell Biol.* 6, 319–327
32 Shi, Y. *et al.* (1995) Abl-interactor-1, a novel SH3 protein binding to the carboxy-terminal portion of the Abl protein, suppresses v-abl transforming activity. *Genes Dev.* 9, 2583–2597
33 Innocenti, M. *et al.* (2003) Phosphoinositide 3-kinase activates Rac by entering in a complex with Eps8, Abi1, and Sos-1. *J. Cell Biol.* 160, 17–23
34 Steffen, A. *et al.* (2004) Sra-1 and Nap1 link Rac to actin assembly driving lamellipodia formation. *EMBO J.* 23, 749–759
35 Miki, H. *et al.* (2000) IRSp53 is an essential intermediate between Rac and WAVE in the regulation of membrane ruffling. *Nature* 408, 732–735
36 Rohatgi, R. *et al.* (1999) The interaction between N-WASP and the Arp2/3 complex links Cdc42-dependent signals to actin assembly. *Cell* 97, 221–231
37 Miki, H. *et al.* (1998) Induction of filopodium formation by a WASP-related actin-depolymerizing protein N-WASP. *Nature* 391, 93–96
38 Suetsugu, S. *et al.* (2002) Sustained activation of N-WASP through phosphorylation is essential for neurite extension. *Dev. Cell* 3, 645–658
39 Cory, G.O. *et al.* (2002) 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
40 Cory, G.O. *et al.* (2003) Phosphorylation of the WASP-VCA domain increases its affinity for the Arp2/3 complex and enhances actin polymerization by WASP. *Mol. Cell* 11, 1229–1239
41 Qualmann, B. and Kessels, M.M. (2002) Endocytosis and the cytoskeleton. *Int. Rev. Cytol.* 220, 93–144
42 Torres, E. and Rosen, M.K. (2003) Contingent phosphorylation/dephosphorylation provides a mechanism of molecular memory in WASP. *Mol. Cell* 11, 1215–1227
43 Eden, S. *et al.* (2002) Mechanism of regulation of WAVE1-induced actin nucleation by Rac1 and Nck. *Nature* 418, 790–793
44 Miki, H. and Takenawa, T. (2002) WAVE2 serves a functional partner of IRSp53 by regulating its interaction with Rac. *Biochem. Biophys. Res. Commun.* 293, 93–99
45 Krugmann, S. *et al.* (2001) Cdc42 induces filopodia by promoting the formation of an IRSp53:Mena complex. *Curr. Biol.* 11, 1645–1655
46 Kitamura, T. *et al.* (1996) Molecular cloning of p125Nap1, a protein that associates with an SH3 domain of Nck. *Biochem. Biophys. Res. Commun.* 219, 509–514
47 Saller, E. *et al.* (1999) Increased apoptosis induction by 121F mutant p53. *EMBO J.* 18, 4424–4437
48 Kobayashi, K. *et al.* (1998) p140Sra-1 (specifically Rac1-associated protein) is a novel specific target for Rac1 small GTPase. *J. Biol. Chem.* 273, 291–295
49 Soderling, S.H. *et al.* (2002) The WRP component of the WAVE-1 complex attenuates Rac-mediated signalling. *Nat. Cell Biol.* 4, 970–975
50 Rogers, S.L. *et al.* (2003) Molecular requirements for actin-based lamella formation in *Drosophila* S2 cells. *J. Cell Biol.* 162, 1079–1088
51 Kunda, P. *et al.* (2003) 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
52 Blagg, S.L. *et al.* (2003) PIR121 regulates pseudopod dynamics and SCAR activity in *Dictyostelium*. *Curr. Biol.* 13, 1480–1487
53 Westphal, R.S. *et al.* (2000) Scar/WAVE-1, a Wiskott–Aldrich syndrome protein, assembles an actin-associated multi-kinase scaffold. *EMBO J.* 19, 4589–4600
54 Dahl, J.P. *et al.* (2003) Characterization of the WAVE1 knock-out mouse: implications for CNS development. *J. Neurosci.* 23, 3343–3352
55 Soderling, S.H. *et al.* (2003) Loss of WAVE-1 causes sensorimotor retardation and reduced learning and memory in mice. *Proc. Natl. Acad. Sci. U. S. A.* 100, 1723–1728
56 Yan, C. *et al.* (2003) WAVE2 deficiency reveals distinct roles in embryogenesis and Rac-mediated actin-based motility. *EMBO J.* 22, 3602–3612
57 Yamazaki, D. *et al.* (2003) WAVE2 is required for directed cell migration and cardiovascular development. *Nature* 424, 452–456
58 Sossey-Alaoui, K. *et al.* (2003) Genomic organization and expression profile of the human and mouse WAVE gene family. *Mamm. Genome* 14, 314–322
59 Suetsugu, S. *et al.* (2003) Differential roles of WAVE1 and WAVE2 in dorsal and peripheral ruffle formation for fibroblast cell migration. *Dev. Cell* 5, 595–609
60 Hahne, P. *et al.* (2001) Scar/WAVE is localised at the tips of protruding lamellipodia in living cells. *FEBS Lett.* 492, 215–220
61 Li, R. (1997) 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
62 Madania, A. *et al.* (1999) The *Saccharomyces cerevisiae* homologue of human Wiskott–Aldrich syndrome protein Las17p interacts with the Arp2/3 complex. *Mol. Biol. Cell* 10, 3521–3538
63 Seastone, D.J. *et al.* (2001) The WASp-like protein scar regulates macropinocytosis, phagocytosis and endosomal membrane flow in *Dictyostelium*. *J. Cell Sci.* 114, 2673–2683
64 Sawa, M. *et al.* (2003) Essential role of the *C. elegans* Arp2/3 complex in cell migration during ventral enclosure. *J. Cell Sci.* 116, 1505–1518
65 Ben-Yaacov, S. *et al.* (2001) Wasp, the *Drosophila* Wiskott–Aldrich syndrome gene homologue, is required for cell fate decisions mediated by Notch signaling. *J. Cell Biol.* 152, 1–13
66 Tal, T. *et al.* (2002) Conserved interactions with cytoskeletal but not signaling elements are an essential aspect of *Drosophila* WASp function. *Dev. Biol.* 243, 260–271
67 Pearson, A.M. *et al.* (2003) Identification of cytoskeletal regulatory proteins required for efficient phagocytosis in *Drosophila*. *Microbes Infect.* 5, 815–824
68 Zallen, J.A. *et al.* (2002) SCAR is a primary regulator of Arp2/3-dependent morphological events in *Drosophila*. *J. Cell Biol.* 156, 689–701
69 Snapper, S.B. *et al.* (1998) Wiskott–Aldrich syndrome protein-deficient mice reveal a role for WASP in T but not B cell activation. *Immunity* 9, 81–91
70 Zhang, J. *et al.* (1999) Antigen receptor-induced activation and cytoskeletal rearrangement are impaired in Wiskott–Aldrich syndrome protein-deficient lymphocytes. *J. Exp. Med.* 190, 1329–1342
71 Snapper, S.B. *et al.* (2001) N-WASP deficiency reveals distinct pathways for cell surface projections and microbial actin-based motility. *Nat. Cell Biol.* 3, 897–904
72 Lommel, S. *et al.* (2001) 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
73 Suzuki, T. *et al.* (2000) Molecular cloning of a novel apoptosis-related gene, human Nap1 (NCKAP1), and its possible relation to Alzheimer disease. *Genomics* 63, 246–254
74 Hudson, A.M. and Cooley, L. (2002) Understanding the function of actin-binding proteins through genetic analysis of *Drosophila* oogenesis. *Annu. Rev. Genet.* 36, 455–488
75 Hummel, T. *et al.* (2000) The *Drosophila* HEM-2/NAP1 homolog KETTE controls axonal pathfinding and cytoskeletal organization. *Genes Dev.* 14, 863–873
76 Soto, M.C. *et al.* (2002) The GEX-2 and GEX-3 proteins are required for tissue morphogenesis and cell migrations in *C. elegans*. *Genes Dev.* 16, 620–632
77 Lundquist, E.A. *et al.* (2001) Three *C. elegans* Rac proteins and several alternative Rac regulators control axon guidance, cell migration and apoptotic cell phagocytosis. *Development* 128, 4475–4488
78 Allen, P.G. (2003) Actin filament uncapping localizes to ruffling lamellae and rocketing vesicles. *Nat. Cell Biol.* 5, 972–979
79 Cory, G.O. and Ridley, A.J. (2002) Cell motility: braking WAVEs. *Nature* 418, 732–733
80 Smith, L.G. and Li, R. (2004) Actin polymerization: riding the wave. *Curr. Biol.* 14, R109–R111
81 Bogdan, S. and Klambt, C. (2003) Kette regulates actin dynamics and genetically interacts with Wave and Wasp. *Development* 130, 4427–4437
82 Winter, D. *et al.* (1999) Activation of the yeast Arp2/3 complex by Bee1p, a WASP-family protein. *Curr. Biol.* 9, 501–504