The Wiskott–Aldrich syndrome protein directs actin-based motility by stimulating actin nucleation with the Arp2/3 complex

Defne Yarar*, Wayne To†, Arie Abo† and Matthew D. Welch*

Actin polymerization at the cell cortex is thought to provide the driving force for aspects of cell-shape change and locomotion. To coordinate cellular movements, the initiation of actin polymerization is tightly regulated, both spatially and temporally. The Wiskott–Aldrich syndrome protein (WASP), encoded by the gene that is mutated in the immunodeficiency disorder Wiskott–Aldrich syndrome [1], has been implicated in the control of actin polymerization in cells [2–5]. The Arp2/3 complex, an actin-nucleating factor that consists of seven polypeptide subunits [6–8], was recently shown to physically interact with WASP [9]. We sought to determine whether WASP is a cellular activator of the Arp2/3 complex and found that WASP stimulates the actin nucleation activity of the Arp2/3 complex *in vitro*. Moreover, WASP-coated microspheres polymerized actin, formed actin tails and exhibited actin-based motility in cell extracts, similar to those behaviors displayed by the pathogenic bacterium *Listeria monocytogenes*. In extracts depleted of the Arp2/3 complex, WASP-coated microspheres and *L. monocytogenes* were non-motile and exhibited only residual actin polymerization. These results demonstrate that WASP is sufficient to direct actin-based motility in cell extracts and that this function is mediated by the Arp2/3 complex. WASP interacts with diverse signaling proteins and may therefore function to couple signal transduction pathways to Arp2/3-complex activation and actin polymerization.

Addresses: *Department of Molecular and Cell Biology, University of California, Berkeley, California 94720, USA. †Onyx Pharmaceuticals, Richmond, California 94806, USA.

Correspondence: Matthew D. Welch or Arie Abo
E-mail: welch@socrates.berkeley.edu or aabo@onyx-pharm.com

Received: 17 March 1999
Revised: 7 April 1999
Accepted: 9 April 1999

Published: 10 May 1999

Current Biology 1999, 9:555–558
http://biomednet.com/elecref/0960982200900555

© Elsevier Science Ltd ISSN 0960-9822

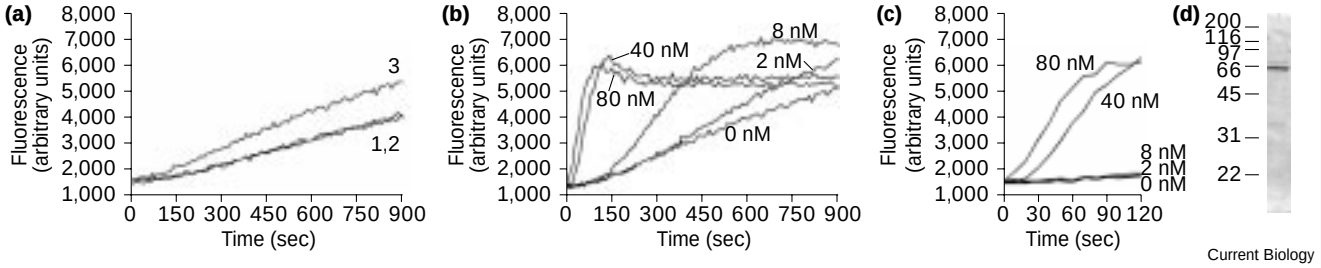
Results and discussion

The actin-nucleating activity of the Arp2/3 complex is stimulated up to 50-fold by the *L. monocytogenes* cell-surface protein ActA [8]. This suggests that a eukaryotic cellular factor possessing an activity similar to that of ActA might regulate actin polymerization during cell motility by modulating the activity of the Arp2/3 complex. No such factor has yet been identified, however.

We tested whether purified recombinant human WASP tagged with the FLAG epitope (FLAG–WASP; Figure 1d) could stimulate the capacity of the Arp2/3 complex to accelerate actin polymerization. Actin-assembly kinetics were monitored using the pyrene–actin assay [10,11]. The polymerization of actin alone was characterized by an initial lag phase, indicative of the kinetic barrier to nucleation, followed by a period of more rapid filament elongation (Figure 1a). Although WASP induces actin polymerization when microinjected into cells [4] and is thought to bind actin monomers *in vitro* [9], FLAG–WASP had no detectable affect on polymerization kinetics under the conditions tested (Figure 1a). As was observed previously, the Arp2/3 complex alone caused a modest acceleration of polymerization relative to the control, but failed to eliminate or reduce the lag phase [7,8]. Addition of increasing amounts of FLAG–WASP with a fixed concentration of the Arp2/3 complex, however, caused a dose-dependent acceleration of actin polymerization (Figure 1b). At the highest concentrations of WASP (a WASP:Arp2/3-complex molar ratio of 4:1), the initial rate of actin assembly was up to 35-fold higher than that in the reactions with the Arp2/3 complex or WASP alone, and the lag phase of polymerization was eliminated (Figure 1c). Thus, WASP stimulates the activity of the Arp2/3 complex, and the Arp2/3 complex and WASP together are sufficient for actin-filament nucleation. This suggests that WASP may influence the spatial and temporal distribution of actin polymerization in cells by modulating the activity of the Arp2/3 complex.

We sought to examine further the function of WASP in a context that more closely resembled the conditions inside a living cell. The effect of WASP on the nucleating activity of the Arp2/3 complex was nearly identical to that of ActA, the *L. monocytogenes* surface protein that is necessary [12,13] and sufficient to direct actin polymerization in cell cytoplasm [14–16]. We therefore examined whether WASP-coated beads were sufficient to induce actin polymerization in cell extracts. FLAG–WASP was bound to 1 μm diameter protein-A-conjugated microspheres coated with anti-FLAG antibody. These microspheres were incubated in bovine-brain-extract high-speed supernatant that was supplemented with rhodamine-labeled actin to allow actin structures to be visualized by fluorescence microscopy. Clouds of polymerized actin were observed at the surface of 59 ± 4% (range 48–69%; n = 417) FLAG–WASP-coated beads (Figure 2a,b). A smaller percentage (34 ± 4%; range

Figure 1



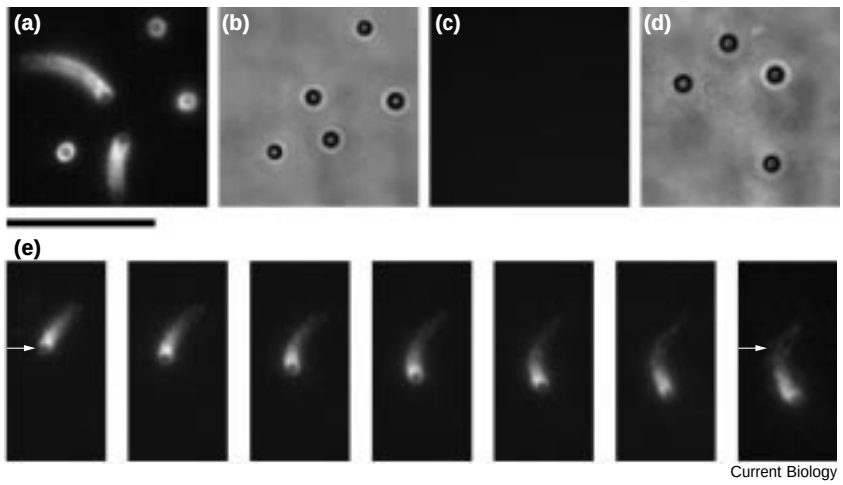
Effects of the Arp2/3 complex and WASP on actin polymerization. (a–c) Graphs of fluorescence intensity versus time after initiating polymerization in the pyrene–actin assay [10,11]. (a) Curve 1, 2 μM actin; curve 2, 2 μM actin with 80 nM FLAG–WASP; curve 3, 2 μM actin with 20 nM Arp2/3. (b) 2 μM actin and 20 nM Arp2/3 with varying concentrations of FLAG–WASP, as indicated. (c) Expanded view of the initial 120 sec of the graph in (b). (d) Purified FLAG–WASP visualized on a 12% polyacrylamide gel containing SDS stained with Coomassie blue.

21–44%) of FLAG–WASP-coated beads formed actin tails (Figure 2a,b), which were similar in morphology to the tails formed by *L. monocytogenes* in the same extracts. Control beads coated with an unrelated FLAG-tagged protein (FLAG–Smad3 [17]) did not induce actin polymerization in extracts (Figure 2c,d), indicating that the ability to polymerize actin is specific to WASP.

Because actin-tail formation accompanies *L. monocytogenes* motility, we used time-lapse microscopy to observe whether tail formation by FLAG–WASP microspheres also correlated with movement. FLAG–WASP microspheres moved at an average rate of 0.2 μm/minute (Figure 2e), which was three times slower than the mean rate of *L. monocytogenes* motility in the extract (0.6 ± 0.1 μm/minute). The discrepancy in motility rates may be caused by differences in the size and shape of the moving particles, by the distribution of WASP or ActA on the surface of the particles, or by the fact that ActA may be more efficient than WASP because it competes with WASP for the Arp2/3 complex. Nevertheless, the observation that WASP-coated microspheres polymerized actin and moved in cell extracts indicates that WASP is sufficient to direct actin-based motility in this context and suggests that WASP functions in this capacity in cells.

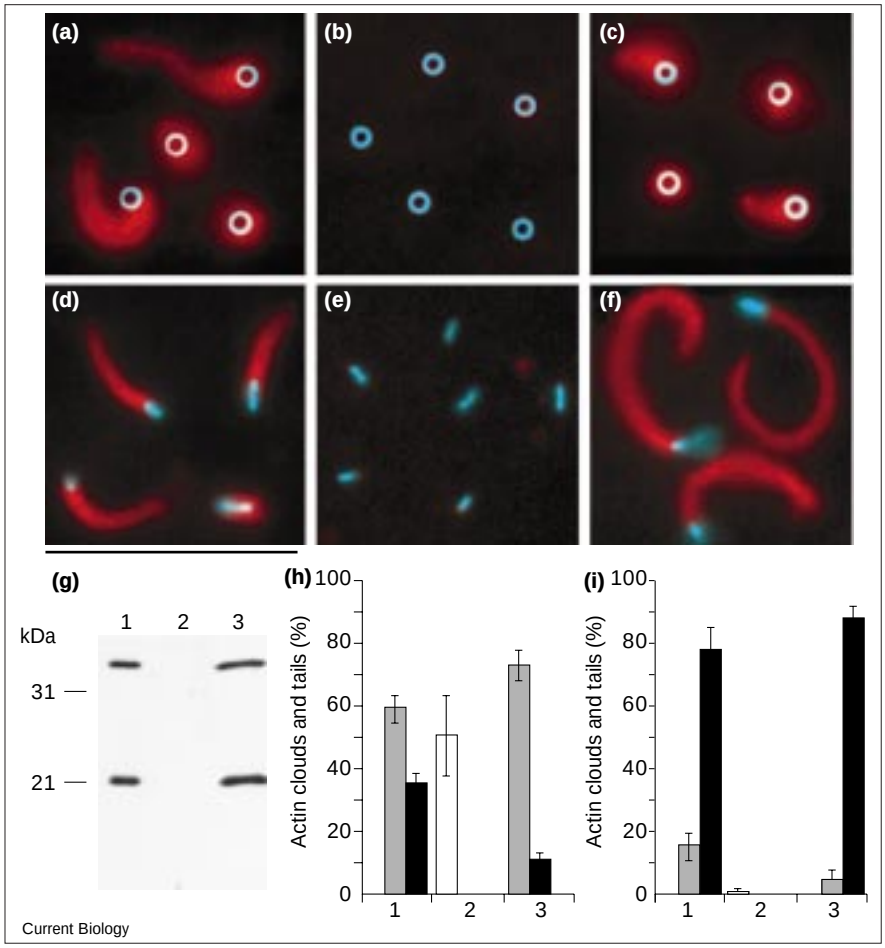
The biochemical interaction between WASP and the Arp2/3 complex *in vitro* suggested that the ability of WASP to induce actin polymerization in cell extracts was mediated by the Arp2/3 complex. Similarly, the Arp2/3 complex has been proposed to mediate the actin polymerization that is induced by *L. monocytogenes* [6,8]. To determine the functional importance of the Arp2/3 complex, it was removed from extracts by immunodepletion using a mixture of affinity-purified antibodies that recognize three of the seven subunits of the complex: Arp3, p41-Arc and p16-Arc. More than 95% of the Arp2/3 complex was removed from the depleted extract when compared with the control extract, which was mock-depleted using

Figure 2



Behavior of WASP–FLAG microspheres in bovine-brain extract. (a,c,e) are fluorescence-microscopy images; (b,d) are phase-contrast images. (a) Composite image of rhodamine-labeled actin structures formed by FLAG–WASP microspheres incubated in bovine-brain extract. (b) The same FLAG–WASP microspheres as in (a). (c) Composite image of rhodamine-labeled actin structures formed by control FLAG–Smad3 microspheres incubated in bovine-brain extract. (d) The same FLAG–Smad3 microspheres as in (c). (e) Time-lapse images of a rhodamine-labeled actin tail trailing behind a moving FLAG–WASP bead in bovine-brain extract. Images were taken at 150 sec intervals. The arrow indicates the original position of the microsphere. The scale bar represents 10 μm.

Figure 3



The effect of Arp2/3-complex depletion on WASP-bead and *L. monocytogenes* motility. (a–c) Composite images of actin structures formed by FLAG–WASP microspheres. WASP microspheres are shown false colored in blue, and rhodamine-labeled actin is shown in red. (a) Actin tails and clouds in control mock-depleted bovine-brain extract; (b) residual actin in Arp2/3-complex-depleted extract and (c) actin tails and clouds in Arp2/3-complex-depleted extract supplemented with pure Arp2/3 complex. (d–f) Composite images of actin structures formed by *L. monocytogenes*. Bacteria are shown labeled with DAPI in blue, and actin labeled with rhodamine in red. (d) Control mock-depleted bovine-brain extract; (e) Arp2/3-complex-depleted extract; (f) Arp2/3-complex-depleted extract supplemented with pure Arp2/3 complex. The scale bar for (a–f) represents 10 μm. (g) Immunoblot of bovine-brain extract probed with antibodies against the Arp2/3 complex components p34-Arc and p21-Arc. Lane 1, mock-depleted extract; Lane 2, Arp2/3-complex-depleted extract; Lane 3, Arp2/3-complex-depleted extract supplemented with pure Arp2/3 complex. (h,i) Graphs illustrating the percentage of (h) WASP beads or (i) *L. monocytogenes* that formed actin clouds (gray), residual clouds (white) and tails (black) in (1) mock-depleted extract, (2) Arp2/3-complex-depleted extract and (3) Arp2/3-complex-depleted extract supplemented with pure Arp2/3 complex.

nonspecific rabbit immunoglobulin G (Figure 3g). In the control extract, a high percentage of FLAG–WASP microspheres (Figure 3a,h) and *L. monocytogenes* (Figure 3d,i) polymerized actin clouds and tails. Depletion of the Arp2/3 complex abolished actin tail formation by FLAG–WASP microspheres (Figure 3b,h) and *L. monocytogenes* (Figure 3e,i). Moreover, depletion greatly reduced the size and fluorescence intensity of actin clouds (Figure 3b,e). Residual actin, which appeared as a faint outline, was observed at the surface of many beads (Figure 3b,h), and a small number of bacteria (Figure 3e,i). The residual actin may have been polymerized by Arp2/3 complex remaining in the extract after depletion (<5% of the endogenous pool), or by other unknown factors. Moreover, in the case of FLAG–WASP microspheres, residual actin may have been bound directly by WASP.

To demonstrate that the effect of Arp2/3-complex depletion on actin polymerization was indeed caused by the removal of the Arp2/3 complex, purified Arp2/3 complex was added back to the depleted extracts. Addition of the Arp2/3 complex at a concentration comparable to endogenous levels (Figure 3g) restored the ability of FLAG–WASP beads to polymerize actin clouds and tails in extracts (Figure 3c,h), although, in general, tails were shorter and less abundant than in the control mock-depleted extract. Addition of Arp2/3 complex completely restored actin-cloud and actin-tail formation by *L. monocytogenes* (Figure 3f,i). The lower level of rescue of FLAG–WASP-microsphere-based actin-tail formation suggests that bead motility may be more sensitive than *L. monocytogenes* motility to removal and replacement of the Arp2/3 complex, perhaps because of a physical property of the beads or a more stringent requirement for other factors in the extract. In either case, these findings indicate that the Arp2/3 complex is necessary for actin-based motility and robust actin polymerization by FLAG–WASP microspheres and *L. monocytogenes* in bovine-brain extracts.

Our results demonstrate that WASP is sufficient to direct actin-polymerization-based motility in cell extracts. Moreover, we have shown that WASP works in concert with the

Arp2/3 complex, both *in vitro* and in cell extracts, to stimulate actin nucleation. WASP may participate indirectly in nucleation by inducing a conformational change in the Arp2/3 complex, or directly by providing an additional binding interface for actin. Regulation of the interaction between WASP and the Arp2/3 complex may be a central control mechanism for modulating the spatial and temporal distribution of actin polymerization in cells. Mutations in the WASP gene cause defects in platelet morphology, macrophage chemotaxis [2,3,5], T-lymphocyte activation and antigen-receptor capping [18]. Actin nucleation by the Arp2/3 complex and WASP may thus be critical for these cellular functions. Diverse WASP-family proteins, which include N-WASP [19] and WAVE/Scar [20,21], may serve to differentially regulate the Arp2/3 complex in distinct cell types. Interestingly, although *L. monocytogenes* ActA may mimic WASP-family proteins, the *Shigella flexneri* equivalent, IcsA/VirG, appears to recruit N-WASP to harness the host's actin cytoskeleton [22].

WASP contains multiple binding sites for proteins involved in signal transduction, suggesting that it may function to integrate information from a diverse set of signals to activate the Arp2/3 complex and actin polymerization. One WASP binding partner is the Rho-family GTPase Cdc42 (reviewed in [23]), which can induce actin polymerization mediated by the Arp2/3 complex at the surface of beads in cell extract [24]. We have been unable to detect any effect of activated Cdc42 on actin nucleation by WASP and the Arp2/3 complex, however (M.D.W. and A.A., unpublished observations). Cdc42 may work to regulate the localization of WASP to the plasma membrane or to sites where rapid polymerization is required. The activity and localization of WASP may also be regulated by signalling pathways involving tyrosine kinases (reviewed in [23,25]). Thus, multiple pathways may act in concert to regulate the activity of WASP-family proteins and the Arp2/3 complex, and delineation of this set of regulatory interactions will help to elucidate the molecular mechanisms of cell morphogenesis, cell locomotion and Wiskott-Aldrich syndrome.

Supplementary material

Additional methodological details are published with this article on the internet.

Acknowledgements

We thank Tim Mitchison for help with antibody production and Shannon Stroschein and Kunxin Luo for FLAG–Smad3 protein. We are indebted to David Drubin, Rebecca Heald, Dan Portnoy, Claire Walczak and Karsten Weis for advice and comments on the manuscript. We are grateful to Zac Cande and his laboratory for help with microscopy. This work was supported by NIH grant GM56478 to A.A., and by funds from the University of California at Berkeley to M.D.W; M.D.W. is a Leukemia Society of America Special Fellow.

References

1. Derry JM, Ochs HD, Francke U: Isolation of a novel gene mutated in Wiskott-Aldrich syndrome. *Cell* 1994, 79:635-644.
2. Ochs HD, Slichter SJ, Harker LA, Von Behrens WE, Clark RA, Wedgwood RJ: The Wiskott-Aldrich syndrome: studies of lymphocytes, granulocytes, and platelets. *Blood* 1980, 55:243-252.
3. Molina IJ, Kenney DM, Rosen FS, Remold-O'Donnell E: T cell lines characterize events in the pathogenesis of the Wiskott-Aldrich syndrome. *J Exp Med* 1992, 176:867-874.
4. Symons M, Derry JM, Karlak B, Jiang S, Lemahieu V, McCormick F, *et al.*: Wiskott-Aldrich syndrome protein, a novel effector for the GTPase CDC42Hs, is implicated in actin polymerization. *Cell* 1996, 84:723-734.
5. Zicha D, Allen WE, Brickell PM, Kinnon C, Dunn GA, Jones GE, *et al.*: Chemotaxis of macrophages is abolished in the Wiskott-Aldrich syndrome. *Brit J Haematol* 1998, 101:659-665.
6. Welch MD, Iwamatsu A, Mitchison TJ: Actin polymerization is induced by the Arp2/3 protein complex at the surface of ***Listeria monocytogenes***. *Nature* 1997, 385:265-269.
7. Mullins RD, Heuser JA, Pollard TD: The interaction of Arp2/3 complex with actin: nucleation, high affinity pointed end capping, and formation of branching networks of actin filaments. *Proc Natl Acad Sci USA* 1998, 95:6181-6186.
8. Welch MD, Rosenblatt J, Skoble J, Portnoy DA, Mitchison TJ: Interaction of human Arp2/3 complex and the ***Listeria monocytogenes*** ActA protein in actin filament nucleation. *Science* 1998, 281:105-108.
9. Machesky LM, Insall RH: Scar1 and the related Wiskott-Aldrich syndrome protein, WASP, regulate the actin cytoskeleton through the Arp2/3 complex. *Curr Biol* 1998, 8:1347-1356.
10. Kouyama T, Mihashi K: Fluorimetry study of N-(1-pyrenyl)iodoacetamide-labelled F-actin: local structural change of actin protomer both on polymerization and on binding of heavy meromyosin. *Eur J Biochem* 1981, 114:33-38.
11. Cooper JA, Walker SB, Pollard TD: Pyrene actin: documentation of the validity of a sensitive assay for actin polymerization. *J Muscle Res Cell Motil* 1983, 4:253-262.
12. Kocks C, Gouin E, Tabouret M, Berche P, Ohayon H, Cossart P: ***L. monocytogenes***-induced actin assembly requires the actA gene product, a surface protein. *Cell* 1992, 68:521-531.
13. Domann E, Wehland J, Rohde M, Pistor S, Hartl M, Goebel W, *et al.*: A novel bacterial virulence gene in ***Listeria monocytogenes*** required for host cell microfilament interaction with homology to the proline-rich region of vinculin. *EMBO J* 1992, 11:1981-1990.
14. Smith GA, Portnoy DA, Theriot JA: Asymetric distribution of the ***Listeria monocytogenes*** ActA protein is required and sufficient to direct actin-based motility. *Mol Microbiol* 1995, 17:945-951.
15. Kocks C, Marchand JB, Gouin E, d'Hauteville H, Sansonetti PJ, Carlier MF, *et al.*: The unrelated surface proteins ActA of ***Listeria monocytogenes*** and IcsA of ***Shigella flexneri*** are sufficient to confer actin-based motility on ***Listeria innocua*** and ***Escherichia coli*** respectively. *Mol Microbiol* 1995, 18:413-423.
16. Cameron LA, Footer MJ, van Oudenaarden A, Theriot JA: Motility of ActA protein-coated microspheres driven by actin polymerization. *Proc Natl Acad Sci USA* 1999, 96:4809-4913.
17. Stroschein S, Wang W, Luo K: Cooperative binding of Smad proteins to two adjacent DNA elements in the plasminogen activator inhibitor-1 promoter mediates TGF-β induced, Smad dependent transcriptional activation. *J Biol Chem* 1999, 274:9431-9441.
18. Snapper SB, Rosen FS, Mizoguchi E, Cohen P, Khan W, Liu CH, *et al.*: Wiskott-Aldrich syndrome protein-deficient mice reveal a role for WASP in T but not B cell activation. *Immunity* 1998, 9:81-91.
19. Miki H, Miura K, Takenawa T: N-WASP, a novel actin-depolymerizing protein, regulates the cortical cytoskeletal rearrangement in a PIP2-dependent manner downstream of tyrosine kinases. *EMBO J* 1996, 15:5326-5335.
20. Miki H, Suetsugu S, Takenawa T: WAVE, a novel WASP-family protein involved in actin reorganization induced by Rac. *EMBO J* 1998, 17:6932-6941.
21. Bear JE, Rawls JF, Saxe CL 3rd: SCAR, a WASP-related protein, isolated as a suppressor of receptor defects in late ***Dictyostelium*** development. *J Cell Biol* 1998, 142:1325-1335.
22. Suzuki T, Miki H, Takenawa T, Sasakawa C: Neural Wiskott-Aldrich Syndrome protein is implicated in the actin-based motility of ***Shigella flexneri***. *EMBO J* 1998, 17:2767-2776.
23. Ramesh N, Anton IM, Martinez-Quiles N, Geha RS: Waltzing with WASP. *Trends Cell Biol* 1999, 9:15-19.
24. Ma L, Rohatgi R, Kirschner MW: The Arp2/3 complex mediates actin polymerization induced by the small GTP-binding protein Cdc42. *Proc Nat Acad Sci USA* 1998, 95:15362-15367.
25. Abo A: Understanding the molecular basis of Wiskott–Aldrich syndrome. *Cell Molec Life Sci* 1998, 54:1145-1153.

Supplementary material

The Wiskott–Aldrich syndrome protein directs actin-based motility by stimulating actin nucleation with the Arp2/3 complex

Defne Yarar, Wayne To, Arie Abo and Matthew D. Welch

Current Biology 10 May 1999, 9:555–558

Supplementary materials and methods

Purification of the Arp2/3 complex and WASP

The Arp2/3 complex was purified as described previously [S1,S2]. Recombinant human FLAG–WASP was expressed in Sf-9 insect cells using the baculovirus system. FLAG–WASP was partially purified from cell lysates by binding to anti-FLAG monoclonal-antibody M2 affinity gel (Eastman Kodak) and eluting with FLAG peptide (Eastman Kodak). Eluted protein was further purified by chromatography on a MonoQ column (Amersham Pharmacia Biotech) and subsequently on a superdex-200 column (Amersham Pharmacia Biotech) equilibrated with control buffer for the pyrene–actin assay [S3].

Pyrene–actin assay

The pyrene–actin assay was carried out as described previously [S3] with the following exception: monomeric actin that was isolated by centrifugation was further purified by chromatography on a superdex-200 column (Amersham Pharmacia Biotech) equilibrated with G buffer [S3].

Preparation of WASP microspheres

Monoclonal M2 anti-FLAG antibody (12 μg) was bound to 10 μl of 1 μm diameter protein-A-conjugated microspheres (Bangs Laboratories). Microspheres were washed into extract buffer (20 mM HEPES pH 7.7, 50 mM KCl, 2 mM $MgCl_2$, 5 mM EGTA, 1 mM EDTA, 100 mM sucrose). Of the 10 μl sample of microspheres, 5 μl were incubated with 5 μg FLAG–WASP and the other 5 μl with 8 μg FLAG–Smad3 protein [S4]. Coated microspheres were washed and resuspended in extract buffer.

WASP-microsphere and L. monocytogenes *assays*

Frozen bovine brain was pulverized and ground in liquid nitrogen, then mixed with an equal volume of extract buffer supplemented with 0.5 mM ATP, 1 mM leupeptin, 1 mM chymostatin, 1 mM pepstatin and 0.5 mM DTT. Brain high-speed supernatant was obtained by centrifugation at 100,000 × *g* for 15 min (final protein concentration 7 mg/ml). Similar extracts have been used to study *L. monocytogenes* motility [S5,S6]. To assay for actin polymerization and motility, microspheres or DAPI-labelled *L. monocytogenes* [S2] were incubated in brain extract supplemented with actin labeled with N-hydroxysuccinimidyl 5-carboxytetramethyl rhodamine (1 μM) as described previously [S1,S2], except that 0.5% methyl cellulose was also added.

Antibody preparation and immunodepletion

A polyclonal antibody that recognizes the isoform of p41-Arc that is expressed in brain, Sop2Hs [S7], was made by immunizing rabbits with the synthetic peptide CKTLESSIQGLRIM (in the single-letter amino-acid code), which corresponds to the carboxy-terminal 13 amino acids of Sop2Hs. An anti-human-Arp3 antibody was made by immunizing rabbits with the peptide CKRTVDARLKLSEELSGGRLKPK, which corresponds to amino acids 340–361 of Arp3. An anti-human-p16-Arc antibody was made by immunizing rabbits with the peptide CKGFESPSDNSSAMLLQ, which corresponds to amino acids 112–127 of p16-Arc. Peptide-conjugation, antibody affinity-purification and antibody storage procedures were performed as described previously [S8].

For immunodepletion, random rabbit IgG (90 μg; Jackson Laboratories), or a combination of anti-Sop2Hs antibody (27 μg), anti Arp3(340–361) antibody (42 μg), and anti-p16(112–127) antibody (35 μg) was bound to 50 μl Affiprep protein-A beads (BioRad Labs). Beads were washed with extract buffer and then incubated with 60 μl bovine-brain high-speed supernatant for 40 min at 4°C. Beads were pelleted and the supernatant was subjected to one or two more rounds of depletion, yielding the depleted extract. For rescue, 0.2–1 μl of purified Arp2/3 complex was added to the depleted extract. The amount of added Arp2/3 complex was quantified by immunoblotting using antibodies to p34-Arc and p21-Arc [S9].

Supplementary references

S1. Welch MD, Iwamatsu A, Mitchison TJ: Actin polymerization is induced by the Arp2/3 protein complex at the surface of ***Listeria monocytogenes***. *Nature* 1997, 385:265-269.

S2. Welch MD, Mitchison TJ: Purification and assay of the platelet Arp2/3 complex. *Methods Enzymol* 1998, 298:52-61.

S3. Welch MD, Rosenblatt J, Skoble J, Portnoy DA, Mitchison TJ: Interaction of human Arp2/3 complex and the ***Listeria monocytogenes*** ActA protein in actin filament nucleation. *Science* 1998, 281:105-108.

S4. Stroschein S, Wang W, Luo K: Cooperative binding of Smad proteins to two adjacent DNA elements in the plasminogen activator inhibitor-1 promoter mediates TGF-β induced, Smad dependent transcriptional activation. *J Biol Chem* 1999, 274:9431-9441.

S5. David V, Gouin E, Troys MV, Grogan A, Segal AW, Ampe C, *et al*.: Identification of cofilin, coronin, Rac and capZ in actin tails using a ***Listeria*** affinity approach. *J Cell Sci* 1998, 111:2877-2884.

S6. Laurent V, Loisel TP, Harbeck B, Wehman A, Grobe L, Jockusch BM, *et al*.: Role of proteins of the Ena/VASP family in actin-based motility of ***Listeria monocytogenes***. *J Cell Biol* 1999, 144:1245-1258.

S7. Balasubramanian MK, Feoktistova A, McCollum D, Gould KL: Fission yeast Sop2p: a novel and evolutionarily conserved protein that interacts with Arp3p and modulates profilin function. *EMBO J* 1996, 15:6426-6437.

S8. Welch MD, DePace AH, Verma S, Iwamatsu A, Mitchison TJ: The human complex is composed of evolutionarily conserved subunits and is localized to cellular regions of dynamic actin filament assembly. *J Cell Biol* 1997, 138:375-384.

S9. Sawin KE, Mitchison TJ, Wordeman LG: Evidence for kinesin-related proteins in the mitotic apparatus using peptide antibodies. *J Cell Sci* 1992, 101:303-313.