es in computing power and a rethinking of the problem have aided the development of new codes that can provide vibrational energies and wave functions far more accurate than the NMA (*4*, *5*). Using our MULTIMODE code (*5*), which allows the user to choose between different levels of accuracy, we have obtained essentially exact results for five- and six-atom molecules and less accurate but still realistic results for much larger molecules.

The basic approach underlying these codes is the representation of the potential as a hierarchical set of mode-mode interactions, such that the full N-mode PES is represented by a sum of two-mode, three-mode, and four-mode interactions and a mean-field (so-called vibrationally self-consistent field) treatment of vibrational interactions (*6*). Gerber and co-workers recently introduced the effect of mode correlation into their code using second-order perturbation theory (*7*). Carter and Bowman's newest code (*8*) treats mode correlations with "configuration mixing" methods that can give essentially exact results. The codes have been linked to popular electronic structure codes, enabling direct calculation of forces and vibrational dynamics (*9*–*11*). For example, calculations of the vibrational energies of the Cl–H_2O complex were able to confirm the results of one experiment over another (*11*). MULTIMODE has also been extended to treat internal rotation in molecules (*12*), which is widespread in large molecules but notoriously difficult to treat computationally.

The stage is thus set for more realistic and accurate calculations of molecular vibrations of fairly complex molecules and molecular systems, such as adsorbates, complexes, and small molecules encapsulated in confined environments. Isomerizing states of molecules are sure to receive more experimental attention in the near future, and the understanding and interpretation of these experiments will benefit from such an accurate theoretical treatment of vibrational motion.

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PERSPECTIVES: SIGNAL TRANSDUCTION

N-WASP Regulation—the Sting in the Tail

James Fawcett and Tony Pawson

The proteins of signaling pathways in eukaryotic cells selectively interact with one another and with small molecules such as phospholipids. These interactions frequently require modular domains that retain their capacity to recognize defined peptide motifs (or phospholipids) when they are expressed separately in cells (*1*). Signaling proteins often possess many of these domains and so are capable of multiple interactions with other proteins and phospholipids. Furthermore, domains within the same protein have the potential to interact with each other, providing a sophisticated means of switching signaling proteins on and off.

Members of the WASP (Wiskott-Aldrich syndrome protein) family regulate the assembly of actin monomers into filaments, and thus are key regulators of the cytoskeletal organization and motility of cells. When activated, many cell surface receptors induce alterations in the organization of intracellular signaling complexes leading to changes in actin assembly (polymerization) and cell motility. But it is still not clear how signals from these myriad receptors are integrated within the cell to yield a coherent cytoskeletal response. Recent findings suggest that a limited number of cytoplasmic proteins including WASP family members provide focal points at which multiple signals converge to control the dynamics of actin polymerization. Three recent papers (*2*–*4*), including the report by Prehoda *et al.* on page 801 of this issue, explore how activation of WASP (found in lymphocytes) and its relative N-WASP (expressed in many cell types) connects several signaling pathways to the initiation of actin assembly.

The carboxyl terminus of WASP and N-WASP contains a conserved VCA region consisting of a verprolin homology region (V), a cofilin homology region (C), and an acidic region (A) (see the figure). The acidic motif and cofilin homology region bind to the actin related protein complex Arp2/3, which initiates actin polymerization by promoting addition of actin monomers to the barbed ends of actin filaments (*5*). The V region binds to monomers of unpolymerized actin, which can then be passed to the neighboring Arp2/3 complex for assembly into filaments (see the figure). The amino-terminal region of WASP contains an EVH1 (WH1) domain, followed by a short basic region and a guanosine triphosphatase (GTPase) binding domain (see the figure). The GTPase binding domain associates with the GTPase Cdc42, which regulates organization of the cytoskeleton. Between the amino- and carboxyl-terminal domains of WASP lies a proline-rich sequence that can bind to profilin and to the Src homology (SH) 3 domains of signaling proteins (such as, Grb2 and Nck) and cytoplasmic tyrosine kinases.

This profusion of interaction motifs suggests that WASP and N-WASP are regulated by multiple molecules that associate with their amino-terminal and central domains, resulting in activation of a single response (actin polymerization) mediated by the carboxyl-terminal VCA region. In the absence of appropriate signals, WASP's activity is blocked by cooperative folding of the amino-terminal GTPase binding domain with the carboxyl-terminal C motif (*6*). This folding may prevent the Arp2/3 complex from binding to WASP's A and C motifs as well as potentially blocking binding of actin monomers to the V region. Binding of GTP-Cdc42 to the GTPase binding domain induces unfolding of WASP and liberation of the VCA region, which is then free to bind and activate Arp2/3, resulting in the initiation of actin assembly (*5*). A more potent way to initiate actin assembly is through the cooperative activation of WASP proteins by GTP-Cdc42 and the phospholipid, phos-

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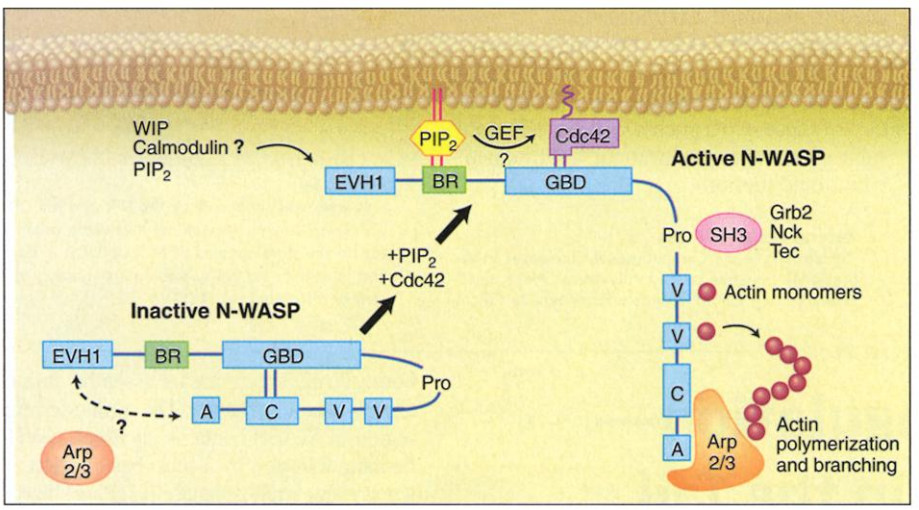
phatidylinositol-4,5-bisphosphate (PIP_2). The three new papers now propose how this cooperation might work.

Not unexpectedly, all three groups show that both the GTPase binding domain and the C and A regions are required for WASP's ability to block its own activation (autoinhibition) (*2*–*4*), consistent with earlier biochemical and nuclear magnetic resonance studies (*6*, *7*). However, analysis of N-WASP has identified a highly basic region of 20 residues preceding the GTPase binding domain that cooperates with this domain to block actin assembly at the VCA motif.



The ties that bind. A protein-protein and a protein-phospholipid interaction activate the N-WASP protein, which coordinates initiation of actin assembly. In its inactive state, N-WASP blocks its own activity by the folding of its GTPase binding domain (GBD) at the amino terminus with its cofilin homology domain (C) at the carboxyl terminus. Cooperative binding of PIP_2 and activated Cdc42 to the basic region (BR) and to the GBD of N-WASP, respectively, results in activation of the Arp2/3 complex. This complex binds to the acidic (A) and C regions of N-WASP and then initiates the assembly of actin monomers (which bind to the verprolin homology domain, V) into polymerized actin filaments.

Although the GTPase binding domain can bind to the C and A regions on its own, it cannot block Arp2/3 activation and actin assembly without the help of the basic region. Adding yet another layer of complexity, one group reports that the EVH1 amino-terminal domain binds weakly to the VCA region (*4*).

The abundance of lysine residues in the basic region suggests that it might serve as a binding site for negatively charged phospholipids. Indeed, this region not only binds specifically to liposomes containing PIP_2, but is essential for the ability of this phosphoinositide to augment the activation of N-WASP by Cdc42 in an assay using purified components (*2*, *4*). These observations indicate that the basic region and the GTPase-binding domain cooperate to maintain N-WASP in an autoinhibited state. The former motif binds PIP_2 and the latter motif binds Cdc42, suggesting that coincident binding of PIP_2 and Cdc42 could reverse autoinhibition by inducing the amino and carboxyl termini to unfold.

In their study, Prehoda *et al.* (*2*) used a mini–N-WASP gene encoding the GTPase-binding domain and the basic motif (which they call the control region) joined to the VCA region through a short linker sequence. The mini–N-WASP protein recapitulates the ability of full-length N-WASP to bind to Cdc42 and PIP_2 and to activate Arp2/3. Indeed, the authors show that Cdc42 and PIP_2 act cooperatively to stimulate Arp2/3-mediated actin assembly, because the concentration of each ligand required to activate mini–N-WASP is reduced by a factor of 10 if they are added in combination, rather than individually. By studying the binding of PIP_2-containing vesicles and fluorescently labeled Cdc42 to mini–N-WASP, Prehoda *et al.* explored how this cooperation might be achieved. They show that both PIP_2 and Cdc42 bind simultaneously to the control region but do not interact with one another. Furthermore, PIP_2 enhances binding of Cdc42 to the control region of mini–N-WASP, and Cdc42 strongly potentiates PIP_2's affinity for the control region. These results suggest that in the absence of an activating signal, N-WASP is in a closed state, with its Cdc42- and PIP_2-binding sites occluded. Perhaps N-WASP is a "coincidence detector" because when it binds to activated Cdc42 it becomes destabilized and primed such that only a small amount of a coincident signal—in this case PIP_2—is needed for it to become fully activated (see the figure).

It is now apparent that the regulation of WASP and N-WASP is extremely complex. For example, although Rohatgi *et al.* (*4*) concur with Prehoda *et al.*'s finding that binding of PIP_2 to the N-WASP basic motif is required to activate Arp2/3, they observed an additional level of N-WASP regulation by PIP_2 in a more biologically relevant system. They reconstituted N-WASP–depleted *Xenopus* egg extracts with either wild-type N-WASP or an N-WASP mutant lacking the basic region. Although the deletion mutant was still autoinhibited, surprisingly it could still be activated by PIP_2 to induce actin assembly, albeit less efficiently than the wild-type protein. Rohatgi *et al.* propose that PIP_2 might also work upstream of Cdc42 by activating a guanine nucleotide exchange factor. Thus, PIP_2 may both control Cdc42 activation and directly enhance its binding to N-WASP.

The mechanism by which folding of N-WASP into the autoinhibited state blocks Arp2/3 activation has yet to be resolved. One possibility is that the intramolecular interactions of the C and A regions with the GTPase binding domain simply occlude the Arp2/3 binding site. Alternatively, as Prehoda *et al.* suggest, the autoinhibited N-WASP may still be capable of binding Arp2/3 through both the C and A regions and the basic motif, but in a way that precludes Arp2/3 activation. In this model, the association of the C and A motifs with the GTPase binding domain may position the basic region so that it can engage Arp2/3 and maintain it in an inactive state, yet in such a way that Arp2/3 is poised to respond to Cdc42 and PIP_2 as soon as they bind to N-WASP. Finally, WASP itself appears to differ in several ways from N-WASP—both in its oligomeric state and in the binding of PIP_2 to the basic region—and it will be important to understand the basis for these differences (*3*).

N-WASP may appear to be a simple molecule: After all, it consists of a series of interaction domains and motifs that accept incoming signals from multiple signaling molecules and channel them into the activation of Arp2/3, which then initiates actin assembly. Yet WASP proteins are structurally exceedingly complex in ways we are only starting to understand. It seems a safe bet that ongoing analyses of WASP proteins will yield new layers of regulation that enable myriad signals to guide subtle changes in the cytoskeleton and motility of cells.

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CREDIT: ADAPTED FROM (*2*) AND (*7*)