Comment

How WASP Regulates Actin Polymerization

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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)[1] (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 NH_2 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 NH_2 terminus GTPase binding domain (GBD; Miki et al., 1998). The NH_2 terminus also binds PIP_2 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 NH_2 Terminus Binds its COOH Terminus to Inhibit Nucleation

To investigate which region of the NH_2 terminus is required for inhibition, different domains were expressed and examined for their ability to bind and inhibit the activity of the VCA fragment. NH_2-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 NH_2-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 NH_2 terminus (Kim et al., 2000; Rohatgi et al., 2000). C and A also bind the Arp2/3 complex; therefore, binding of the NH_2 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 NH_2-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 NH_2 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 PIP_2 and Cdc42; yet their responses differ to either alone. N-WASP is partially activated by either PIP_2 or Cdc42; WASP is activated by PIP_2, 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 PIP_2 differs. A fragment of N-WASP's NH_2 terminus including the basic region adjacent to GBD (but not the EVH1 do-

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[1]*Abbreviations used in this paper:* CRIB, Cdc42/Rac interactive binding; EVH1, Ena Vasp homology 1; GBD, GTPase-binding domain; N-WASP, neural Wiskott-Aldrich Syndrome protein; PRD, proline-rich domain; VCA, verprolin homology, cofilin homology, and acidic region; WASP, Wiskott-Aldrich Syndrome protein; WIP, WASP interacting protein.

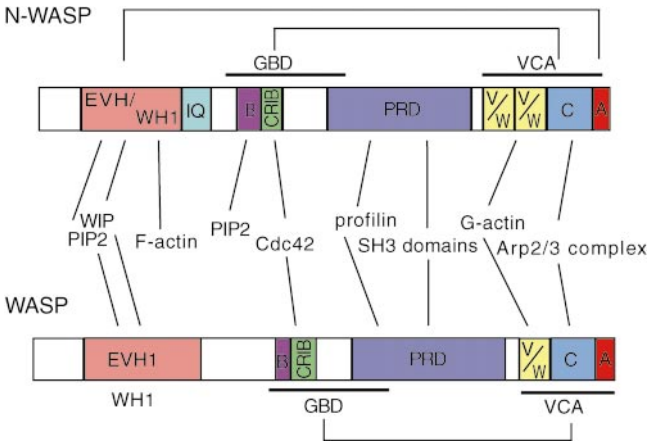

Figure 1. Domain structure of WASP and N-WASP with sites of interaction between COOH- and NH_2-termini, and binding by other factors. The NH_2 terminus of WASP and N-WASP contain an EVH1/WH1 domain that binds the proline-rich protein, WIP (Ramesh et al., 1997). The NH_2 terminus of N-WASP also binds PIP_2, 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 GDP-Cdc42. In N-WASP, the basic sequence (B) binds PIP_2 (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 PIP_2; Miki et al., 1996), responds to PIP_2 by decreased binding to VCA (Rohatgi et al., 2000). However, WASP's comparable NH_2-terminal fragment containing the basic domain, but not the EVH1 domain, does not respond to PIP_2 (Higgs and Pollard, 2000). Thus, WASP's basic domain is probably insufficient for the response to PIP_2, but it remains to test an NH_2-terminal fragment containing the EVH1 domain.

As yet, it is still risky to assign these different responses to PIP_2 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 PIP_2 liposomes and the molar ratio of PIP_2 to NH_2-terminal fragment also differed. Furthermore, the role of PIP_2 is complicated in cell extracts where PIP_2-evoked actin polymerization requires Cdc42 (Chen et al., 2000). Possibly in extracts, PIP_2 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 NH_2 and COOH termini. Alternatively, the PRD may extend the molecule, to allow intermolecular binding between the NH_2 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, PIP_2, 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.

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