Biochemistry (Moscow), Vol. 70, No. 12, 2005, pp. 1305-1309. Translated from Biokhimiya, Vol. 70, No. 12, 2005, pp. 1587-1592.

MINI-REVIEW

WASP Family Proteins Act between Cytoskeleton and Cellular Signaling Pathways

S. N. Samarin

Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, 142290 Pushchino, Moscow Region, Russia; fax: (7-0967) 790-553; E-mail: stassam@mail.ru

Received September 28, 2004
Revision received November 15, 2004

Abstract—This review considers the proteins of the WASP (Wiskott–Aldrich syndrome protein) family and their role in the regulation of actin-based motility. It contains detailed classification of the WASP family proteins and data on their subcellular localization. Impairments of expression of the WASP family proteins cause certain cell pathologies. The review also deals with domain organization of these proteins and proteins interacting with various domains of the WASP proteins. Special attention is given to analysis of the role of the WASP family proteins in initiating directed actin assembly in the leading edge of the migrating cell and on the surface of some bacteria. Putative pathways of regulation of WASP proteins by various protein ligands and their links with cell signaling systems are considered.

Key words: WASP family proteins, Arp2/3 complex, Rho family GTPases, PIP_2, Grb2, Nck, intracellular signaling

There are many recent experimental data that help better understanding of molecular mechanisms underlying actin-based cell motility. However, one of the major problems of cell biology is understanding how a cell integrates external signals from various receptors and responds to them by directed actin polymerization at a certain time and at a particular site. The solution of this problem requires identification of all components of the signaling chain from cell receptors to particular protein effectors localized in sites of active actin dynamics. Significant success has been achieved in understanding the regulatory mechanism of actin polymerization by WASP (Wiskott–Aldrich syndrome protein) family proteins. This mechanism is universal for all eukaryotic cells. The WASP family proteins integrate various signals accepted from receptor tyrosine kinases and GTPases such as Cdc42 (cell division cycle 42) and Rac (ras-related C3 botulinum toxin substrate) and initiate directed actin polymerization due to local activation of Arp2/3 complex.

ACTIN-BASED CELL MOTILITY

Locomotion is an ultimate precondition for normal functioning of most eukaryotic cells. Accepting various external signals (e.g., light, nutrients, growth factors, chemoattractants, repellents, etc.), a cell responds to them by directed locomotion. How does the cell move?

Good evidence now exists that cell movement is mainly determined by directed and regulated actin polymerization at the cell cortex [1]. Data of electron microscopy demonstrate that a narrow zone (about 1 μm) of leading edge of the moving cell is filled with actin filaments oriented by their rapidly growing plus-ends towards the cytoplasmic membrane and organized into mechanically rigid, dichotomous branched structures [2]. The protein complex Arp2/3 plays the structure-forming role in this actin network [3]. Two subunits of this complex referred to as Arp2 and Arp3 (actin-related protein 2 and 3, respectively) share high structural homology with actin subunit. Due to this homology Arp2/3 complex inserts between actin filaments attaching minus-end of one filament to the other filament at the angle of 70° [2]. Growth of such filaments at the plus-end pushes the cell membrane forward [4], thus forming characteristic pseudopodia known as filopodia and lamellipodia. Pseudopodia attached to substrate determine subsequent direction of cell movement, and the migrating cell acquires extended polarized shape with flat leading edge and stable posterior margin (Fig. 1b).

How does a cell accept various external signals, transduce them, and begin assembly of actin filaments at the cell cortex at a particular requested place?

Recent studies revealed that Arp2/3 complex is the protein component regulating initial assembly of actin filaments; in response to interaction with protein activators the Arp2/3 complex induces actin polymerization by

0006-2979/05/7012-1305

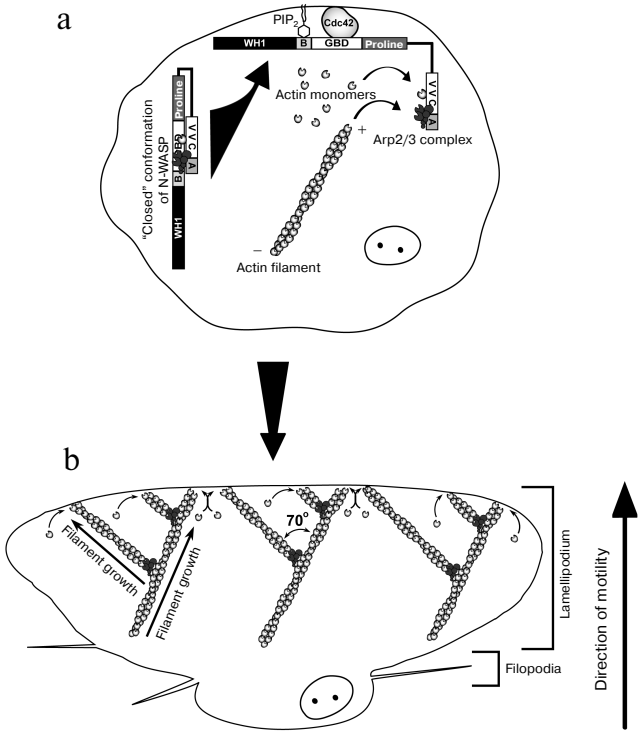

Fig. 1. Scheme of regulation of actin-based cell motility by N-WASP. a) In the absence of protein activators N-WASP exists in folded conformation in which Arp2/3 cannot interact with actin filaments. Cooperative binding of PIP_2 and Cdc42 leads to unfolding of the molecule and release of active actin–N-WASP–Arp2/3 complex, which interacts with existing actin filaments and forms new filaments as the branches from the existing filaments. b) This results in formation of a branched network of actin filaments oriented at an angle of 70° versus each other under the cell cortex. This network is stabilized by Arp2/3 complex positioned at branching nodes. Subsequent growth of active filaments at plus-ends oriented toward the cell plasma membrane pushes the cell membrane forward and forms flat lamellipodia and hair-like filopodia. Plus (+) and minus (–) designate rapidly growing (plus-end) and slowly growing (minus-end) actin filament ends.

binding with pre-existing actin filaments and creating new active filaments as branches of the initial ones [5, 6]. In such a way, Arp2/3 complex forms a branched actin network (Fig. 1). In eukaryotic cells, the role of activators of the Arp2/3 complex belongs to proteins of the WASP family. These are universal cell messengers linking Arp2/3 complex to various signaling systems of the cell.

PROTEINS OF THE WASP FAMILY

Human representatives of the WASP protein family include WASP, several isoforms of N-WASP, and Scar/WAVE. The Wiskott–Aldrich syndrome protein (WASP) is expressed in hemopoietic cells only; however, its closest relative, N-WASP, is widely distributed [7-9]. The suppressor of cAMP receptor (Scar) was identified by means of genetic screening directed to the search of mutation suppressors of *Dictyostelium* cAMP receptors [7]. Four Scar homologs denominated as Hs-Scar1, Hs-Scar2, Hs-Scar3, and Hs-Scar4 have been identified in man [7]. One nucleotide sequence, denominated as WAVE (WASP family Verprolin-homologous protein), has been found in the human genome [8]. In yeast there is a WASP homolog known as Bee1 or Las17 [10].

The Wiskott–Aldrich syndrome protein (WASP) was denominated by the name of a gene carrying a mutation that causes a disease known as the Wiskott–Aldrich syndrome. This pathological state is referred as the primary X-linked immunodeficiency characterized by impairments of cytoskeleton in T-cells, platelets, and phagocytes followed by impairments in functioning of T-cells, platelet damage, and defects in chemotaxis of neutrophils and macrophages. This explains such major symptoms of the Wiskott–Aldrich syndrome as thrombocytopenia, eczema, and recurrent infection caused by attenuation of the immune response to polysaccharide antigens [11-13].

Data on cellular localization of these proteins seem to underline the important role of the WASP family proteins in the regulation of actin based cell motility. For example, immunofluorescent analysis revealed that the WASP family proteins are positioned in the places of active actin dynamics, preferentially within the protruding margin of the moving cell. Scar1 is located in the anterior extremity of cellular lamellipodia [14], whereas N-WASP is detected in the anterior extremity, cytoplasm, and nucleus [15].

STRUCTURE OF WASP FAMILY PROTEINS

The N-terminal site of all members of the WASP family consists of EVH1 (WH1) (Ena/VASP homology 1 or WASP homology 1) domain followed by a short site (B) enriched with basic amino acids; the latter can bind phosphatidylinositol-4,5-bisphosphate (PIP_2) [16-18]. WASP and N-WASP contain an amino acid sequence of 14 residues that follows the site enriched with basic amino

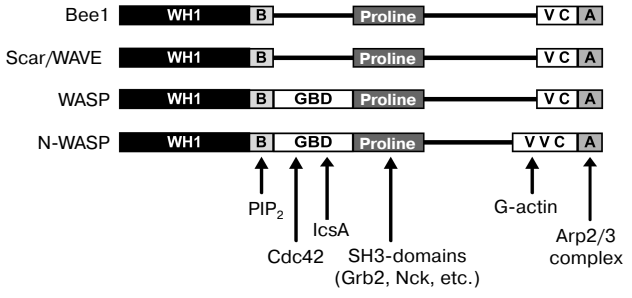

Fig. 2. Domain organization of WASP family proteins and their ligands (see explanations in the text).

acid residues. It is denominated as GBD or CRIB domain (abbreviations of G-protein binding domain or Cdc42/Rac interactive binding). This is responsible for binding of Rho family GTPases (Rac-homologous). In contrast to WASP and N-WASP, the Scar1/WAVE and Bee1/Las17 proteins lack the GBD/CRIB domain [19] (Fig. 2).

The presence of a central proline-rich site interacting with profilin and with SH3-domain (Src homology 3 domains) containing proteins such as signaling proteins Grb2 (growth factor receptor binding protein 2) [20] and Nck (non-catalytic region of tyrosine kinase adaptor protein) [21] is a common feature of all members of the WASP family.

The C-terminus of WASP family proteins contains a conservative VCA domain (also defined as WA domain); this domain consists of veprolin (V) homology domain, cofilin (C) homology domain, and a negatively charged site (A) [16]. In contrast to other members of this family, N-WASP contains two veprolin homology domains. The V-site interacts with monomeric actin [22] and CA site binds Arp2/3 complex [23] (Fig. 2).

WASP FAMILY PROTEINS ARE UNIVERSAL CELL MESSENGERS

WASP family proteins can interact with various molecules and due to this feature they represent a universal tool integrating various intracellular signals and initiating actin polymerization in response to such signal in a particular place and at a requested time. For example, simultaneous binding of Arp2/3 complex and monomeric actin at the VCA site of WASP causes activation of Arp2/3 complex followed by production of new actin filaments *in vitro*. These data were confirmed by experiments where Arp2/3 complex was depleted from lamellipodia of mammalian cells by overexpression of WASP VCA fragment. Such delocalization of Arp2/3 complex results in loss of lamellipodia and stress fibers [23].

Comparison of Arp2/3 complex activation by N-WASP and its VCA fragment revealed that the presence of VCA-domain is the ultimate (and sufficient) precondition for activation of Arp2/3 complex. Under these conditions full length N-WASP was two orders of magnitude less active with respect to Arp2/3 complex [24]. Functional activity of the wild type N-WASP molecule is blocked due to cooperative interaction of the GBD/CRIB domain at the N-end with the C-terminal VCA domain [9]. According to a complex model of allosteric regulation of N-WASP activity proposed by Prehoda et al. [18], GBD/CRIB domain and the basic domain B maintain VCA–Arp2/3 complex in inactive ("closed") state. This state is characterized by poor binding of Rho GTPases and PIP_2 with GBD/CRIB and B sites, respectively. Binding of either GTPase or PIP_2 thermodynamically destabilizes the folded ("closed") conformation of the N-WASP molecule and facilitates cooperative binding of the second activator resulting in full unfolding of N-WASP and susceptibility of VCA–Arp2/3 complex [18]. Thus, VCA–Arp2/3 complex becomes available for interaction with actin and so promotes assembly of new actin filaments (Fig. 1a).

Experimental data obtained *in vitro* confirm the hypothesis of Prehoda et al. [18]. For example, it was shown that independent action of Cdc42 and PIP_2 insignificantly increased activity of WASP and N-WASP, whereas their concerted effect completely restored protein activity up to the level of VCA [24, 25]. Interestingly, synergistic effect of Cdc42 and PIP_2 requires the presence of both activators on the surface of lipid membrane. Full activation of WASP on the surface of PIP_2 micelles or PIP_2 containing vesicles was only possible in the case of prenylated Cdc42 (i.e., Cdc42 contained a hydrophobic tail) [25].

The family of Rho GTPases plays a key role in intracellular signal transduction by coupling various external signals with actin dynamics in various organisms and cell types [26]. The Rho family proteins as other Ras-like GTPases can exist in inactive GDP-bound and active GTP-bound forms. Many various molecules are involved in regulation of Rho family proteins. These include guanine nucleotide exchange factors (GEF), which catalyze exchange of GDP for GTP and thus activate Rho proteins. Proteins increasing GTPase activity of Rho proteins (GAPs; GTPase-activating proteins) cause their transition into inactive GDP-bound form. Finally, guanine nucleotide dissociation inhibitors (GDIs) maintain Rho family proteins in inactive GDP-bound state [27, 28].

The N-WASP GBD/CRIB-domain preferentially interacts with GTP-Cdc42, and it also binds GTP-Rac (although with lower affinity) but not Rho [19, 29, 30]. *In vivo* data also suggest direct involvement of Rho proteins into initiation of actin polymerization in cells. For example, activation of Cdc42 in COS7 cells results in filopodia formation [9], and injection of Cdc42 or Rac into macrophages induces formation of filopodia and lamellipodia, respectively [31]. GTP-Cdc42 initiated actin polymerization in neutrophil supernatants [32]. Immobilization of activated Cdc42 (GTP-Cdc42) on membrane receptors of intact cells induced actin polymerization leading to plasma membrane protrusion [33]. Rho proteins can be activated in response to chemoattractant with cell membrane receptors. For example, interaction of N-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP) with serpentine membrane receptors leads to Rac2 activation in intact neutrophils [34, 35], whereas Rac2 knockout mice are characterized by weak chemotaxis response to FMLP, interleukin-8, and leukotriene B4 [36].

Activation of WASP family proteins may involve interaction of their central site containing polyproline

sequences with SH3 domain proteins such as Src and Src-like tyrosine kinases, phospholipase Cγ (PLCγ), Nck, and Grb2 [37]. For example, binding of Grb2 (SH3-SH2-SH3 adaptor protein) with N-WASP increased activation of Arp2/3 complex *in vitro* in dose dependent manner. Grb2 activated N-WASP in synergic manner with GTP-Cdc42 [38]. Grb2 specifically interacts via its SH2 domain with phosphorylated tyrosine residue, and this is important for signal transduction from receptor tyrosine kinases such as epidermal growth factor receptor (EGFR) to WASP family proteins [20]. Similar data have also been obtained for Nck; this protein containing three SH3 domains and one SH2 domain binds to activated platelet-derived growth factor receptor (PDGFR) and represents an important protein adaptor involved in signal transduction from this receptor [39]. It was demonstrated that Nck can directly activate WASP in synergic manner with PIP_2 *in vitro* [40].

ACTIN POLYMERIZATION AND MOTILITY OF SOME BACTERIA

Transmembrane protein IcsA (VirG) [41] of the Gram-negative bacterium *Shigella flexneri* [42] is another ligand of N-WASP [43]. Being a facultative intracellular parasite, *Sh. flexneri* employs directed actin polymerization for motility inside the infected cell. IcsA interacts with N-WASP GBD-domain [44] (see also Fig. 2) of the host cells and activates it. This results in N-WASP-mediated initiation of Arp2/3-based polymerization of cell actin on the surface of bacteria similarly to that in cell lamellipodia under normal conditions. Growing actin filaments form a characteristic bacterial "tail" pushing *Sh. flexneri* forward [41].

The gram-positive bacterium *Listeria monocytogenes* is another parasite using host cell actin machinery for its movement [45]. In contrast to IcsA, its membrane ActA protein [46] is a functional homolog of WASP [47], so it can directly activate Arp2/3 complex of the infected cell and therefore polymerization of cell actin on the surface of the bacterial cell as occurs in the case of motility of *Sh. flexneri* [48, 49]. Thus, both *Sh. flexneri* and *L. monocytogenes* use cytoskeleton proteins of the host cell, bypassing cell signaling pathways which involve N-WASP activation. This feature of both bacteria is successfully employed by researchers as a convenient model for studies of actin-based cell motility. Interestingly, polystyrene microspheres with adsorbed N-WASP [50, 51] or ActA [52, 53] can move in cell extracts and artificial media that contain purified cytoskeleton proteins, like *Shigella* and *Listeria*.

The activity of WASP family proteins can be regulated by various molecules followed by activation of Arp2/3 complex representing the universal response to these different stimuli. Consequently, WASP family proteins act as "instruments" accepting various input signals and precisely localizing initiation of actin polymerization in response to these signals in requested sites of the cell membrane. However, in spite of evident progress, complete understanding of the molecular mechanisms responsible for WASP family protein functioning requires solution of many problems. For example, the molecular mechanism of activation of Arp2/3 complex by WASP family proteins still remains unclear. The mechanism underlying transition of active N-WASP conformation into inactive (folded) form is unknown. The hypothesis of Prehoda et al. [18] requires more specifications and experimental confirmations. It remains unclear whether WH1-domain of N-WASP binds actin filaments [44] *in vivo*, and if it does what is the purpose of such interaction. The mechanism of Scar activation remains unknown (in contrast to WASP and N-WASP this protein lacks GBD-domain). Several highly qualified laboratories in Europe, USA, and Japan are now trying to solve these problems, and it is possible that a comprehensive picture will be drawn in the foreseeable future.

REFERENCES

1. Malawista, S. E., and De Boisfleury, C. A. (1982) *J. Cell. Biol.*, **95**, 960-973.
2. Svitkina, T. M., and Borisy, G. G. (1999) *J. Cell Biol.*, **145**, 1009-1026.
3. Machesky, L. M., Atkinson, S. J., Ampe, C., Vandekerckhov, E. J., and Pollard, T. D. (1994) *J. Cell Biol.*, **127**, 107-115.
4. Mogilner, A., and Oster, G. (1996) *Biophys. J.*, **71**, 3030-3045.
5. Higgs, H. N., Blanchoin, L., and Pollard, T. D. (1999) *Biochemistry*, **38**, 15212-15222.
6. Machesky, L. M., Mullins, R. D., Higgs, H. N., Kaiser, D. A., Blanchoin, L., May, R. C., Hall, M. E., and Pollard, T. D. (1999) *Proc. Natl. Acad. Sci. USA*, **96**, 3739-3744.
7. Bear, J. E., Rawls, J. F., and Saxe, C. L., 3rd. (1998) *J. Cell Biol.*, **142**, 1325-1335.
8. Miki, H., Suetsugu, S., and Takenawa, T. (1998) *EMBO J.*, **17**, 6932-6941.
9. Miki, H., Sasaki, T., Takai, Y., and Takenawa, T. (1998) *Nature*, **391**, 93-96.
10. Li, R. (1997) *J. Cell Biol.*, **136**, 649-658.
11. Derry, J. M., Ochs, H. D., and Francke, U. (1994) *Cell*, **78**, 635-644.
12. Zicha, D., Allen, W. E., Brickell, P. M., Kinnon, C., Dunn, G. A., Jones, G. E., and Thrasher, A. J. (1998) *Br. J. Haematol.*, **101**, 659-665.
13. Nonoyama, S., and Ochs, H. D. (1998) *Curr. Opin. Immunol.*, **10**, 407-412.
14. Hahne, P., Sechi, A., Benesch, S., and Small, J. V. (2001) *FEBS Lett.*, **492**, 215-220.
15. Bear, J. E., Krause, M., and Gertler, F. B. (2001) *Curr. Opin. Cell Biol.*, **13**, 158-166.

16. Miki, H., Miura, K., and Takenawa, T. (1996) *EMBO J.*, **15**, 5326-5335.
17. Rohatgi, R., Ho, H. Y., and Kirschner, M. W. (2000) *J. Cell Biol.*, **150**, 1299-1310.
18. Prehoda, K. E., Scott, J. A., Mullins, R. D., and Lim, W. A. (2000) *Science*, **290**, 801-806.
19. Bi, E., and Zigmond, S. H. (1999) *Curr. Biol.*, **9**, R160-R163.
20. She, H., Rockow, S., Tang, J., Nishimura, R., Skolnik, E. Y., Chen, M., Margolis, B., and Li, W. (1997) *Mol. Biol. Cell*, **8**, 1709-1721.
21. Rivero-Lezcano, O. M., Marcilla, A., Sameshima, J. H., and Robbins, K. C. (1995) *Mol. Cell Biol.*, **15**, 5725-5731.
22. Miki, H., and Takenawa, T. (1998) *Biochem. Biophys. Res. Commun.*, **243**, 73-78.
23. Machesky, L. M., and Insall, R. H. (1998) *Curr. Biol.*, **8**, 1347-1356.
24. Rohatgi, R., Ma, L., Miki, H., Lopez, M., Kirchhausen, T., Takenawa, T., and Kirschner, M. W. (1999) *Cell*, **97**, 221-231.
25. Higgs, H. N., and Pollard, T. D. (2000) *J. Cell Biol.*, **150**, 1311-1320.
26. Hall, A. (1998) *Science*, **279**, 509-514.
27. Van Aelst, L., and D'Souza-Schorey, C. (1997) *Genes Dev.*, **11**, 2295-2322.
28. Mackay, D. J. G., and Hall, A. (1998) *J. Biol. Chem.*, **273**, 20685-20688.
29. Symons, M., Derry, J. M., Karlak, B., Jiang, S., Lemahieu, V., McCormick, F., Francke, U., and Abo, A. (1996) *Cell*, **84**, 723-734.
30. Aspenstrom, P., Lindberg, U., and Hall, A. (1996) *Curr. Biol.*, **6**, 70-75.
31. Allen, W. E., Jones, G. E., Pollard, J. W., and Ridley, A. J. (1997) *J. Cell Sci.*, **110**, 707-720.
32. Zigmond, S. H., Joyce, M., Yang, C., Brown, K., Huang, M., and Pring, M. (1998) *J. Cell Biol.*, **142**, 1001-1012.
33. Castellano, F., Montcourrier, P., Guillemot, J. C., Gouin, E., Machesky, L., Cossart, P., and Chavrier, P. (1999) *Curr. Biol.*, **9**, 351-360.
34. Benard, V., Bohl, B. P., and Bokoch, G. M. (1999) *J. Biol. Chem.*, **274**, 13198-13204.
35. Akasaki, T., Koga, H., and Sumimoto, H. (1999) *J. Biol. Chem.*, **274**, 18055-18059.
36. Roberts, A. W., Kim, C., Zhen, L., Lowe, J. B., Kapur, R., Petryniak, B., Spaetti, A., Pollock, J. D., Borneo, J. B., Bradford, G. B., Atkinson, S. J., Dinauer, M. C., and Williams, D. A. (1999) *Immunity*, **10**, 183-196.
37. Mayer, B. J., and Baltimore, D. (1993) *Trends Cell Biol.*, **3**, 8-13.
38. Carlier, M.-F., Nioche, P., Broutin-L'Hermite, I., Boujemaa, R., Le Clainche, C., Egile, C., Garbay, C., Ducruix, A., Sansonetti, P., and Pantaloni, D. (2000) *J. Biol. Chem.*, **275**, 21946-21952.
39. Nishimura, R., Kashishian, W. L. A., Mondino, A., Zhou, M., Cooper, J., and Schlessinger, J. (1993) *Mol. Cell Biol.*, **13**, 6889-6896.
40. Rohatgi, R., Nollau, P., Ho, H. Y. H., Kirschner, M. W., and Mayer, B. J. (2001) *J. Biol. Chem.*, **276**, 26448-26452.
41. Bernardini, M. L., Mounier, J., d'Hauteville, H., Coquis-Rondon, M., and Sansonetti, P. J. (1989) *Proc. Natl. Acad. Sci. USA*, **86**, 3867-3871.
42. Sansonetti, P. J. (2001) *Am. J. Physiol. Gastrointest. Liver Physiol.*, **280**, G319-G323.
43. Suzuki, T., Miki, H., Takenawa, T., and Sasakawa, C. (1998) *EMBO J.*, **17**, 2767-2776.
44. Egile, C., Loisel, T. P., Laurent, V., Li, R., Pantaloni, D., Sansonetti, P. J., and Carlier, M.-F. (1999) *J. Cell Biol.*, **146**, 1319-1332.
45. Cossart, P., and Bierne, H. (2001) *Curr. Opin. Immunol.*, **13**, 96-103.
46. Kocks, C., Gouin, E., Tabouret, M., Berche, P., Ohayon, H., and Cossart, P. (1992) *Cell*, **68**, 521-531.
47. Boujemaa-Paterski, R., Gouin, E., Hansen, G., Samarin, S., Le Clainche, C., Didry, D., Dehoux, P., Cossart, P., Kocks, C., Carlier, M.-F., and Pantaloni, D. (2001) *Biochemistry*, **40**, 11390-11404.
48. Welch, M. D., Iwamatsu, A., and Mitchison, T. J. (1997) *Nature*, **385**, 265-269.
49. Welch, M. D., Rosenblatt, J., Skoble, J., Portnoy, D. A., and Mitchison, T. J. (1998) *Science*, **281**, 105-108.
50. Yarar, D., To, W., Abo, A., and Welch, M. D. (1999) *Curr. Biol.*, **9**, 555-558.
51. Wiesner, S., Helfer, E., Didry, D., Ducouret, G., Lafuma, F., Carlier, M.-F., and Pantaloni, D. (2003) *J. Cell Biol.*, **160**, 1-13.
52. Cameron, L. A., Footer, M. J., van Oudenaarden, A., and Theriot, J. A. (1999) *Proc. Natl. Acad. Sci. USA*, **96**, 4908-4913.
53. Samarin, S., Romero, S., Kocks, C., Didry, D., Pantaloni, D., and Carlier, M.-F. (2003) *J. Cell Biol.*, **163**, 131-142.