How WASP Regulates Actin Polymerization

Sally H. Zigmond

Biology Department, University of Pennsylvania, Philadelphia, Pennsylvania 19104-6018

Protrusion of lamellipodia and filopodia from the cell surface requires that actin polymerize locally. Actin polymerization is initiated by numerous agonists, including growth factors, chemoattractants, extracellular matrix, and phagocytic particles. The signaling pathways from the corresponding receptors converge on Rho family GTPases, especially Rac and Cdc42, which induce actin polymerization through a family of proteins called WASP (Wiskott-Aldrich Syndrome protein) (Higgs and Pollard 1999). In mammals, the family includes WASP (specific to hematopoietic cells), N-WASP (neural WASP, which is actually ubiquitous), and at least four forms of WAVE (WASP verprolin homologous protein). The conserved COOH terminus of these proteins stimulates the Arp2/3 complex to nucleate actin filaments, which then elongate at their free barbed ends (Machesky et al., 1999).

Two papers in this issue (Higgs and Pollard, 2000; Rohatgi et al., 2000) advance our knowledge of how WASP proteins regulate actin. First, although it was previously known that recombinant N-WASP can be stimulated by Cdc42 to activate nucleation, recombinant WASP, however, is constitutively active and thus is not regulated by a Rho-GTPase (Yarar et al., 1999). Now Higgs and Pollard (2000) have isolated native WASP from thymus and shown that it is indeed inactive until stimulated. Second, the known binding of N-WASP’s COOH terminus by its NH₂ terminus has now been shown to inhibit the ability of the COOH terminus to activate actin nucleation; this illuminates the molecular basis of this regulation (Higgs and Pollard, 2000; Rohatgi et al., 2000).

Knowing that WASP stimulates actin polymerization, a key question is: what regulates WASPs? Recent results suggest that WASPs, like many proteins, are self-regulating, i.e., they contain both effector and regulatory domains (Fig. 1). The effector is the COOH-terminal VCA (verprolin homology, cofilin homology, acidic) domain, which is sufficient to activate nucleation (Machesky et al., 1999). A likely regulator is the NH₂ terminus GTase binding domain (GBD; Miki et al., 1998). The NH₂ terminus also binds PIP₂ and WASP interacting protein (WIP; Miki et al., 1996; Ramesh et al., 1997). Between the GBD and VCA lies a proline-rich domain (PRD) that binds profilin as well as several proteins containing src homology 3 (SH3) domains (Brunnell et al., 1996; Finan et al., 1996). Any of these factors binding to a WASP might enhance or inhibit its activity.

WASP’s NH₂ Terminus Binds its COOH Terminus to Inhibit Nucleation

To investigate which region of the NH₂ terminus is required for inhibition, different domains were expressed and examined for their ability to bind and inhibit the activity of the VCA fragment. NH₂-terminal fragments that include the GBD bind VCA and inhibit its activity; furthermore Cdc42 relieves this inhibition (Higgs and Pollard 2000; Rohatgi et al., 2000). At least for N-WASP, binding to VCA is decreased when the NH₂-terminal fragment lacks the Ena Vasp homology 1/WASP homology 1 (EVH1/WH1) domain. Indeed, N-WASP constructs lacking the EVH1 domain are partially active, showing increased basal activity in a purified system, and also when expressed in cells (Moreau et al., 2000; Rohatgi et al., 2000).

By a similar strategy it was shown that both the C and A components of VCA bind the WASP NH₂ terminus (Kim et al., 2000; Rohatgi et al., 2000). C and A also bind the Arp2/3 complex; therefore, binding of the NH₂ terminus to VCA explains, at least partly, the inhibition of nucleation (Rohatgi et al., 2000). On the other hand, VCA binding of monomeric actin, assayed by Western blots, is not blocked by NH₂-terminal fragments; Western blots also suggest that intact N-WASP binds monomeric actin (Rohatgi et al., 2000). However, this disagrees with evidence that VCA sequesters monomeric actin better than intact N-WASP (Miki et al., 1998; Egile et al., 1999). Thus, the NH₂ terminus probably inhibits VCA’s interactions, with Arp2/3 and with actin, both essential for nucleation.

WASP and N-WASP are both activated optimally by the combination of PIP₂ and Cdc42; yet their responses differ to either alone. N-WASP is partially activated by either PIP₂ or Cdc42; WASP is activated by PIP₂, but not Cdc42 (Higgs and Pollard, 2000). With both WASP and N-WASP, Cdc42 releases binding of GBD containing fragments to VCA; however, again the response to PIP₂ differs. A fragment of N-WASP’s NH₂ terminus including the basic region adjacent to GBD (but not the EVH1 do-
Figure 1. Domain structure of WASP and N-WASP with sites of interaction between COOH- and NH2-termini, and binding by other factors. The NH2 terminus of WASP and N-WASP contain an EVH1/WH1 domain that binds the proline-rich protein, WIP (Ramesh et al., 1997). The NHQ terminus of N-WASP also binds PIP2, F-actin, and, through its IQ domain, calmodulin (Miki et al., 1996; Egile et al., 1999). The GTPase-binding domain (GBD) includes a Cdc42/Rac interactive binding (CRIB) motif and surrounding sequences. The GBD preferentially binds Cdc42 over Rac and GTP-Cdc42 over GDF-Cdc42. In N-WASP, the basic sequence (B) binds PIP2; (Rohatgi et al., 2000). The PRD binds profilin, as well as several SH3-containing proteins, including: adaptors Nck and Grb2, tyrosine kinases, PLCγ1, and syndapin I (Brunnell et al., 1996; Finan et al., 1996; Qualmann et al., 1999). The VCA/WA (WASP homology II and acidic region) domain is the minimal fragment able to activate nucleation by the Arp2/3 complex (Machesky et al., 1999). The V motif binds monomeric actin (G-actin), whereas the CA motif binds the Arp2/3 complex (Higgs and Pollard, 1999; Machesky et al., 1999).

main shown to bind PIP2; Miki et al., 1996), responds to PIP2 by decreased binding to VCA (Rohatgi et al., 2000). However, WASP’s comparable NH2-terminal fragment containing the basic domain, but not the EVH1 domain, does not respond to PIP2 (Higgs and Pollard, 2000). Thus, WASP’s basic domain is probably insufficient for the response to PIP2, but it remains to test an NH2-terminal fragment containing the EVH1 domain.

As yet, it is still risky to assign these different responses to PIP2 to differences between WASP and N-WASP because many conditions differed between the studies. For example, the constructs used were not identical, and for N-WASP (but not WASP), the constructs used were fusion proteins; the composition of the PIP2 liposomes and the molar ratio of PIP2 to NH2-terminal fragment also differed. Furthermore, the role of PIP2 is complicated in cell extracts where PIP2-evoked actin polymerization requires Cdc42 (Chen et al., 2000). Possibly in extracts, PIP2 acts upstream of Cdc42 by activating an exchange factor for Cdc42 or acts in parallel with Cdc42 to activate N-WASP. This will require further study.

Other Activators

In addition to the Rho GTPases, WASPs interact with proteins from several other signaling pathways (Fig. 1). Thus, WASP is poised to integrate information from multiple pathways. Such integration likely serves T cell differentiation and platelet half-life, both of which are disrupted in patients with Wiskott-Aldrich Syndrome. Recent studies suggest that WASP and N-WASP also integrate signals for actin polymerization. Thus, both Grb2 and profilin, which bind the PRD, enhance nucleation (Carlier et al., 2000; Yang et al., 2000). This suggests that the PRD also regulates nucleation. It will be interesting to see if other proteins binding this region also affect nucleation. WASPs undergo phosphorylation that may also contribute to activation. Finally, some proteins that bind WASP serve to localize it in the cell. For example, in Vaccinia infection, Nck and WIP bring N-WASP to the virus (Moreau et al., 2000), and for the EGF receptor, Grb2 brings N-WASP (She et al., 1997).

Structure of Native WASPs

The PRD in native WASP may form a hinge, folding the molecule back on itself to allow intramolecular binding between the NH2 and COOH termini. Alternatively, the PRD may extend the molecule, to allow intermolecular binding between the NH2 and COOH termini of two WASP molecules. Results differ between labs: recombinant, His-tagged N-WASP and isolated WASP behave as a dimer or a multimer (Carlier et al., 2000; Higgs and Pollard, 2000); whereas recombinant, untagged N-WASP behaves as a monomer (Rohatgi et al., 2000). WASP family proteins are notoriously sticky, probably because they can oligomerize between the EVH1 and the PRDs, as well as between GBD and VCA domains. Preparations of recombinant N-WASP vary in the extent to which they are inactive without Cdc42 and PIP2, and it has been impossible (so far) to obtain recombinant WASP in an inactive form. We need to better understand the native (inactive) structure: perhaps it is stabilized by covalent modification or cofactors.

Role of Clustering

A filopodium is essentially a point of protruding membrane, i.e., it is one-dimensional. Therefore, when Cdc42 induces a filopodium via N-WASP (Miki et al., 1998), actin polymerization must be activated at a point. Somehow the cell must regulate not only the level of actin nucleation, but also its spatial distribution. This could occur by clustering WASP. Such clustering in vivo apparently enhances actin polymerization (Castellano et al., 1999). Furthermore, clusters due to overexpression of WASP/N-WASP colocalize with polymerized actin (Kato et al., 1999). Clustering may also contribute to activation in vitro. Nucleation induced by WASP is better with GST-VCA (glutathione S-transferase fused to VCA) than with plain VCA, and it is better with prenylated Cdc42 than with nonprenylated Cdc42 (Higgs and Pollard, 2000). Because GST dimerizes, it can dimerize VCA, and prenylation can cause clumping of Cdc42. Monomeric VCA binds Arp2/3, and nonprenylated Cdc42 binds WASP; therefore, clustering may increase nucleation. Finally, PIP2, which also activates nucleation, is essentially always present as a multimer and its activity likely requires this. Perhaps enhanced
nucleation by clusters of activated WASP provide the spatial regulation of filopodial protrusion.

Actin polymerization is essential for many cellular functions. Since WASP family members are key intermediates in signaling pathways leading to polymerization, the increased understanding of how they are regulated provided by the two papers in this issue (Higgs and Pollard, 2000; Rohatgi et al., 2000) represent an important advance.

I thank Drs. J. Lackie, M. Pring, M. Rosen, P. Sterling, and C. Yang for reading a draft.

S.H. Zigmond is supported by the National Institutes of Health grant AI-19883.

Submitted: 25 August 2000
Revised: 25 August 2000
Accepted: 25 August 2000

References

Brunell, S.C.P., A. Henry, R. Kolluri, T. Kirchhausen, R.J. Rickles, and L.J. Berg. 1996. Identification of Itk/Tsk src homology 3 domain ligands. J. Biol. Chem. 271:25646–25656.

Carlier, M-F., P. Nicoche, I. Broutin-L’Hermite, R. Boujemaa, C. Le Caimche, C. Egile, C. Garbay, A. Ducruix, P. Sansonetti, and D. Pantaloni. 2000. Grb2 links signalling to actin assembly by enhancing interaction of neural Wiskott-Aldrich syndrome protein (N-WASP) with actin-related proteins (Arp2/3) complex. J. Biol. Chem. 275:21969–21974.

Castellano, F., P. Montcourrier, J-C. Guillemot, E. Guin, L. Machesky, P. Cos-sart, and P. Chavrier. 1999. Inducible recruitment of Cdc42 or WASP to a cell-surface receptor triggers actin polymerization and filopodium formation. Curr. Biol. 9:551–560.

Chen, F., L. Ma, M.C. Parrini, X. Mao, M. Lopez, C. Wu, P.W. Marks, L. Davidson, D.J. Kwiatkowski, T. Kirchhausen, et al. 2000. Cdc42 is required for PI(3,4,5)P3-induced actin polymerization and early development but not for cell viability. Curr. Biol. 10:758–765.

Egile, C., T.P. Loisel, V. Laurent, R. Li, D. Pantaloni, P.J. Sansonetti, and F-F. Carlier. 1999. Activation of the Cdc42 effector N-WASP by the Shigella flexneria IcsA protein promotes actin nucleation by Arp2/3 complex and bacterial actin-based motility. J. Cell Biol. 146:1319–1332.

Finan, P.M., C.J. Soames, L. Wilson, D.L. Nelson, D.M. Stewart, O. Truong, J.J. Hsuan, and S. Kellie. 1996. Identification of regions of the Wiskott-Aldrich Syndrome protein responsible for association with selected Src homology 3 domains. J. Biol. Chem. 271:26291–26295.

Higgs, H.N., and T.D. Pollard. 1999. Regulation of actin polymerization by Arp2/3 complex and the WASp/Scarb proteins. J. Biol. Chem. 274:32531–32534.

Higgs, H.N., and T.D. Pollard. 2000. Activation by Cdc42 and PI(3,4,5)P3 of Wiskott-Aldrich Syndrome protein (WASP) stimulates actin nucleation by Arp2/3 complex. J. Cell Biol. 150:1311–1320.

Kato, M., H. Miki, K. Imai, S. Nonoyama, T. Suzuki, C. Sasakawa, and T. Take-nawa. 1999. Wiskott-Aldrich Syndrome protein induces actin clustering without direct binding to Cdc42. J. Biol. Chem. 274:27225–27230.

Kim, A.S., L.T. Kakalis, N. Abdul-Manan, G.A. Liu, and M.K. Rosen. 2000. Autoinhibition and activation mechanisms of the Wiskott-Aldrich Syndrome protein. Nature. 404:151–158.

Machesky, L., 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 dendritic nucleation of actin filaments by the Arp2/3 complex. Proc. Natl. Acad. Sci. USA. 96:3739–3744.

Miki, H., K. Miura, and T. Takenawa. 1996. N-WASP, a novel actin-depolymerizing protein, regulates the cortical cytoskeletal rearrangement in a PIP2-dependent manner downstream of tyrosine kinases. EMBO (Eur. Mol. Biol. Organ.) J. 15:5326–5335.

Miki, H., T. Sasaki, Y. Takai, and T. Takenawa. 1998. Induction of filopodium formation by a WASP-related actin-depolymerizing protein N-WASP. Nature. 391:93–96.

Moreau, V., F. Fischnecht, I. Reckmann, R. Vincentelli, G. Rabut, D. Stewart, and M. Way. 2000. A complex of N-WASP and WIP integrates signalling cascades that lead to actin polymerization. Nat. Cell Biol. 2:441–448.

Qualmann, B., J. Roos, P.J. DiGregorio, R.B. Kelly. 1999. Syndapin I, a synap-tic dynamin-binding protein that associates with the neural Wiskott-Aldrich Syndrome protein. Mol. Biol. Cell. 10:501–513.

Ramesh, N., L.M. Anton, J.H. Hartwig, and R.S. Gieha. 1997. WIP, a protein associated with Wiskott-Aldrich syndrome protein, induces actin polymerization and redistribution in lymphoid cells. Proc. Natl. Acad. Sci. USA. 94:14671–14676.

Rohatgi, R., H.-y.H. Ho, and M.W. Kirschner. 2000. Mechanism of N-WASP activation by Cdc42 and phosphatidylinositol 4,5-bisphosphate. J. Cell Biol. 150:1299–1309.

She, H.-y., S. Rockow, J. Tang, R. Nishimure, E.Y. Skolnik, M. Chen, B. Margolis, and W. Li. 1997. Wiskott-Aldrich Syndrome protein is associated with the adaptor protein Grb2 and the epidermal growth factor receptor in living cells. Mol. Biol. Cell. 8:1709–1721.

Yang, C., M. Huang, J. DelBasso, M. Pring, M. Joyce, H. Miki, T. Takenawa, and S.H. Zigmond. 2000. Profilin enhances Cdc42-induced nucleation of actin polymerization. J. Cell Biol. 150:1001–1012.

Yarar, D., W. To, A. Asbo, and M.D. Welch. 1999. The Wiskott-Aldrich syn-drome protein directs actin-based motility by stimulating actin nucleation with the Arp2/3 complex. Curr. Biol. 9:555–558.