JCB Mini-Review

Regulation of WASP/WAVE proteins: making a long story short

Guillaume Bompard[1] and Emmanuelle Caron[2]

[1]School of Biosciences, Division of Molecular Cell Biology, University of Birmingham, Birmingham B15 2TT, England, UK
[2]Centre for Molecular Microbiology and Infection and Department of Biological Sciences, Imperial College London, London SW7 2AZ, England, UK

Despite their homology, the regulation of WASP and WAVE, activators of Arp2/3-dependent actin polymerization, has always been thought to be different. Several recent studies have revealed new aspects of their regulation, highlighting its complexity and the crucial role of post-translational modifications. New data also suggest additional functions for WASP family proteins, pushing us to reconsider existing models.

Actin dynamics play an essential role in cell biology. They control cell morphology, cell shape change, cytokinesis, and cell motility. These processes are regulated by members of the Rho family of small GTPases and by the Arp2/3 complex. Rho proteins, specifically Rac and Cdc42, are activated in response to many extracellular signals and are thought to orchestrate the proper spatio-temporal activation of the Arp2/3 complex, the main regulator, described to date, of actin polymerization. Once activated, Arp2/3 initiates the growth of a branching network of actin filaments that generates a protruding force. Branching has been proposed to occur by either polymerization from the sides of preexisting actin filaments or from their barbed end (for reviews see Pollard and Borisy, 2003; Millard et al., 2004). Activation of Rac and Cdc42 is relayed to Arp2/3 via the Wiskott-Aldrich syndrome proteins (WASPs) (Pollard and Borisy, 2003). WASP, the prototype of this family, was initially identified as the product of the gene mutated in patients with Wiskott-Aldrich syndrome, an X chromosome–linked disease characterized by immune disorders and thrombocytopenia (Thrasher, 2002). Five WASP family members exist in mammals: WASP, N-WASP, and three WASP family verprolin homologous (WAVE) proteins. WAVE proteins are also known as suppressor of cAMP receptor (SCAR) proteins. A recent flurry of exciting papers highlights how structural differences within the WASP family appears to result in major differences in their modes of regulation, and hence their effects on Arp 2/3 activation. In this paper we will review this recent work.

WASP family proteins are ~500 amino acids long and have a common molecular organization (see Figs. 1 and 2). They are characterized by the presence of three independent domains at the carboxy terminus: the verprolin homology (V) (a.k.a., WASP homology 2/WH2 domain), central (C), and acidic (A) regions. Together, these domains form the so-called VCA module that is necessary and sufficient to activate Arp2/3-dependent actin polymerization in vitro. The V and A regions bind actin monomers and Arp2/3, respectively. The C region also binds Arp2/3 and induces crucial changes in its tertiary and quaternary structure, thereby regulating its ability to induce actin polymerization (Panchal et al., 2003). Another feature common to all WASP family proteins is the presence upstream of the VCA module of a basic region followed by a proline-rich stretch known to bind SH3 and WW domains. In spite of these similarities, the five mammalian WASP family members can be divided into two categories based on primary sequence homology and functional data: the WASP-like proteins, including hematopoietic WASP and ubiquitous N-WASP, and the WAVE proteins. WASP and N-WASP possess a central GTPase binding domain (GBD) and an NH_2-terminal WASP homology domain 1 (WH1), also called an Ena-VASP homology domain 1 (EVH1) (Callebaut et al., 1998). By contrast, WAVE proteins lack these domains but possess a highly specific WAVE homology domain (WHD), also known as a SCAR homology domain (SHD). This structural divergence translates to significant variation in the activity and regulation of WASP family proteins.

The primary difference in the activity of the WASP and the WAVE proteins can be explained by their specificity for small GTPases. WASP and N-WASP are direct, specific effectors of Cdc42 and are thought to mediate most of the cytoskeletal effects of active Cdc42. By contrast,

Address correspondence to Guillaume Bompard, School of Biosciences, Division of Molecular Cell Biology, University of Birmingham, Birmingham B15 2TT, England, UK. Tel.: 44-121-414-2508. Fax: 44-121-414-5925. email: G.Bompard@bham.ac.uk

Abbreviations used in this paper: Abi, Abl interactor; GBD, GTPase binding domain; Nap1, Nck-associated protein; PIR121, p53 inducible messenger RNA; SCAR, suppressor of cAMP receptor; WASP, WAVE, WASP family verprolin homologous; Wiskott-Aldrich syndrome protein; WHD, WASP homology domain.

The Journal of Cell Biology, Volume 166, Number 7, September 27, 2004 957–962
http://www.jcb.org/cgi/doi/10.1083/jcb.200403127

The Journal of Cell Biology

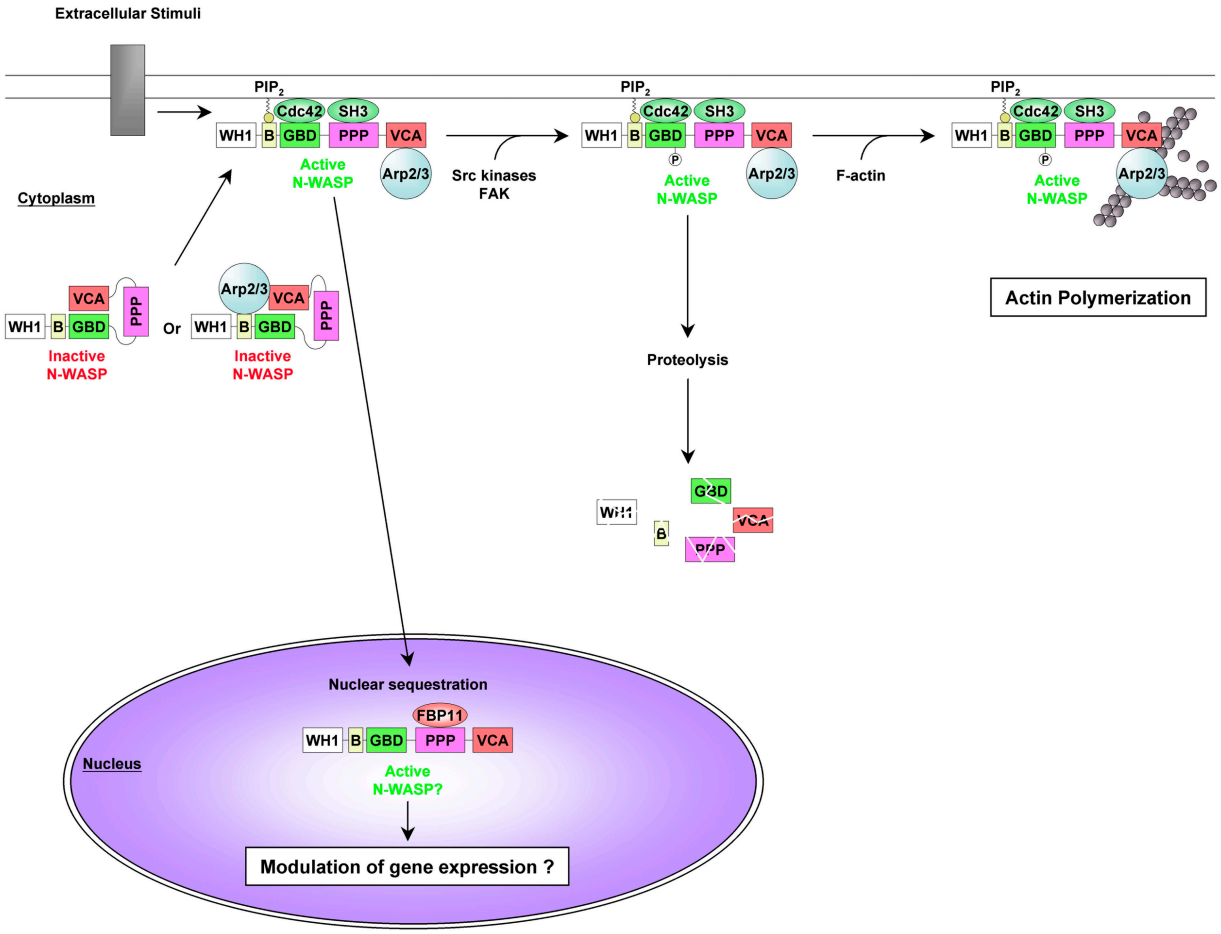

Figure 1. **Model for N-WASP regulation.** Under resting conditions, N-WASP is maintained in an inactive state in the cytosol. Autoinhibition is via interaction of the GBD and the C domain of the VCA module. Alternatively, direct interaction with the basic-rich region could inhibit the activity of a prebound Arp2/3 complex. In response to extracellular stimuli, N-WASP autoinhibition is relieved by the binding of GTP-bound Cdc42 and PtdIns(4,5)P_2 (PIP_2) or SH3 containing proteins (SH3). In the open conformation, N-WASP can be imported into the nucleus, where it may be retained by FBP11 and regulate gene expression. Alternatively, active N-WASP can stay in the cytosol. Phosphorylation by tyrosine kinases enhances the ability of N-WASP to activate the Arp2/3 complex in cooperation with F-actin, prevents its nuclear import, and may induce—in certain models—its degradation through the proteasome pathway.

WAVE proteins play a major role in Rac-induced actin dynamics. WASP and N-WASP bind directly to GTP-bound Cdc42, through their GBD, whereas WAVE proteins, which do not contain a GBD, bind to Rac indirectly (for reviews see Caron, 2003; Pollard and Borisy, 2003). Regulation of WASP/N-WASP and WAVE protein activity is also the result of structural variation. Under resting conditions, WASP/N-WASP activity is autoinhibited via intramolecular interaction between the GBD and the VCA modules, preventing binding and/or activation of the Arp2/3 complex. Alternatively, when in a closed conformation, direct binding of the basic-rich region to VCA bound Arp2/3 inhibits activation (Fig. 1, Prehoda et al., 2000). In either scenario, relief from inhibition involves the cooperative binding of active Cdc42 and phosphatidyl inositol 4,5-bisphosphate (PtdIns[4,5]P_2) to the GBD and the basic-rich region, respectively (Fig. 1; Higgs and Pollard, 2001; Pollard and Borisy, 2003). By striking contrast, WAVE proteins, which lack a GBD domain, are constitutively active in vitro and when overexpressed in cells (Machesky et al., 1999; Miki et al., 1998). How then is WAVE activity regulated, both in resting conditions and upon activation of Rac signaling?

The first protein thought to link WAVE2 specifically to Rac was IRSp53 (Miki et al., 2000). However, IRSp53 was found to have no major effect on WAVE2 activity in vitro (Miki et al., 2000) but rather to be involved in the Cdc42-induced, WAVE2-independent formation of filopodia (Krugmann et al., 2001; Suetsugu et al., 2003). Recently, WAVE1 purified from bovine brain extract was found to belong to a pentameric complex, suggesting new models for its regulation. The complex includes PIR121 (p53 inducible messenger RNA), Nap1 (Nck-associated protein), Abi (Abl interactor), and HSPC300 (Eden et al., 2002). Independent studies have confirmed that in vivo, WAVE1 and WAVE2 are engaged in a PIR121/Nap1/Abi/HSPC300 complex. Moreover, both complexes can be reconstituted in vitro (Gautreau et al., 2004; Innocenti et al., 2004; Steffen et al., 2004). PIR121 is probably the link between the pentameric complex and Rac, as it bears homology to p140Sra-1 (specifically Rac1-associated protein), a Rac effector (Kobayashi et al., 1998, Fig. 2). PIR121 is also likely to mediate the indi-

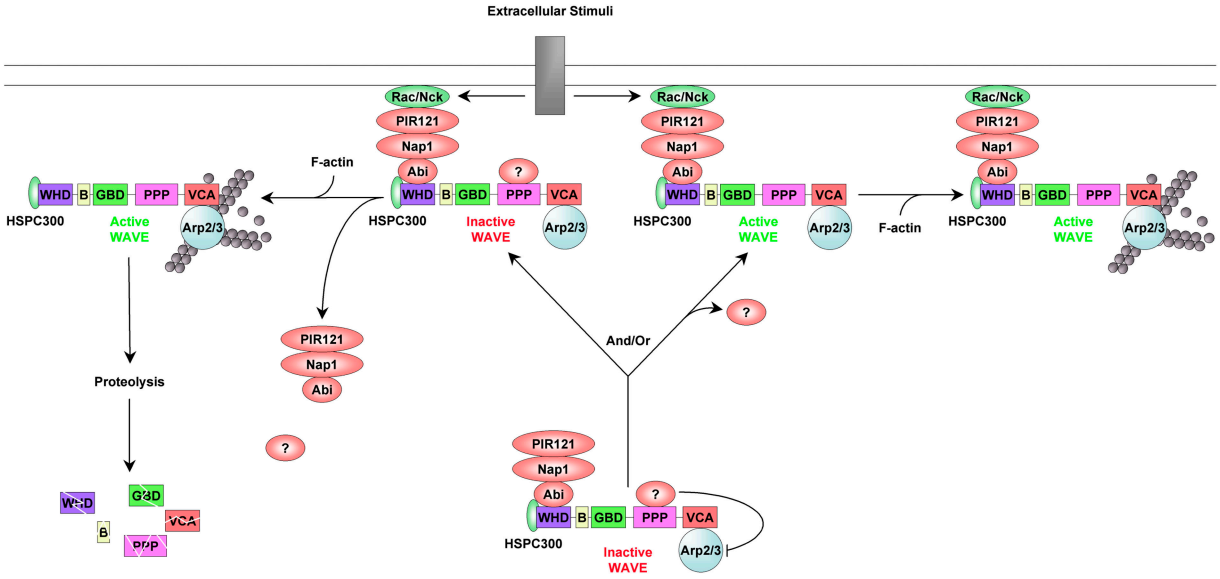

Figure 2. **Model for WAVE regulation.** In this model, WAVE constitutively interacts with the Arp2/3 complex. However, in resting conditions Arp2/3 is not activated, either because binding of WAVE to the PIR121-Nap1-Abi-HSPC300 complex prevents the interaction of WAVE-Arp2/3 with an essential coactivator (e.g., F-actin) or because it is mislocalized. The involvement of an inhibitory protein, similar to Sla1 and Bbc1 (indicated by ?, see text for details), has not been ruled out. Extracellular stimuli, through the activation or Rac or the mobilization of Nck, recruit the pentameric complex to the plasma membrane. WAVE activation requires its proper localization plus, in this model, the presence of F-actin and/or the release of the additional protein(s). Alternatively, a complex composed of WAVE, HSPC300, and Arp2/3 is released first, and then activation takes place in the presence of F-actin and the signal is terminated by the degradation of free WAVE proteins.

rect interaction between Nck and Nap1 (Kitamura et al., 1996), which itself directly interacts with Abi (Gautreau et al., 2004; Yamamoto et al., 2001). Finally, Abi links PIR121/Nap1 to WAVE proteins through binding to their WHD (Echarri et al., 2004; Gautreau et al., 2004; Innocenti et al., 2004). HSPC300 also interacts with Abi and with the WHD of WAVE (Fig. 2; Gautreau et al., 2004).

The incorporation of WAVE proteins into the PIR121/Nap1/Abi/HSPC300 complex has been proposed to maintain their stability (Fig. 2). In *Drosophila* cells, RNAi-mediated ablation of any member of the complex, except HSPC300, correlates with the degradation of WAVE via the proteasome pathway (Kunda et al., 2003). Similarly, p140Sra-1, Nap1, and Abi1 knock-down induce WAVE2 degradation in mammalian cells (Innocenti et al., 2004; Steffen et al., 2004). Furthermore, the WAVE1 level is reduced in mouse embryo fibroblasts null for Abi2 and heterozygous for Abi1 (Echarri et al., 2004). Knocking out PIR121 in *Dictyostelium discoideum* also induces WAVE degradation (Blagg et al., 2003). Surprisingly, however, WAVE2 is not degraded when reexpressed at physiological concentration in p140Sra-1 knock-down cells (Steffen et al., 2004). Although this may result from an aberrant turn-over of the tagged protein, validation of the degradation mechanism in more physiological conditions will have to await further studies.

It was originally reported that within the pentameric complex WAVE1 cannot stimulate Arp2/3 nucleation activity in vitro; actin polymerization only occurred in the presence of Rac or of the adaptor protein Nck, whose binding resulted in the dissociation of PIR121, Nap1, and Abi from a WAVE1/HSPC300 subcomplex (Fig. 2; Eden et al., 2002). However, new data strongly challenge this transinhibition model. Two studies independently reported that the WAVE-containing complexes remain stable in vivo, even after Rac activation (Innocenti et al., 2004; Steffen et al., 2004). Furthermore, the PIR121/Nap1/Abi complex did not inhibit the ability of WAVE1 or WAVE2 to activate the Arp2/3 complex in vitro. Abi1 binding to WAVE2 even increased its basal activity, whereas Rac had no measurable effect (Innocenti et al., 2004). The apparent discrepancies between these two possible mechanisms of activation, dissociation versus stable association, could be due to the different experimental set-ups used: the WAVE-associated complex was purified from tissue in one case (Eden et al., 2002) and reconstituted from recombinant proteins in the other (Innocenti et al., 2004). Potential post-translational modification of members of the complex, or additional molecules, could be crucial for the regulation of WAVE function and could be missed in the latter system (Fig. 2).

As mentioned above, lowering the expression of any member of the pentameric complex induces WAVE degradation. This was originally suggested by the fact that depletion of Abi, Nap1, and PIR121 homologues in *Drosophila* S2 cells by RNAi induced a phenotype similar to the loss of WAVE (Kunda et al., 2003; Rogers et al., 2003). Similarly, depletion of p140Sra-1, Nap1, or Abi1 in mammalian cells strongly inhibited Rac-induced lamellipodia and ruffle formation (Innocenti et al., 2004; Steffen et al., 2004). However, reexpression of WAVE by inhibiting proteasomes in Abi-depleted S2 cells, or by transfecting WAVE2 into p140Sra-1-deficient cells, was not associated with aberrant F-actin structures as usually observed in WAVE-overexpressing cells (Kunda et al., 2003; Steffen et al., 2004). There-

fore, the integrity of the pentameric complex appears to be required for the stimulus-induced, localized activation of the Arp2/3 complex by WAVE proteins. This notion of integrity is also supported by the fact that Abi1 knock-down not only induces the degradation of WAVE2 but also PIR121 and Nap1 (Innocenti et al., 2004).

Crucially, in cells depleted for one constituent of the complex, the remaining proteins (and particularly PIR121 in Nap1 or Abi1 depleted cells) are not recruited to the cell cortex upon Rac activation (Innocenti et al., 2004; Steffen et al., 2004). This strongly suggests that WAVE-containing complex is intrinsically active and that its recruitment is the major regulatory step in WAVE-induced actin polymerization in vivo. Surprisingly, despite a clear decrease in WAVE protein levels, uncontrolled actin polymerization has been associated with a PIR121 knock-out and Nap1 mutant cells, respectively in *Dictyostelium* and *Drosophila* (Hummel et al., 2000; Blagg et al., 2003). These contradictory results could be the result of cellular context, for example the nature or potency of the WAVE-activating stimuli or varying availabilities of coregulators, e.g., actin filaments, in the various systems. Certainly, much more work still needs to be done in order to understand fully the function and regulation of this complex.

Additional WAVE-binding partners that may well contribute to the regulation of WAVE activity in cells have been described. The basic-rich region of WAVE2 was recently shown to bind PtdIns $(3,4,5)P_3$ (Oikawa et al., 2004). Mutagenesis of this region or Wortmannin treatment abolished WAVE translocation to the leading edge and Rac-induced ruffling suggesting that this could be a physiological regulator. However, pharmacological inhibition of PtdIns $(3,4,5)P_3$ production should be interpreted with care since it may affect the recruitment of additional proteins involved in lamellipodia formation (e.g., Rac guanine nucleotide exchange factors [GEF] and cytoskeletal proteins). Furthermore, these experiments did not test the effect of mutagenesis or PtdIns $(3,4,5)P_3$ binding on the integrity or activity of the WAVE-containing complex. A WAVE-associated Rac-specific GTPase activating protein (GAP) that binds to the proline-rich region of WAVE1 has been identified and proposed to be a signal termination factor for Rac, and thus WAVE1, signaling (Soderling et al., 2002). The regulatory subunit (RII) of cAMP-dependent kinase (PKA) interacts with the V domain of WAVE1 in an isoform-specific manner (Westphal et al., 2000). As binding of G-actin and RII are mutually exclusive, this interaction could also inhibit WAVE1 activity locally. Lastly, it is interesting to note that Las17/Bee1, the budding yeast WASP homologue that, like WAVE proteins, lacks a GBD and the autoinhibition mechanism, is inhibited through binding of two SH3-domain containing proteins, Sla1 and Bbc1 (Rodal et al., 2003). Altogether, these data indicate that intermolecular interactions play a major, role in regulating the activity of WASP family proteins toward the Arp2/3 complex.

Phosphorylation, another post-translational modification, also regulates the activity of WASP-like proteins (for review see Caron 2003). WASP is constitutively phosphorylated by caseine kinase 2 at the junction of the C and A domains of the VCA module (Ser 483 and 484). Serine phosphorylation strongly increases the affinity of the VCA module for the Arp2/3 complex in vitro; it is also necessary for the Cdc42-dependent induction of actin polymerization by full-length WASP (Cory et al., 2003). Interestingly, these serine residues are partially conserved in WAVE proteins and, indeed, WAVE1 is constitutively phosphorylated on serine/threonine residues in mouse fibroblasts (Miki et al., 1999). Moreover, PDGF treatment induces WAVE1 phosphorylation on uncharacterized serine/threonine residue(s) in a MAP kinase–dependent manner. The impact of such an inducible phosphorylation on WAVE1 activity is unknown, although it inhibits binding of the SH3 domain of Grb2, suggesting that the phosphorylated residue(s) may lie within the proline-rich region of WAVE1 and therefore outside the VCA module (Miki et al., 1999). WASP and N-WASP (but not WAVE proteins so far) are also phosphorylated on a tyrosine residue (Y291 and Y256, respectively) located within the GBD region in response to a large number of different extracellular signals (Fig. 1). Tyrosine phosphorylation requires that relief of autoinhibition (e.g., through Cdc42 binding) has taken place and it is predicted to stabilize the open conformation of WASP and N-WASP, significantly enhancing their activity toward the Arp2/3 complex (Caron, 2003). Indeed, when overexpressed, a Y256E N-WASP mutant is able to induce microspikes in COS-7 cells and neurites in primary neurons (Suetsugu et al., 2002). Additionally, phosphorylation on Y256/Y291, by Src, Btk, or focal adhesion kinase (Wu et al., 2003), provides a docking site for SH2 domain–containing proteins. This binding is sufficient for WASP-induced actin polymerization in vitro (Torres and Rosen, 2003). Several recent reports have suggested additional roles for this post-translational modification in the regulation of N-WASP stability and subcellular distribution (Fig. 1). In neuronal cells, pharmacological inhibitors of the proteasome induce the accumulation of Y256-phosphorylated N-WASP. Parallel experiments show the Src kinase-dependent ubiquitination of N-WASP, suggesting that tyrosine-phosphorylated N-WASP is degraded by the proteasome pathway. However, this seemingly simple regulation is complicated by the observation that phosphorylated, ubiquitinated N-WASP is only degraded slowly in cells growing neurites (Suetsugu et al., 2002). Additional experiments should clarify the relationship between tyrosine phosphorylation, ubiquitination and degradation of WASP proteins. Phosphorylation of Y256 has also been claimed to regulate exclusion of N-WASP from the nucleus (Suetsugu and Takenawa, 2003). N-WASP is normally found both in the cytosol and the nucleus, whereas the Y256E mutant is essentially cytosolic (Suetsugu et al., 2002). Why cytosolic mutants would be as active as physiologically activated, membrane-localized N-WASP is puzzling. As phosphorylation potentiates the activity of open WASP/N-WASP molecules (Cory et al., 2002; Suetsugu et al., 2002), it is possible that only a small, undetectable fraction of the Y256E mutants reaches the plasma membrane in resting conditions, whereas the majority remains as an inactive, cytosolic pool capable of mobilization upon stimulation. Nuclear localization and export sequences (NLSs and NESs, respectively) have been identified immediately upstream of N-WASP Y256 and both have been shown to be functional through mutagenesis ex-

periments (Suetsugu and Takenawa, 2003). NESs are conserved in WASP, although their role has not been studied yet. As expected from structural studies, exposure of the NLS/NES sequences and localization of N-WASP to the nucleus requires relief from autoinhibition. This exposes a putative importin α NPI-1 binding site (Wu et al., 2003). Phosphorylation of tyrosine 256 would weaken the interaction between open N-WASP and NPI-1 (Wu et al., 2003); it would also expose the NES (Suetsugu and Takenawa, 2003), preventing N-WASP accumulation in the nucleus.

What could be the role of N-WASP in the nucleus? Nuclear sequestration of N-WASP would clearly hinder its activity toward the actin cytoskeleton. However actin, actin-binding proteins, and Arp-related proteins have been found in the nucleus and proposed to regulate chromatin remodelling and RNA processing (Shumaker et al., 2003). It is thus conceivable that nuclear WASP proteins could affect gene expression by regulating the activity of these proteins. In line with the latter, N-WASP has recently been shown to interact with FBP11, a nuclear formin-binding protein involved in spliceosome function (Mizutani et al., 2004). Nuclear N-WASP has also been proposed to regulate gene expression through interaction with the transcription factor HSTF1, but the underlying mechanisms remain unclear (Suetsugu and Takenawa, 2003).

Over the past few years, four major, partly overlapping, levels of regulation of the activity of WASP family proteins have been uncovered: interaction with binding partners; stability/degradation; phosphorylation; and subcellular distribution. We now have a better understanding of how these regulatory mechanisms are integrated to control the localized activation of Arp2/3. Importantly, major progress has been made in the understanding of WAVE regulation. WAVE belongs to a conserved, multiprotein complex that needs to be recruited to the cell cortex to mediate Rac-induced, Arp2/3-dependent actin polymerization. The debate currently focuses on whether the WAVE-containing complex can intrinsically activate Arp2/3 in vivo. The pentameric complex clearly plays a crucial role in the activation of WAVE proteins in response to stimuli. However, this same complex could inhibit WAVE activity in resting conditions, by sequestering WAVE proteins from coactivators (e.g., F-actin). The exact function of individual WAVE isoforms remains another unresolved issue. Isoform-specific differences must exist, as WAVE1 and WAVE2 are involved in dorsal and peripheral ruffle formation, respectively (Suetsugu et al., 2003; Yamazaki et al., 2003; Yan et al., 2003). Future work on the mechanisms controlling the localization and composition of the WAVE-associated complexes should clarify these differences. In addition, investigating the role played by each member of the complex on the activity of WAVE proteins should help clarify isoform-specific differences and may reveal exciting new aspects of WAVE regulation. Finally, study of the function and regulation of WAVE3 could also help establish the common regulatory features of the three WAVE isoforms.

In parallel, additional functions have been proposed for WASP/WAVE proteins, which may or may not prove to be related to Arp2/3 and actin polymerization. Both N-WASP, WAVE, and Abi-1 can accumulate in the nucleus in certain conditions, possibly to regulate nucleocytoskeleton dynamics and thus gene expression (Echarri et al., 2004; Suetsugu and Takenawa, 2003; Westphal et al., 2000). Interestingly, kinases involved in the tyrosine phosphorylation of N-WASP—and thus in its retention in the cytosol—are mostly proto-oncogenes. Prevention of nuclear accumulation of N-WASP could potentially be important for cell transformation induced by these kinases. Nonetheless, understanding both the mechanisms underlying the shuttling of WASP/WAVE proteins between nucleus and cytosplasm and their nuclear function will be a major task.

Despite many differences regarding the regulation of WASP and WAVE proteins, both categories of proteins effect the localized activation of the Arp2/3 complex by Cdc42 and Rac, respectively. Nonetheless, the number of proteins that WASP/N-WASP and WAVE interact with suggests multiple levels of regulation. The new data regarding the regulation of WASP family proteins, the involvement of post-translational modifications, and the possible existence of Arp2/3-independent functions for these proteins not only challenge many of our thoughts, they also open up new fields of challenging research.

We wish to thank the authors who kindly accepted to disclose unpublished results and Laura M. Machesky for fruitful discussions and critical reading of the manuscript.

G. Bompard is supported by a Fondation pour la Recherche Médicale (FRM) post-doctoral fellowship. Research in the Caron laboratory is supported by grants from the Wellcome Trust and the BBSRC.

Submitted: 22 March 2004
Accepted: 10 August 2004

References

Blagg, S.L., M. Stewart, C. Sambles, and R.H. Insall. 2003. PIR121 regulates pseudopod dynamics and SCAR activity in Dictyostelium. *Curr. Biol.* 13: 1480–1487.

Callebaut, I., P. Cossart, and P. Dehoux. 1998. EVH1/WH1 domains of VASP and WASP proteins belong to a large family including Ran-binding domains of the RanBP1 family. *FEBS Lett.* 441:181–185.

Caron, E. 2003. Regulation by phosphorylation. Yet another twist in the WASP story. *Dev. Cell.* 4:772–773.

Cory, G.O., R. Cramer, L. Blanchoin, and A.J. Ridley. 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.

Cory, G.O., R. Garg, R. Cramer, and A.J. Ridley. 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.

Echarri, A., M.J. Lai, M.R. Robinson, and A.M. Pendergast. 2004. Abl interactor 1 (Abi-1) wave-binding and SNARE domains regulate its nucleocytoplasmic shuttling, lamellipodium localization, and Wave-1 levels. *Mol. Cell. Biol.* 24: 4979–4993.

Eden, S., R. Rohatgi, A.V. Podtelejnikov, M. Mann, and M.W. Kirschner. 2002. Mechanism of regulation of WAVE1-induced actin nucleation by Rac1 and Nck. *Nature.* 418:790–793.

Gautreau, A., H.Y. Ho, J. Li, H. Steen, S.P. Gygi, and M.W. Kirschner. 2004. Purification and architecture of the ubiquitous Wave complex. *Proc. Natl. Acad. Sci. USA.* 101:4379–4383.

Higgs, H.N., and T.D. Pollard. 2001. Regulation of actin filament network formation through ARP2/3 complex: activation by a diverse array of proteins. *Annu. Rev. Biochem.* 70:649–676.

Hummel, T., K. Leifker, and C. Klambt. 2000. The *Drosophila* HEM-2/NAP1 homolog KETTE controls axonal pathfinding and cytoskeletal organization. *Genes Dev.* 14:863–873.

Innocenti, M., A. Zucconi, A. Disanza, E. Frittoli, L.B. Areces, A. Steffen, T.E.

Stradal, P.P. Fiore, M.F. Carlier, and G. Scita. 2004. Abi1 is essential for the formation and activation of a WAVE2 signalling complex. *Nat. Cell Biol.* 6:319–327.

Kitamura, T., Y. Kitamura, K. Yonezawa, N.F. Totty, I. Gout, K. Hara, M.D. Waterfield, M. Sakaue, W. Ogawa, and M. Kasuga. 1996. Molecular cloning of p125Nap1, a protein that associates with an SH3 domain of Nck. *Biochem. Biophys. Res. Commun.* 219:509–514.

Kobayashi, K., S. Kuroda, M. Fukata, T. Nakamura, T. Nagase, N. Nomura, Y. Matsuura, N. Yoshida-Kubomura, A. Iwamatsu, and K. Kaibuchi. 1998. p140Sra-1 (specifically Rac1-associated protein) is a novel specific target for Rac1 small GTPase. *J. Biol. Chem.* 273:291–295.

Krugmann, S., I. Jordens, K. Gevaert, M. Driessens, J. Vandekerckhove, and A. Hall. 2001. Cdc42 induces filopodia by promoting the formation of an IRSp53:Mena complex. *Curr. Biol.* 11:1645–1655.

Kunda, P., G. Craig, V. Dominguez, and B. Baum. 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.

Machesky, L.M., R.D. Mullins, H.N. Higgs, D.A. Kaiser, L. Blanchoin, R.C. May, M.E. Hall, and T.D. Pollard. 1999. Scar, a WASp-related protein, activates nucleation of actin filaments by the Arp2/3 complex. *Proc. Natl. Acad. Sci. USA.* 96:3739–3744.

Miki, H., M. Fukuda, E. Nishida, and T. Takenawa. 1999. Phosphorylation of WAVE downstream of mitogen-activated protein kinase signaling. *J. Biol. Chem.* 274:27605–27609.

Miki, H., S. Suetsugu, and T. Takenawa. 1998. WAVE, a novel WASP-family protein involved in actin reorganization induced by Rac. *EMBO J.* 17:6932–6941.

Miki, H., H. Yamaguchi, S. Suetsugu, and T. Takenawa. 2000. IRSp53 is an essential intermediate between Rac and WAVE in the regulation of membrane ruffling. *Nature.* 408:732–735.

Millard, T.H., S.J. Sharp, and L.M. Machesky. 2004. Signalling to actin assembly via the WASP-family proteins and the Arp2/3 complex. *Biochem. J.* 380:1–17.

Mizutani, K., S. Suetsugu, and T. Takenawa. 2004. FBP11 regulates nuclear localization of N-WASP and inhibits N-WASP-dependent microspike formation. *Biochem. Biophys. Res. Commun.* 313:468–474.

Oikawa, T., H. Yamaguchi, T. Itoh, M. Kato, T. Ijuin, D. Yamazaki, S. Suetsugu, and T. Takenawa. 2004. PtdIns(3,4,5)P(3) binding is necessary for WAVE2-induced formation of lamellipodia. *Nat Cell Biol*. In press.

Panchal, S.C., D.A. Kaiser, E. Torres, T.D. Pollard, and M.K. Rosen. 2003. A conserved amphipathic helix in WASP/Scar proteins is essential for activation of Arp2/3 complex. *Nat. Struct. Biol.* 10:591–598.

Pollard, T.D., and G.G. Borisy. 2003. Cellular motility driven by assembly and disassembly of actin filaments. *Cell.* 112:453–465.

Prehoda, K.E., J.A. Scott, R.D. Mullins, and W.A. Lim. 2000. Integration of multiple signals through cooperative regulation of the N-WASP-Arp2/3 complex. *Science.* 290:801–806.

Rodal, A.A., A.L. Manning, B.L. Goode, and D.G. Drubin. 2003. Negative regulation of yeast WASp by two SH3 domain-containing proteins. *Curr. Biol.* 13:1000–1008.

Rogers, S.L., U. Wiedemann, N. Stuurman, and R.D. Vale. 2003. Molecular requirements for actin-based lamella formation in *Drosophila* S2 cells. *J. Cell Biol.* 162:1079–1088.

Shumaker, D.K., E.R. Kuczmarski, and R.D. Goldman. 2003. The nucleoskeleton: lamins and actin are major players in essential nuclear functions. *Curr. Opin. Cell Biol.* 15:358–366.

Soderling, S.H., K.L. Binns, G.A. Wayman, S.M. Davee, S.H. Ong, T. Pawson, and J.D. Scott. 2002. The WRP component of the WAVE-1 complex attenuates Rac-mediated signalling. *Nat. Cell Biol.* 4:970–975.

Steffen, A., K. Rottner, J. Ehinger, M. Innocenti, G. Scita, J. Wehland, and T.E. Stradal. 2004. Sra-1 and Nap1 link Rac to actin assembly driving lamellipodia formation. *EMBO J.* 23:749–759.

Suetsugu, S., M. Hattori, H. Miki, T. Tezuka, T. Yamamoto, K. Mikoshiba, and T. Takenawa. 2002. Sustained activation of N-WASP through phosphorylation is essential for neurite extension. *Dev. Cell.* 3:645–658.

Suetsugu, S., and T. Takenawa. 2003. Translocation of N-WASP by nuclear localization and export signals into the nucleus modulates expression of HSP90. *J. Biol. Chem.* 278:42515–42523.

Suetsugu, S., D. Yamazaki, S. Kurisu, and T. Takenawa. 2003. Differential roles of WAVE1 and WAVE2 in dorsal and peripheral ruffle formation for fibroblast cell migration. *Dev. Cell.* 5:595–609.

Thrasher, A.J. 2002. WASp in immune-system organization and function. *Nat. Rev. Immunol.* 2:635–646.

Torres, E., and M.K. Rosen. 2003. Contingent phosphorylation/dephosphorylation provides a mechanism of molecular memory in WASP. *Mol. Cell.* 11:1215–1227.

Westphal, R.S., S.H. Soderling, N.M. Alto, L.K. Langeberg, and J.D. Scott. 2000. Scar/WAVE-1, a Wiskott-Aldrich syndrome protein, assembles an actin-associated multi-kinase scaffold. *EMBO J.* 19:4589–4600.

Wu, X., S. Suetsugu, L.A. Cooper, T. Takenawa, and J.L. Guan. 2003. Focal adhesion kinase regulation of N-WASP subcellular localization and function. *J. Biol. Chem*. 279:9565–9576.

Yamamoto, A., T. Suzuki, and Y. Sakaki. 2001. Isolation of hNap1BP which interacts with human Nap1 (NCKAP1) whose expression is down-regulated in Alzheimer's disease. *Gene.* 271:159–169.

Yamazaki, D., S. Suetsugu, H. Miki, Y. Kataoka, S. Nishikawa, T. Fujiwara, N. Yoshida, and T. Takenawa. 2003. WAVE2 is required for directed cell migration and cardiovascular development. *Nature.* 424:452–456.

Yan, C., N. Martinez-Quiles, S. Eden, T. Shibata, F. Takeshima, R. Shinkura, Y. Fujiwara, R. Bronson, S.B. Snapper, M.W. Kirschner, et al. 2003. WAVE2 deficiency reveals distinct roles in embryogenesis and Rac-mediated actin-based motility. *EMBO J.* 22:3602–3612.