J. Biochem. **134**, 309–313 (2003)
DOI: 10.1093/jb/mvg146

Regulation of Actin Dynamics by WASP Family Proteins

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Received May 14, 2003; accepted May 21, 2003

Rapid reorganization of the actin cytoskeleton underlies morphological changes and motility of cells. WASP family proteins have received a great deal of attention as the signal-regulated molecular switches that initiate actin polymerization. The first member, WASP, was identified as the product of a gene of which dysfunction causes the human hereditary disease Wiskott-Aldrich syndrome. There are now five members in this protein family, namely WASP, N-WASP, WAVE/Scar1, 2, and 3. WASP and N-WASP have functional and physical associations with Cdc42, a Rho family small GTPase involved in filopodium formation. In contrast, there is evidence that links the WAVE/Scar proteins with another Rho family protein, Rac, which is a regulator of membrane ruffling. All WASP family members have a VCA domain at the C-terminus through which Arp2/3 complex is activated to nucleate actin polymerization. Analyses of model organisms have just begun to reveal unexpected functions of WASP family proteins in multicellular organisms.

Key words: actin cytoskeleton, Arp2/3 complex, Rho family, WASP, WAVE/Scar.

Abbreviations: EVH (Ena/VASP-homology), GBD/CRIB (GTPase binding domain/Cdc42-Rac interactive binding), PIP2 (phosphatidylinositol-4,5-bisphosphate), Scar (suppressor of cAMP receptor), SHD/WHD (Scar/WAVE-homology domain), VCA (verprolin-homology, cofilin-homology, and highly acidic), WASP (Wiskott-Aldrich Syndrome Protein), WAVE (WASP-family verprolin-homologous protein), and WIP (WASP-interacting protein).

Identification of family members and their binding partners

Wiskott-Aldrich syndrome (WAS) is an X-chromosome–linked hereditary disease that is characterized by thrombocytopenia, eczema, and immunodeficiency. In 1994, Derry *et al*. (*1*) identified the gene that is mutated in WAS patients and named it *Wiskott-Aldrich Syndrome Protein* (*WASP*). Northern blotting analysis indicated that *WASP* is expressed exclusively in hematopoietic cells. Two years later, a novel protein with ~50% amino acid identity to the *WASP* gene product (WASP) was reported as a binding partner for the Grb2/Ash adapter protein (*2*). In contrast to WASP, this protein was expressed ubiquitously, but strongest expression was observed in nerve cells, and thus it was named Neural-WASP (N-WASP). As shown in Fig. 1, WASP and N-WASP are very similar to each other and possess the same functional domains and motifs, including an EVH1 (or WH1) domain, a highly basic region, a GBD/CRIB motif, a proline-rich region, and a VCA region. The EVH1 domain binds the evolutionarily conserved WASP-interacting protein (WIP) family proteins such as WIP, CR16, and WICH in mammalian cells (*3*–*6*). The crystal structure of the binding interface between N-WASP and WIP was recently reported and revealed an unusual mode of interaction that requires a long ~20 amino acid peptide in WIP (*7*). The WIP family proteins are essential functional partners for WASP and N-WASP, probably through the regulation of their localization (*8*–*10*). The basic region and the GBD/CRIB motif regulate activation of WASP/N-WASP through binding to PIP2 and activated Cdc42, respectively (*11*–*15*). The proline-rich region also contributes to activation by binding several SH3 domain-containing proteins such as Grb2/Ash (*16*), Nck (*17*), and WISH (*18*). The C-terminal VCA region was first described as a verprolin-homology (V), cofilin-homology (C), and highly acidic (A) region (*2*). The V region binds directly to monomeric actin, and the CA regions together bind Arp2/3 complex (*19*–*22*). This VCA region is the minimum essential domain for activation of Arp2/3 complex to nucleate actin polymerization (*21*, *22*).

A database search for novel proteins with sequences similar to that of the actin-binding V region identified an uncharacterized mRNA that had been deposited as KIAA0269. This mRNA encoded a novel protein that was named WAVE for WASP-family verprolin-homologous protein (*23*). Shortly before the identification of WAVE, Bear *et al*. (*24*) described a new gene essential for actin reorganization during chemotactic movement of *Dictyostelium* cells in response to cAMP stimulation and named it *Scar* for *suppressor of cAMP receptor*. The *Scar* gene encodes the *Dictyostelium* homolog of WAVE. Sequence comparison with WASP/N-WASP showed that the C-terminal VCA region and the proline-rich region are also present in WAVE, but that its N-terminal region was not similar to that of WASP/N-WASP (Fig. 1). Since the N-terminal region of WASP/N-WASP is important for localization and activity, this suggested that WAVE is regulated differently from WASP/N-WASP. An extensive database search then identified two additional mRNAs that encode WAVE-related proteins (*25*). cDNAs encoding full-length proteins were isolated and named WAVE2

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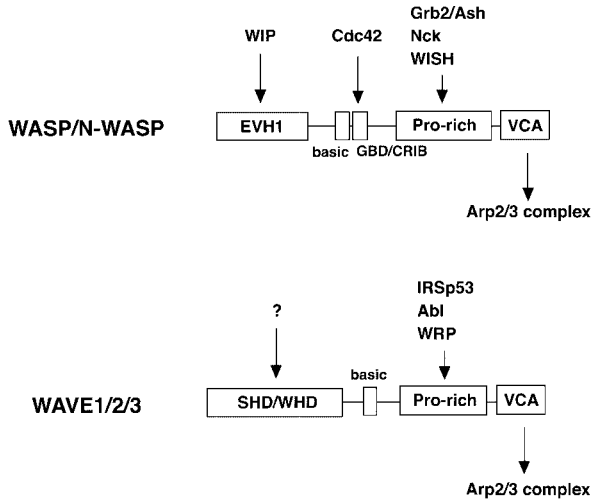

Fig. 1. **WASP family proteins and their binding partners. Structures of WASP/N-WASP and WAVE1/WAVE2/WAVE3.** The blocks indicate functional domains or motifs, which include the EVH1 domain, SHD/WHD, a highly basic region, the GBD/CRIB motif, a proline-rich region, and the VCA region. Binding partner proteins are also indicated. Details are described in the main text.

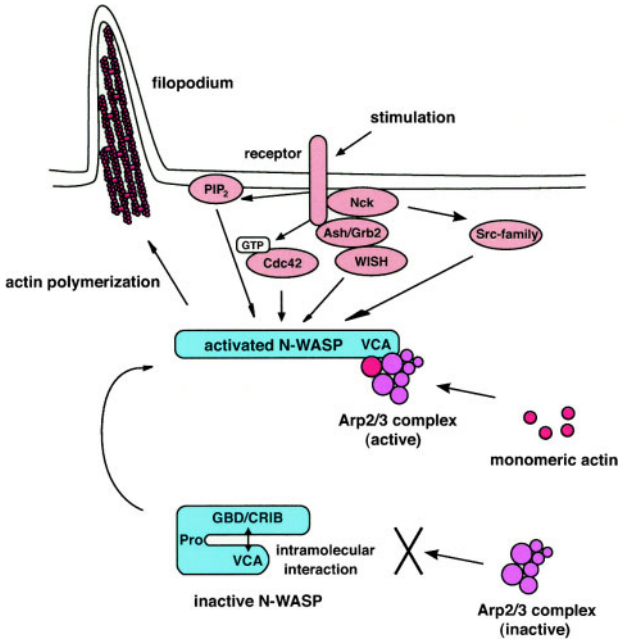

Fig. 2. **Mechanism of activation of N-WASP.** During the resting state, N-WASP is kept inactive by autoinhibition. Various stimuli induce activation of N-WASP, which results in actin polymerization through the Arp2/3 complex. Cdc42 binds directly to the GBD/CRIB motif and disrupts the autoinhibited structure. Various SH3 proteins, such as Grb2/Ash, Nck, and WISH, bind to the proline-rich region and induce a structural change in N-WASP that probably releases the folded conformation of inactive N-WASP. Src-family tyrosine kinases activate N-WASP through phosphorylation.

and WAVE3 (the original WAVE was then renamed WAVE1). WAVE1, 2, and 3 contain a novel homologous domain (SHD/WHD), which consists of ~200 amino acid residues, at the N-terminus. Several SH3 domain proteins, including Abl (*26*), IRSp53 (*27*), and WRP (*28*), have been shown to associate with the proline-rich region of WAVEs, but a ligand for the SHD/WHD, which is most highly conserved in WAVEs, has not been reported.

Mechanism of WASP/N-WASP regulation

The activity of N-WASP on Arp2/3 complex is autoinhibited by an intramolecular interaction. This autoinhibition was first proposed based on the observation that the VCA region binds to the N-WASP fragment containing the GBD/CRIB motif (*12*). Later, a structural analysis was performed on the complex of the two fragments (*29*). Interestingly, the structure of the GBD/CRIB motif in the complex was quite different from that when it was bound to activated Cdc42, indicating that Cdc42-binding induces a structural change in the GBD/CRIB motif that releases the inhibition caused by the intramolecular interaction with the VCA region. This release is believed to be the basis of N-WASP activation.

Biochemical evidence of the autoinhibition of N-WASP came from an *in vitro* actin polymerization assay with Arp2/3 complex and full-length N-WASP. Rohatgi *et al.* (*21*) performed a kinetic analysis of actin polymerization and found that the isolated VCA fragment of N-WASP quite strongly stimulates actin polymerization in the presence of Arp2/3 complex. This result indicates that the VCA region has an Arp2/3 complex-activating function. In contrast, full-length N-WASP stimulated actin polymerization very weakly, reflecting the autoinhibition in the full-length protein. They then examined the effect of Cdc42 (GTP-form) and PIP2, both of which associate directly with N-WASP. In this case, even full-length N-WASP activated Arp2/3 complex at a level comparable to that by the VCA region alone. In addition, it was also reported that the endogenous WASP proteins purified from bovine thymus were activated by addition of activated Cdc42 and PIP2 (*14*).

In addition to Cdc42 and PIP2, several SH3 domain-containing proteins, including Grb2/Ash (*16*), Nck (*17*), and WISH (*18*), have been shown to activate N-WASP. WISH is a very potent activator of N-WASP, because addition of WISH or the SH3 domain fragment of WISH activates full-length N-WASP to a level comparable to that of the VCA region alone. These SH3 proteins bind directly to the proline-rich region of N-WASP, clearly indicating that the proline-rich region can receive signals from upstream regulators. In addition, a novel mechanism of WASP/N-WASP activation was proposed recently. WASP (*30*) and N-WASP (*31*) are phosphorylated by Src-family kinases on tyrosine residues located near the GBD/CRIB motif. This phosphorylation activates WASP/N-WASP, probably through release of the autoinhibition. Indeed, a phospho-mimicking mutant (Tyr to Glu mutant) was constitutively active in Arp2/3 complex-mediated actin polymerization. Taken together, these data suggest that WASP and N-WASP are the platforms where multiple signals for the generation of actin filaments are integrated (Fig. 2).

Functional and physical connections between Rac and WAVEs

In contrast to WASP and N-WASP, a functional connection between WAVEs and Rac has been suggested (*23*). Three pieces of experimental evidence supported this

possibility. First, endogenous WAVE1 accumulates at significant levels in areas where membrane ruffling occurs. Second, expression of a WAVE1 mutant lacking the V region selectively suppresses Rac-induced membrane ruffling. Third, when WAVE1 and activated Rac are co-expressed, they form a protein complex in cells. However, direct interaction between Rac and WAVEs was not observed, and thus the mechanism by which Rac regulates WAVEs remained unclear.

One biochemical link between Rac and WAVEs is IRSp53, which was originally identified as a substrate for the insulin receptor. IRSp53 contains an SH3 domain at its C-terminus, through which it associates specifically with WAVE2 (*27*). In addition, the N-terminal region of IRSp53 binds directly to activated Rac to form a Rac/IRSp53/WAVE2 complex, which presumably links the Rac signal to activation of the Arp2/3 complex. In contrast, several reports noted a specific interaction of IRSp53 with Cdc42 but not with Rac (*32*, *33*). In these cases, activated Cdc42 bound to the partial CRIB motif located in the central part of IRSp53. These contradictory results were then reconciled by the finding that an intramolecular interaction in full-length IRSp53 yields a structure that inhibits the interaction with Rac, but formation of the complex with WAVE2 releases the inhibitory interaction (*34*).

Recently, two reports appeared that described the functional connection between Rac and WAVE1. Both describe attempts to purify endogenous WAVE1 proteins by column chromatography or immunoprecipitation. Eden *et al*. (*35*) found that endogenous WAVE1 exists as a large protein complex with PIR121, Nap125, and HSPC300. They also reported that the complex containing WAVE1 had very weak ability to activate Arp2/3 complex, whereas recombinant WAVE1 protein, which was expressed with baculoviruses and then purified, was a strong activator of Arp2/3 complex. Interestingly, when activated Rac or the adapter protein Nck was added to the WAVE1-complex, PIR121 and Nap125 were released from the complex, and the remaining WAVE1 and HSPC300 potently activated Arp2/3 complex. Thus, endogenous WAVE1 appears to be kept inactive through interactions with other proteins. In contrast, Soderling *et al*. (*28*) identified a novel GTPase-activating protein specific for Rac in anti-WAVE1 immunoprecipitates and named it WRP. WRP contains an SH3 domain and associates directly with the proline-rich region of WAVE1. Ectopic expression analysis indicated that WRP functions as a negative regulator of Rac in cells.

These results clearly indicate that at least WAVE1 and WAVE2 have biochemical links to Rac. However, several important questions remain. The mechanism underlying inhibition of the WAVE1 activity and its release by Rac should be focus of future analyses. More detailed characterization of WAVEs will be needed to understand the physiological functions of WAVEs.

Functional analyses in multicellular organisms

Thus far, gene disruption analyses of the *WASP* (*36*), *N-WASP* (*37*, *38*), and *WAVE1* (*39*) genes have been done in mice. *WASP*–/– mice showed several phenotypes similar to characteristics of the human WAS patients, including defective T cell activation. A slight reduction in the number of platelets, though not as severe as that in human WAS patients, was also observed. *N-WASP*–/– was an embryonic lethal mutation. This result is reasonable, because N-WASP is expressed ubiquitously. Analyses of fibroblasts obtained from knockout embryos confirmed various N-WASP functions, including intracellular movement of pathogens. The importance of N-WASP in filopodium formation was also confirmed in one study (*37*). Generation of *WAVE1*–/– mice was reported very recently (*39*). Knockout mice showed sensorimotor retardation and defects in learning and memory, which reflect the restricted expression of *WAVE1* in brain. These studies on knockout mice have had significant impacts as clear demonstrations of the physiological functions of WASP, N-WASP, and WAVE1. However, most of the results were within the scope of expectation.

In contrast, analyses of *Drosophila* mutants of the WASP family genes yielded several unexpected findings regarding the importance of these molecules in multicellular organisms. In the *Drosophila* genome, there are only one *WASP*-homologous gene and one *WAVE/Scar*-homologous gene. Mutant flies lacking the functional *WASP* gene were viable, but abnormal differentiation of neurons, which was caused by the defect in asymmetric cell division and resulted in the generation of excess numbers of neurons, occurred (*40*). In addition, the authors reported a genetic link between the *WASP* gene and components of the Notch-signaling pathway, which has an established connection with neural differentiation, and concluded that WASP is a critical signal transducer of the Notch-signaling pathway. The mutant flies for the *WAVE/Scar* gene had a more severe phenotype than those for the *WASP* gene (*41*). The phenotype was caused by a generalized defect in the organization of the actin cytoskeleton during early development. Interestingly, this phenotype was very similar to those of mutants for the genes encoding subunits of the Arp2/3 complex (*42*), suggesting that the "main" activator of Arp2/3 complex during early development is WAVE/Scar and not WASP. In contrast, a mosaic analysis showed that WAVE/Scar is dispensable in neural cell fate decisions that require both WASP and Arp2/3 complex.

In conclusion, analyses of *Drosophila* mutants revealed several unexpected functions of WASP family members in a multicellular organism. The relevance of these findings in mammalian cells will be the focus of future studies.

Conclusion

In this minireview, we have summarized recent advances in research on WASP family proteins. As described at the end of each section, unresolved and interesting questions remain. The answers to these questions will undoubtedly contribute to our understanding of the general principles that govern regulation of cell morphology and motility. They may also have significant impacts on areas of research such as cell differentiation and development.

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