WAVE signalling: from biochemistry to biology

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Abstract

The small GTPases Rho, Rac and Cdc42 (cell-division cycle 42) function as molecular switches to modulate the actin cytoskeleton. They achieve this by modulating the activity of downstream cellular targets. One group of Rho GTPase effectors, WAVE (Wiskott–Aldrich syndrome protein verprolin homologous)-1, WAVE-2 and WAVE-3, function as scaffolds for actin-based signalling complexes. The present review highlights current knowledge regarding the biochemistry of the WAVE signalling complexes and their biological significance.

Introduction

Actin reorganization regulates key biological events, including cell migration, neurite extension, synapse remodelling and endocytosis. Restructuring of the actin cytoskeleton is achieved via the WASP (Wiskott–Aldrich syndrome protein) family of scaffolding proteins, which includes WASP, N-WASP (neural WASP), WAVE (WASP verprolin homologous)-1, WAVE-2 and WAVE-3. This family function as molecular platforms for the co-ordination of actin polymerization and branching catalysed by the Arp2/3 (actin-related protein 2/3) complex [1,2]. The Arp2/3 complex is composed of seven subunits, two of which are actin-related proteins. On its own, Arp2/3 inefficiently nucleates actin polymerization; however, actin branching is potently stimulated by the WASP family of proteins in response to signalling events downstream of the Rho family of GTPases. How the WASP family is regulated by Rho GTPases has recently received much attention, and two general mechanisms have emerged. WASP and N-WASP are activated by a direct interaction with the GTPase Cdc42 (cell-division cycle 42) and PIP_2 [PtdIns(4,5)P_2], which relieves an auto-inhibitory state to allow Arp2/3 activation [3–5]. In contrast, the GTPase Rac regulates WAVE-1, WAVE-2 and WAVE-3, albeit by a mechanism that is poorly understood. This review discusses the existing information on how WAVEs assemble signalling complexes, how these interacting proteins regulate their activity and some recent genetic evidence that points to the biological processes that the WAVE proteins regulate.

Key words: actin remodelling, A-kinase anchoring protein (AKAP), cytoskeletal organization, kinase anchoring, Wiskott–Aldrich syndrome protein verprolin homologous protein (WAVE protein).

Abbreviations used: Abi, Abl-interacting protein; Arp2/3, actin-related protein 2/3; Cdc42, cell-division cycle 42; E, embryonic day; HSPC300, heat-shock protein C300; GAP, GTPase-activating protein; Nap1, Nck-associated protein; PI3K, phosphoinositide 3-kinase; PIP_3, PtdIns(3,4,5)P_3; PKA, protein kinase A; RNAi, RNA interference; SH, Src homology; Sra-1, specifically Rac-associated protein 1; VCA, verprolin/cofilin/acidic; WASP, Wiskott–Aldrich syndrome protein; N-WASP, neural WASP; WAVE, WASP verprolin homologous; WHD, WAVE homology domain; WRP, WAVE-associated GAP.

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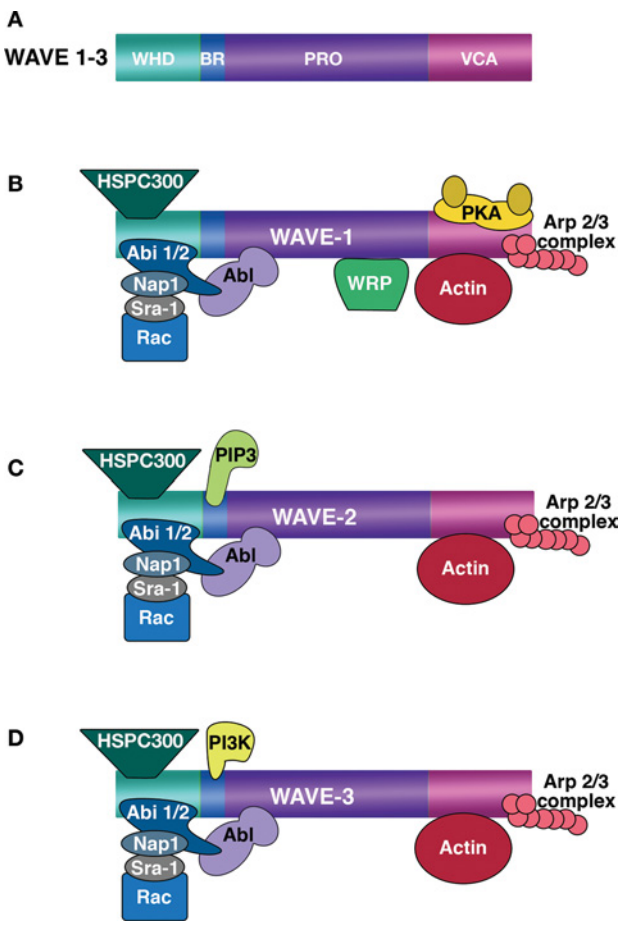
WAVE proteins assemble signalling complexes

The WAVE proteins are composed of multiple conserved domains, some of which are thought to stimulate the Arp2/3 complex [6–8]. These conserved domains include an N-terminal WHD (WAVE homology domain), a region of basic amino acids, a proline-rich domain and a C-terminal VCA (verprolin/cofilin/acidic) domain (Figure 1A). It is the VCA domain that binds both monomeric actin and the Arp2/3 complex. The presence of multiple domains, the finding that the WAVE proteins do not couple to Rac directly and the observation that recombinant WAVE is constitutively active all support the notion that WAVE proteins are regulated by interacting signalling factors.

Several studies now strongly support this hypothesis. Purification of endogenous WAVE-1 from brain extracts results in the co-purification of four tightly associated proteins: Sra-1 (specifically Rac-associated protein 1), Nap1 (Nck-associated protein), Abi2 (Abl-interacting protein 2) and HSPC300 (heat-shock protein C300) [9]. *In vitro* actin polymerization assays demonstrated that this complex alone failed to stimulate the Arp2/3 complex. In contrast, the addition of Rac to the complex potently stimulated Arp2/3, thus triggering actin polymerization. Activation was associated with the disassembly of Sra-1, Nap1 and Abi2, which subsequently released a WAVE-1–HSPC300 subcomplex. These observations led to a proposed model whereby WAVE is maintained in an inactive state by the co-ordinated binding of Sra-1, Nap1 and Abi2. Rac then releases this inhibition by disassembling the complex. Subsequent mapping studies defined the configuration of this complex (Figures 1B–1D) [10–12]. This work has led to the proposal of three links that are necessary for the activation of the WAVE-1 complex: (i) WAVE-1 directly binds to both HSPC300 and Abi2 via the N-terminal WHD domain, (ii) Abi2 recruits Nap1, which binds Sra-1, and (iii) Sra-1 binds activated Rac [13]. Thus Sra-1 provides the missing link between WAVE and Rac, thereby explaining how Rac GTPase activity regulates WAVE. This model is analogous to an elegant structural model that has been put forward to explain how WASP and N-WASP are modulated by their interaction with a related GTPase, Cdc42 [14].

Figure 1 | Architecture of the WAVE signalling complexes

WAVE proteins are composed of multiple protein interaction domains. (**A**) Diagram of the domains conserved in WAVE-1, -2 and -3. Each WAVE isoform interacts with both common and specific signalling partners. WHD, WAVE homology domain; BR, basic region; PRO, proline-rich domain. (**B**–**D**) Schematic diagram of the signalling complexes for the individual WAVE isoforms.



WAVE activation, however, seems to be a more sophisticated mechanism involving additional proteins.

Despite the attractiveness of the above model, it does not explain how WAVE-1 translocates to the leading edge of motile cells upon its activation. Thus this initial model for WAVE activation was called into question by studies that identified a parallel protein complex associated with WAVE-2 [15,16] (Figure 1C). In contrast with the initial postulate, these studies concluded that the Sra-1, Nap1 and Abi complex does not dynamically inhibit WAVE-2. Rather, WAVE-2 remains associated with its interacting partners in both the dormant and active states. In fact, cell-based assays demonstrated that, upon stimulation, an intact WAVE-2 complex migrates to the leading edge of the lamellipodia. Thus the central role for WAVE-2 in this alternative model is to co-ordinate subcellular targeting to the leading edge in response to activated Rac.

Although it is clear that all three WAVE isoforms assemble a core complex with Abi, HSPC300, Nap1 and Sra-1 [12], the discrepancy between these models is currently difficult to reconcile. An attractive possibility is that other associated factors or post-translational modifications alter the activity of the WAVEs and these may explain the apparent disparities in the experimental models.

For example, WAVE-1 also associates with a Rho GAP (GTPase-activating protein), WRP [WAVE-associated GAP; also called MeGAP (mental-disorder-associated GAP protein)/SrGAP3 (SLIT-ROBO Rho GAP 3)] [17] (Figure 1B). Rho GAPs function to inhibit Rho GTPases by enhancing their intrinsic GTPase activity and returning them to their inactive state. Biochemical and *in vivo* studies demonstrate that WRP is specific for inactivating Rac and that it binds directly to the proline-rich region of WAVE-1 via a C-terminal SH3 (Src homology 3) domain. Thus WRP probably functions in a negative-feedback loop that inactivates the Rac associated with WAVE-1.

WAVE family members also assemble multi-kinase complexes. For instance, WAVE-1 binds both the non-receptor tyrosine kinase, Abl, as well as the serine/threonine kinase PKA (protein kinase A) [18] (Figure 1C). The Abl SH3 domain associates with a proline-rich region in the central core of WAVE-1, whereas PKA binds WAVE-1 through an amphipathic helix located in the VCA domain. Interestingly, Abl also binds the Abi proteins, suggesting that there may be multiple contacts between the tyrosine kinase and members of the WAVE-1 signalling complex [19,20].

In addition to WAVE-1, Abl also associates with and regulates WAVE-2 [21] (Figure 1C). Abl phosphorylates WAVE-2 at a conserved site, Tyr^{150}, via a mechanism that is dependent on the presence of Abi1. This phosphorylation event may enhance WAVE-2's ability to activate the Arp2/3 complex. Future experiments will be necessary to determine whether similar results hold for WAVE-1 and WAVE-3, which also contain regions homologous with the Tyr^{150} site.

Kinase anchoring by WAVE proteins may be a common regulatory theme. This is suggested by the addition of the lipid kinase, PI3K (phosphoinositide 3-kinase), to the WAVE-3 complex. PI3K is composed of both a p110 catalytic subunit and a p85 regulatory subunit. It is well known that PI3K regulates cell migration and Rac activation by generating membrane gradients of PIP_3 [PtdIns(3,4,5)P_3] [22]. Yeast two-hybrid and co-immunoprecipitation analyses demonstrate that WAVE-3 associates with PI3K, while lipid-binding assays show that WAVE-2 interacts at nanomolar affinity with the product of PI3K activity, PIP_3 [23,24]. The interaction between WAVE-3 and PI3K occurs directly between the basic domain of WAVE-3 and the C-terminal SH2 domain of the p85 regulatory subunit of PI3K. Interestingly, PIP_3 binding maps to the basic region of WAVE-2, the mutation of which disrupts the localization of WAVE-2 to the lamellipodium. Furthermore, WAVE-2 co-localizes with membrane-bound p110 in the presence of dominant-negative Rac or the actin-disrupting agent latrunculin. These results suggest that PIP_3 is sufficient to localize WAVE-2 to

the leading edge of motile cells. Thus PI3K and PIP_3 are likely to be important for regulating the WAVE complex, either by localizing WAVE to the leading edge or by coupling WAVE to signals linked to Rac activation.

Mouse genetic analysis of WAVE

Although the biochemistry of the WAVE complex has been studied in detail, less is known about the biological consequences of WAVE signalling. In order to begin to address these questions, mouse genetics have been used to functionally disrupt both WAVE-1 and WAVE-2. The phenotype of the targeted disruption of WAVE-1 was characterized almost simultaneously by two groups. In the first study, WAVE-1 was disrupted by homologous recombination, deleting a portion of the fourth coding exon, including the splice donor site [25]. Immunohistochemistry and Western blot analysis demonstrated that WAVE-1 was neural-specific and that the knockout animals lacked expression of WAVE-1. WAVE-1-knockout mice were not embryonic lethal, but post-natal lethality of approx. 30% was noted, with the surviving mice displaying a reduced body size in the first 8 weeks. Since WAVE-1 expression is restricted to the central nervous system, extensive behavioural analysis was performed on adult littermates. Compared with wild-type littermates, WAVE-1-knockout mice exhibit defects in balance and co-ordination, reduced anxiety, and deficits in learning and memory. These data suggest that signalling through WAVE-1 to the actin cytoskeleton is important for normal neural function.

In support of these findings, related genetic studies of WAVE-1-associated proteins report overlapping phenotypes. For example, Abi2-knockout mice also display learning and memory deficits, which are consistent with a role for the WAVE–Abi interaction in the regulation of cognitive behaviour [26]. Additionally, WRP, which regulates WAVE-1-based signalling to the actin cytoskeleton, is also implicated in a form of human mental retardation, 3p− syndrome [27]. Patients suffering from this condition display severe cognitive disabilities, as well as deficits in motor-co-ordination function.

In a second WAVE-1-knockout study, expression was disrupted by a retroviral LacZ-gene trap within the intron immediately upstream of the first coding exon [28]. Western blot analysis demonstrated a lack of protein expression in null animals. β-Galactosidase staining in heterozygous mice suggested that WAVE-1 was ubiquitously expressed at E9 (embryonic day 9), but became neural-restricted by E18. WAVE-1-null animals exhibited a reduced body size after birth, as well as post-natal lethality. Lethality was observed to be 100% by post-natal day 26. Histological examination found that the hippocampus and cortex were properly formed, but the lateral ventricles were enlarged, and the corpus callosum appeared thin. Interestingly, knockout mice also exhibited hindlimb weakness as well as a resting tremor, which are consistent with the neurological defects in these animals. Currently, it is unknown why differences exist between the phenotypes in the two WAVE-1-knockout models. It is likely to be a reflection of either the differences in the strategies employed to disrupt WAVE-1 expression or differences in the genetic background of the mouse strains.

Two WAVE-2-knockout models have also been characterized, and both are embryonic lethal. In the first study, embryonic death was estimated to occur around E10.5. These embryos also displayed dilation of the pericardial cavities, small and incompletely looped hearts, and, in many cases, extensive haemorrhaging [29]. Immunohistochemical analysis of WAVE-2 at this stage demonstrated expression in endocardial cells, as well as in endothelial cells within the dorsal aorta. Defects in angiogenic remodelling and sprouting occurred in the null embryos, along with morphological defects in endothelial cells, as revealed by scanning electron microscopy. Importantly, cultured endothelial cells from WAVE-2-deficient embryos exhibit defects in capillary formation *in vitro* compared with cells from wild-type littermates. This is significant in that it probably explains the observed defects in angiogenesis.

Together, these data demonstrate that WAVE-2 plays an important role in the developing embryo, particularly within endothelial cells during angiogenesis. Similar results were also observed in a second line of WAVE-2-null mice, with some notable differences [30]. In this study, embryonic lethality was observed around E12.5. Knockout embryos also show evidence of haemorrhaging, were smaller overall and exhibited malformation of the head and caudal extremities. Specifically, both the forebrain and hindbrain ventricles lacked symmetry and were unusually small. Surprisingly, organogenesis of the heart was normal, which contrasts with the initial WAVE-2 study. Cultured mouse embryonic fibroblasts from knockout embryos were used to examine the functional role of WAVE-2 in Rac-dependent actin reorganization. Extracts from these fibroblasts are deficient for Rac, but not Cdc42-dependent actin polymerization, an effect that could be rescued by re-expression of WAVE-2. Furthermore, in response to application of PDGF (platelet-derived growth factor), knockout cells elicited a dramatic reduction in lamelliopodia, ruffling and cell migration in comparison with wild-type cells. These studies confirmed, at the cellular level, the essential role of WAVE-2 signalling downstream of growth factor stimulation.

Conclusions and future questions

Understanding how WAVE proteins assemble and organize signalling complexes to regulate the actin cytoskeleton remains a fascinating topic for future research. Perhaps the most important question regarding WAVE signalling is the molecular mechanism underlying Arp2/3 activation by the WAVE proteins. This question may be partially addressed by RNAi (RNA interference) studies. Interestingly, knockdown of WAVE-associated proteins leads to the concomitant reduction of WAVE levels [15,16,31]. This not only complicates the interpretation of RNAi results, but may also suggest that the half-life of WAVE proteins depends on the integrity of the WAVE complex. Finally, emerging evidence suggests

that Abi not only regulates WAVE, but also interacts with and modifies WASP and N-WASP [32,33]. Thus future studies of WAVE complex members will also have to take into account their contribution to regulating the actin cytoskeleton outside of WAVE signalling.

Although progress has been made using mouse knockout models to study WAVE function, many exciting questions remain. Behavioural evidence from WAVE-1-knockout mice indicates that it plays an important role in regulating neuronal function. What is the relationship between these behavioural defects and the regulation of actin at the cellular level? Even the larger role of Arp2/3-mediated signalling in neurons remains unclear [34]. Also, WAVE-2 obviously plays an important role during development, but its contribution at later stages remains unknown. Future studies using conditional alleles of WAVE-2 will probably be necessary to address these questions. The biological roles of WAVE-3 from knockout studies also await to be discovered. Finally, can genetic studies be paired with biochemical evidence to bring an integrated view to the biology of WAVE signalling? This is perhaps the greatest challenge that we face in the future.

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