The Journal of Experimental Biology 206, 1977-1984

doi:10.1242/jeb.00240

Review

Actin comet tails, endosomes and endosymbionts

Kammy Fehrenbacher*, Thomas Huckaba*, Hyeong-Cheol Yang, Istvan Boldogh and Liza Pon†

Department of Anatomy and Cell Biology, Columbia University, New York, NY, USA

*These individuals contributed equally to this manuscript

†Author for correspondence (e-mail: lap5@columbia.edu)

Accepted 15 January 2003

Summary

The Arp2/3 complex consists of seven highly conserved and tightly associated subunits, two of which are the actin-related proteins Arp2 and Arp3. One of the best-studied functions of the Arp2/3 complex is to stimulate actin nucleation and force production at the leading edge of motile cells. What is now clear is that Arp2/3-complex-mediated force production drives many intracellular movements, including movement of bacterial pathogens in infected host cells, internalization of extracellular materials *via* phagocytosis and endocytosis, and movement of mitochondria during cell division in budding yeast. Here, we describe recent advances in the mechanisms underlying Arp2/3 complex-driven intracellular movement.

Key words: actin, cytoskeleton, endocytosis, mitochondria, organelle movement, pathogen.

The Arp2/3 complex

The Arp2/3 complex was first isolated from *Acanthomoeba castellanii* on the basis of its affinity for the actin binding protein, profilin (Machesky et al., 1994). Subsequently, the complex has been identified in and purified from many different organisms such as humans (Welch et al., 1997b), other vertebrates (Ma et al., 1998; Rohatgi et al., 1999) and the yeast *Saccharomyces cerevisiae* (Winter et al., 1997). Based on X-ray crystallography data, the two largest subunits, Arp2 and Arp3, are thought to form a heterodimer template for actin filament formation after activation of the entire complex (Robinson et al., 2001; Kelleher et al., 1995). This Arp2/3 complex plays two important roles in the cell: nucleation of new actin filaments in response to upstream factors (Ma et al., 1998; Mullins and Pollard, 1999; Rohatgi et al., 1999), and cross-linking newly formed actin filaments into dendritic arrays containing branch angles of 70° (Blanchoin et al., 2000; Mullins et al., 1998). In the latter case, the Arp2/3 complex is positioned at the branch point between filaments and stabilizes the cross-link both *in vivo* and *in vitro* (Svitkina and Borisy, 1999; Volkmann et al., 2001). It is thought that these two activities coordinate in the cell to create new actin filaments and to connect them into a higher-order dendritic actin array.

The Arp2/3 complex is regulated by a host of nucleation promoting factors (NPFs; Table·1) that are quite diverse in both structural organization and the proteins with which they interact in the cell. All NPFs contain a conserved Arp2/3 binding sequence, or CA region, consisting of a short stretch of basic (connector region or C) and acidic (acidic region or A) amino acids that is necessary for the Arp2/3 complex activation. Binding of the CA region of NPFs to the Arp2/3 complex induces a conformation change in the complex that facilitates actin nucleation activity. However, the CA region is not sufficient for activation of the Arp2/3 complex. The NPF must also have either a G-actin binding site (Class I NPFs) or an F-actin binding site (Class II NPFs), adjacent to the CA element. In the case of Class I NPFs, presentation of G-actin by the NPF to Arp2/3 complex may facilitate formation of a nucleus for actin polymerization. The role of the F-actin binding site of Class II NPFs in activation of the Arp2/3 complex remains to be determined.

Roles of the Arp2/3 complex

Movement of bacterial pathogens

The earliest studies implicating the Arp2/3 complex in intracellular movements focused on the bacterial pathogens *Listeria*, *Shigella* and the spotted fever group of *Rickettsia*, which exploit the host cytoskeleton for propulsion both intracellularly and between cells (Goldberg, 2001). Although many pathogens utilize the host cytoskeleton for uptake and consequent infection, these three are distinct. All three escape the phagosomal membrane shortly after uptake. Thereafter, a ‘comet tail’, consisting of F-actin and various actin binding proteins, develops on the surface of the bacteria (Fig.·1). The comet tail drives movement of bacterial pathogens both within the cytoplasm of the infected host cells and from one host cell to the next.

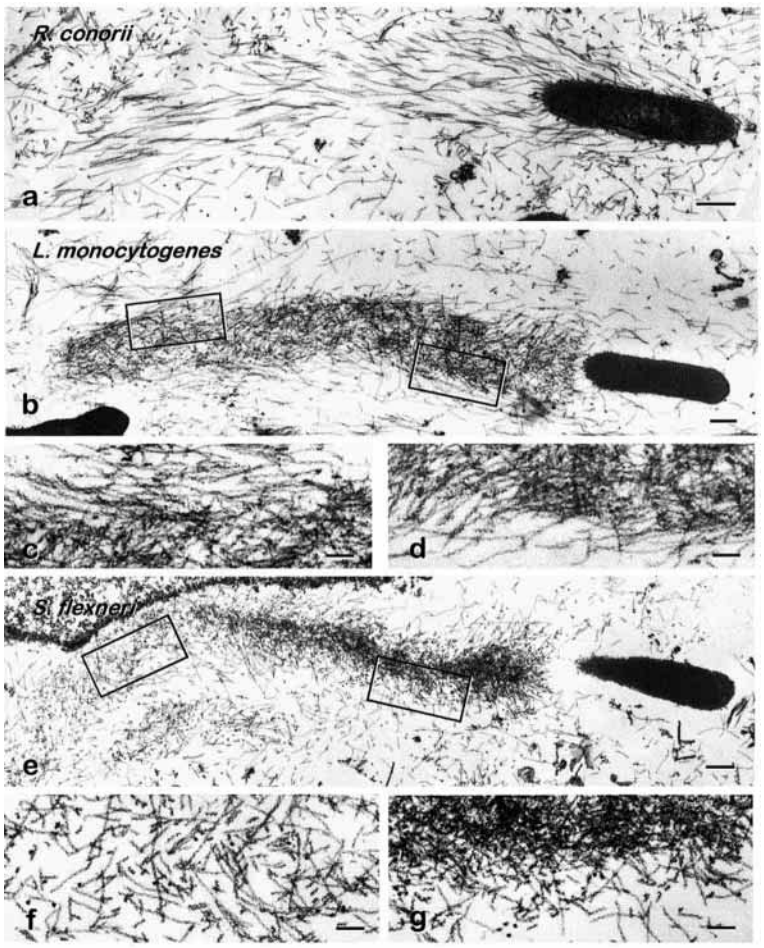

Fig. 1. Electron microscopy of myosin S1-decorated actin tails of *R. conorii* (a), *L. monocytogenes* (b) and *S. flexneri* (e) in Hep2 cells. The boxes in b and e are shown at higher magnification in (c,d) and (f,g), respectively. Scale bars: 0.2 μm (a,b); 0.4 μm (e); 50 nm (c,d,f,g). Reproduced from Gouin et al. (1999), with permission from Company of Biologists, Ltd.

Decoration of the comet tails with the S1 fragment of myosin revealed that the barbed ends of the filaments are oriented toward the bacterial surface of each organism, indicating that polymerization is taking place on or near the bacteria (Tilney et al., 1990; Gouin et al., 1999; Heinzen et al., 1999; Van Kirk et al., 2000). However, the rates of movement of these bacteria, their direction of movement, the morphology of their comet tails, and the organization of F-actin and actin-binding protein composition in the actin comet tail vary in different species. *L. monocytogenes* and *S. flexneri* have comparable rates of movement, while *R. conorii* and *R. rickettsii* move at about one-third that rate (Gouin et al., 1999; Heinzen et al., 1999). In addition, *L. monocytogenes* often moves in tight circles, while *R. rickettsii* tends to track along straight lines, changing direction only when it comes in contact with other structures in the cell (Heinzen et al., 1999). Finally, while the comet tails of *Listeria* and *Shigella* tend to be shorter and composed of highly F-actin branched structures, the tails of the spotted fever *Rickettsia* are longer and contain unbranched bundles of actin filaments (Fig.·1). Moreover, the half-life of the actin filaments in the comet tail of *Rickettsia* is three times that of *Listeria* (Tilney and Portnoy, 1989; Gouin et al., 1999; Heinzen et al., 1999; Van Kirk et al., 2000).

The recruitment of different actin-binding proteins likely contributes to the morphological differences and distinct turnover rates observed in the actin tails of the three microorganisms. Only VASP and α-actinin localize to the actin tail of *R. conorii* by immunofluorescence, while the actin tails of *L. monocytogenes* and *S. flexneri* contain additional proteins. These include the Arp2/3 complex, profilin, gelsolin, ezrin, coronin, capping protein and ADF/cofilin (Loisel et al., 1999; Smith et al., 1996; Theriot et al., 1994; Gouin et al., 1999; Laine et al., 1997; Carlier et al., 1997; Rosenblatt et al., 1997).

The mechanism of actin comet tail formation is best understood in *L. monocytogenes* and *S. flexneri*. *Listeria monocytogenes* and its closely related species *Listeria ivanovii* produce the surface proteins ActA and iActA, respectively.

Table·1. *Arp2/3 complex nucleation promoting factors (NPFs)*

NPF	Reference
ActA protein of *Listeria monocytogenes*	Welch et al., 1998
WASP (Wiskott–Aldrich syndrome protein)	Winter et al., 1999b; Yarar et al., 1999
N-WASP	Rohatgi et al., 1999
Suppressor of cAR (Scar/WASP family)	Machesky et al., 1999
Verprolin homologous proteins (WAVE)	Machesky et al., 1999
Myosin I proteins of yeast	Evangelista et al., 2000; Lechler et al., 2000; Lee et al., 2000
Metazoan cortactin	Urono et al., 2001; Weaver et al., 2001; Weed et al., 2000
Capping protein of amoeba (Arp213)	Jung et al., 2001
Myosin linker (CARMIL)	Jung et al., 2001
Abp1p of yeast	Goode et al., 2001
Pan1p of yeast	Duncan et al., 2001

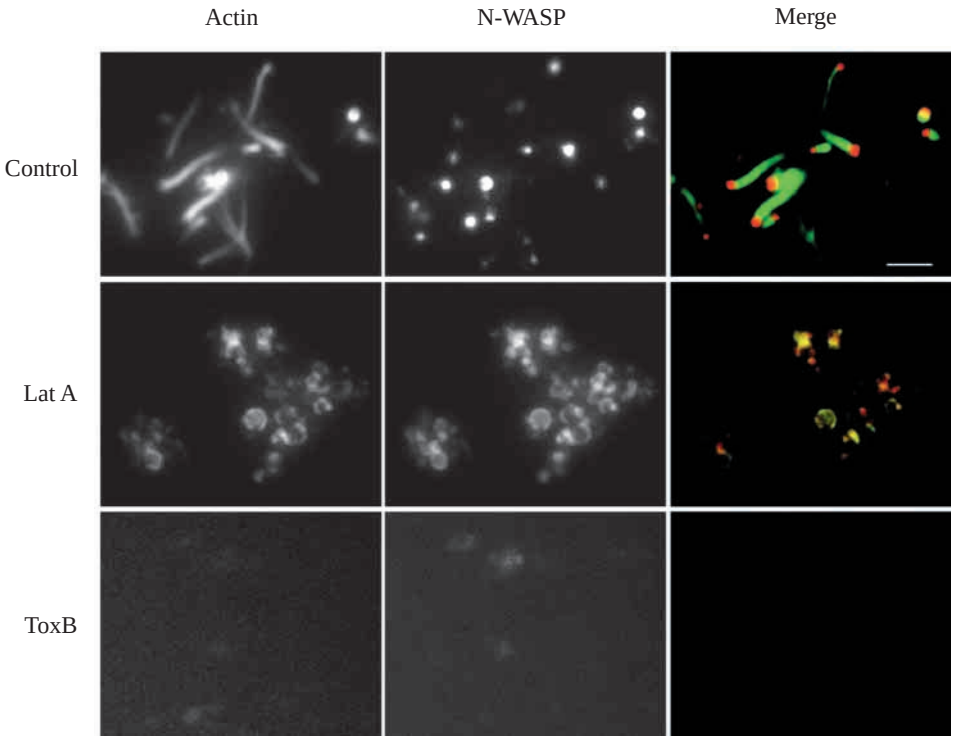

Fig.·2. PMA (PKC activator phorbol myristate acetate) stimulates the recruitment of N-WASP to vesicles associated with actin comet tails. Cell-free vesicle motility assays containing *Xenopus* cytosol, membranes and 1·μmol·l^{-1} PMA were fixed in perfusion chambers and immunolabeled with affinity-purified anti-N-WASP antibodies followed by Texas Red-labeled secondary antibodies and FITC–phalloidin. N-WASP and F-actin recruitment occurred in the presence of 75·μmol·l^{-1} latrunculin A (Lat A), but comet tails were not observed. ToxB treatment prevented both N-WASP and F-actin recruitment, although nonspecific labeling of fixed membranes could be observed with long exposure times. Bar, 5·μm. Reproduced from Taunton et al. (2000), with permission from the Rockefeller University Press.

The ActA protein is the only bacterial protein that is required for actin-based movement of *Listeria monocytogenes*. Mammalian cells transfected with the ActA gene have a greater amount of cellular F-actin (Pistor et al., 1994). In addition, non-motile bacteria exhibit actin-based motility in cell extracts upon expression of ActA (Smith et al., 1995; Kocks et al., 1995). Most significantly, ActA-coated polystyrene beads generate directional movement in cytoplasmic extracts (Cameron et al., 1999).

The ActA protein shares some homology with WASP family members and stimulates actin nucleation activity of the Arp2/3 complex (May et al., 1999; Welch et al., 1997a). ActA has VASP binding activity and colocalizes with VASP on the surface of *Listeria* (Chakraborty et al., 1995). This suggests that the ActA protein recruits the Arp2/3 complex and stimulates Arp2/3 complex actin nucleating activity, perhaps synergistically with VASP on the surface of *Listeria*. By activating the Arp2/3 complex specifically at one pole these bacteria generate directional movement *via* continuous actin assembly and the resultant formation of an actin comet tail.

In *Shigella*, IcsA, previously identified as VirG (Lett et al., 1989), is the pathogen-encoded protein responsible for Arp2/3 complex-driven movement. Deletion of the *IcsA* gene causes a loss of actin comet tail assembly (Bernardini et al., 1989). *E. coli* expressing IcsA are able to form actin comet tails in cytoplasmic extract (Kocks et al., 1995). In addition, IcsA-coated silica particles form actin comet tails in cytoplasmic extract (Goldberg, 2001). Taken together, these data indicate that the IcsA protein is the only *Shigella* protein that is necessary to induce actin polymerization and directed movement.

IcsA binds N-WASP *via* a series of glycine-rich repeats near the N terminus of IcsA and recruits N-WASP to the trailing edge of *Shigella* nearest the comet tail (Suzuki et al., 1998). N-WASP$^{-/-}$ *Shigella* cells do not form actin comet tails, further supporting the role of N-WASP recruitment by IcsA in actin comet tail formation (Snapper et al., 2001). N-WASP then binds to and activates the Arp2/3 complex, bringing the active actin nucleator to the bacterial surface. Surprisingly, the *in vitro* interaction between IcsA and N-WASP is a more potent stimulator of Arp2/3 activation than Cdc42 (Egile et al., 1999).

Much less is known about the mechanism whereby the spotted fever group of *Rickettsia* (including *R. conorii*, *R. montana*, *R. parkeri*, *R. akari*, *R. australis* and *R. rickettsii*) control actin polymerization. A member of the typhus group, *R. typhi*, also forms comet tails, although these are much shorter than in the spotted fever group. It is unclear whether the Arp2/3 complex is even involved, as constituents of the Arp2/3 complex have not been detected in the actin tails of *R. conorii* (Gouin et al., 1999). In fact, VASP and α-actinin were the only actin-binding proteins found in the comet tails. What is known is that *Rickettsial* protein synthesis is necessary for actin-based motility, so it may yet be that an ActA/IcsA-type protein will be found on the surface of *Rickettsia* (Heinzen et al., 1993).

Endocytosis

Imaging studies on cells not infected with bacterial or viral pathogens revealed that subunits of the Arp2/3 complex localize to the leading edge of motile cells and to punctate intracellular structures (Welch et al., 1997b). This suggested

that the function of the Arp2/3 complex was not restricted to extension of lamellopodia. Indeed, recent studies implicate the Arp2/3 complex and actin polymerization during endocytosis.

Endocytosis occurs in five basic steps: membrane invagination, coated pit formation, coated pit sequestration, detachment of a newly formed vesicle, and movement of the new endocytic compartment away from the plasma membrane into the cytosol. A growing body of evidence suggests that the actin cytoskeleton plays a role in each endocytic step. First, an actin cytoskeleton underlying the plasma membrane could localize the endocytic machinery to specific regions in the plasma membrane by providing a diffusion barrier or by anchoring components directly (Gaidarov et al., 1999). The actin cytoskeleton could also deform or invaginate the plasma membrane, providing a membrane curvature, which could facilitate the coating machinery in pinching off the membrane. Moreover, it has been shown that a rigid cortical actin cytoskeleton can inhibit membrane traffic (Trifaró and Vitale, 1993), and actin fibers are essentially absent in regions immediately surrounding clathrin-coated pits (Fujimoto et al., 2000). Finally, actin could participate in membrane fission events, responsible for freeing endocytosed vesicles from the plasma membrane (Lamaze et al., 1997).

Recent studies indicate that the Arp2/3 complex may participate in multiple steps during endocytosis. The Arp2/3 complex has been implicated in membrane internalization in *Dictyostelium* and budding yeast. Phagocytosis and macropinocytosis are actin-dependent mechanisms. Arp2/3 complex subunits have been localized at the site of particle attachment during phagocytosis, at the phagocytotic cup, to endosomes at early stages (i.e. uptake), and at late and post-lysosomal stages after internalization in *Dictyostelium* (Insall et al., 2001). In *Saccharomyces cerevisiae*, mutations in the Arp2 subunit of the Arp2/3 complex, or in type I myosins, proteins that colocalize with and activate Arp2/3 complex nucleation *in vitro*, also show defects in endocytosis. In addition, *arp2-1* mutants were synthetically lethal with *end3-1*, a known endocytosis mutant (Moreau et al., 1997). The interaction of Arp2/3 complex nucleation-promoting factors and endocytic proteins suggests a model in which actin polymerization might provide the force behind plasma membrane invagination or the ‘pinching off’ of endocytic vesicles.

Taunton et al. (2000) provided evidence that Arp2/3 complex participates in another step in endocytosis, namely in endosome movement. First, they observed formation of actin comet tails on endosomes and movement of these vesicles in *Xenopus* extract and eggs. Consistent with this, they found that Rho GTPase inhibitors blocked the observed formation of actin comet tails, and that actin comet tails were restored upon treatment with Cdc42, a protein that stimulates WASP- and Arp2/3 complex-mediated actin assembly. Finally, the group showed that N-WASP colocalizes with the membrane-proximal ends of comet tails *in vitro*, and that recruitment of N-WASP to endosomes is blocked by the Rho GTPase inhibitor ToxB but not by an agent that inhibits actin polymerization (Figs·2, 3). Taken together, these data suggest a mechanism for endosome movement whereby Cdc42 drives recruitment of an Arp2/3 complex activator to the endosome. Arp2/3 complex-mediated actin nucleation then drives actin comet tail formation and endosome movement.

The link between the Arp2/3 complex and endocytosis is supported by findings in many cell systems. First, actin comet tails have been observed on endosomes or lysosomes in a number of other cell systems including HeLa cell extracts, cultured mast cells and NIH 3T3 cells (Merrifield et al., 1999; Kaksonen et al., 2000). Second, cortactin, Abp1p and Pan1p have been implicated in endocytosis (Duncan et al., 2001; Goode et al., 2001; Urono et al., 2001; Weaver et al., 2001). All of these NPFs fall outside of the SCAR/ WASP (Class I NPF) family of proteins, and appear to activate Arp2/3 complex by a mechanism that is different from that of the SCAR/ WASP proteins. Finally, recent studies using GFP fused to the pheromone receptor, Ste2p, as a marker for endosomes, provide preliminary evidence that endosomal motility in budding yeast depends on the Arp2/3 complex activator, Las17p/Bee1p, and F-actin polymerization (Chang and Blumer, 2002).

Mitochondrial movement and inheritance in budding yeast

Mitochondria are essential organelles that cannot be synthesized *de novo*. Therefore, these organelles must be transferred from mother to developing daughter cell during cell division. In budding yeast, mitochondria exist as a branched network at the cell cortex that undergoes remodeling by fission, fusion and movement (Stevens, 1977; Nunnari et al., 1997; Simon et al., 1997). Time-lapse imaging of mitochondria in living yeast revealed a series of cell-cycle-dependent motility events that contribute to segregation of the organelle between mother cells and buds. There are two key events in this mitochondrial inheritance process: (1) vectorial movement of the organelle from mother cell to bud and (2) retention of mitochondria at specific positions within both mother and bud (Yang et al., 1999).

Mounting evidence suggests a role for the actin cytoskeleton in mitochondrial motility in *S. cerevisiae*. First, destabilization of the actin cytoskeleton by mutations in the actin-encoding *ACT1* gene resulted in defects in mitochondrial morphology and transfer of mitochondria from mother cells to buds (Drubin et al., 1993; Lazzarino et al., 1994). Second, destabilization of the actin cytoskeleton by treatment with Latrunculin-A (an actin-monomer sequestering agent) blocks mitochondrial movement (Simon et al., 1995). Third, mitochondria colocalize with actin cables, bundles of actin filaments that align along the mother-bud axis during polarized growth (Drubin et al., 1993; Lazzarino et al., 1994). Consistent with this, disruption of actin cables by deletion of the tropomyosin-encoding gene (*TPM1*) results in a loss of polarized mitochondrial movement into the bud (Simon et al., 1997). These observations support the model that mitochondria use actin cables as tracks for movement from the mother cell to the bud.

Although type V myosin proteins have been implicated as the motor molecules that drive movement of cargo along actin

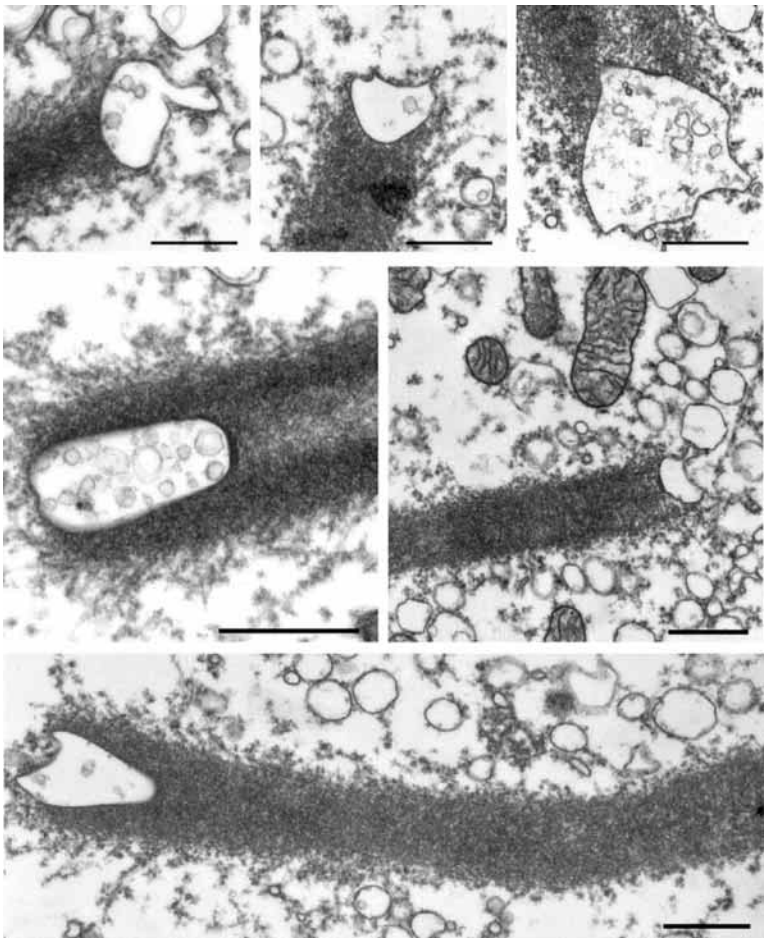

Fig. 3. Thin-section electron microscopic analysis of vesicles associated with comet tails on endosomes. Cell-free reactions were fixed in perfusion chambers and processed for electron microscopy. A gallery of representative images was assembled to highlight the odd shapes, tubular processes, and multivesicular lumens of vesicles associated with tails. Note the clear circular profiles and mitochondria, none of which are associated with comet tails. Bars, 500 nm. Reproduced from Taunton et al. (2000), with permission from the Rockefeller University Press.

cables in yeast, recent studies suggest a role for the Arp2/3 complex and actin polymerization in mitochondrial movement and inheritance in yeast (Boldogh et al., 2001). First, mitochondrial movement requires constant actin assembly and disassembly, and is impaired by an agent that perturbs actin dynamics. Second, Arp2/3 complex subunits colocalize with mitochondria in intact cells (Fig.·4) and are tightly associated with the surface of isolated yeast mitochondria. Third, Arp2/3 complex-dependent actin nucleation activity is observed in isolated yeast mitochondria and associated with mitochondria in living yeast. Finally, mutations in Arp2/3 complex subunits inhibit mitochondrial movement, but have no obvious effect on colocalization of mitochondria with actin cables. These observations indicate that the Arp2/3 complex is associated with yeast mitochondria and that the Arp2/3-complex-driven actin assembly provides the driving force for mitochondrial movement.

Although yeast mitochondria and *L. monocytogenes* require Arp2/3 complex and actin dynamics for movement, there are fundamental differences in their mechanism of movement. First, yeast mitochondria and *L. monocytogenes* display different patterns of movement. Arp2/3 complex-mediated movement of *L. monocytogenes* and endosomes has no obvious direction or track dependence. In contrast, yeast mitochondria colocalize with actin cables, and use actin cables as tracks for linear, polarized movement from the mother cell to the bud. Second, there are no obvious actin comet tails on yeast mitochondria. However, since mitochondria are associated with actin cables, which are linear, F-actin containing structures, comet tails on mitochondria may not be distinguishable from actin cables. This interpretation is supported by the finding that destabilization of actin cables results in generation of Arp2/3 complex-dependent 'clouds' of F-actin on mitochondria that resemble intermediates in actin comet tail assembly observed on *L. monocytogenes in vitro* (Boldogh et al., 2001).

What mediates association of mitochondria to actin cables in yeast? Previous studies revealed that yeast mitochondria contain an actin binding activity that may be distinct from the Arp2/3 complex. Using a sedimentation assay to study direct interactions between mitochondria and F-actin, Boldogh et al. (1998) showed that a protein(s) on the mitochondrial surface binds to F-actin. This actin-binding activity is ATP-sensitive, reversible, saturable, and requires two integral mitochondrial outer membrane proteins (Mmm1p and Mdm10p). Collectively, these proteins are referred to as the mitochondrial actin binding particle (mABP; Boldogh et al., 1998).

Three lines of evidence suggest that mABP is distinct from the Arp2/3 complex. First, although the Arp2/3 complex has F-actin binding activity, binding of the Arp2/3 complex to the lateral surface of F-actin occurs in the presence of ATP (Mullins et al., 1998). In contrast, F-actin binding by mABP is ATP-sensitive (Lazzarino et al., 1994). Thus, F-actin binding by mABP and the Arp2/3 complex show different biochemical properties. Second, mABP activity is observed in mitochondria isolated from yeast carrying mutations in the Arp2/3 complex (I. Boldogh and L. Pon, unpublished observations). Thus, mABP-mediated binding of mitochondria to F-actin does not require the Arp2/3 complex. Third, mitochondria colocalize with actin cables in yeast carrying mutations in Arp2/3 complex (Boldogh et al., 2001). Thus, association of mitochondria with actin cables does not require the Arp2/3 complex.

The model that emerges from these studies invokes mABP as the mediator for binding of mitochondria to actin cables. Since mABP-mediated binding of mitochondria to F-actin is

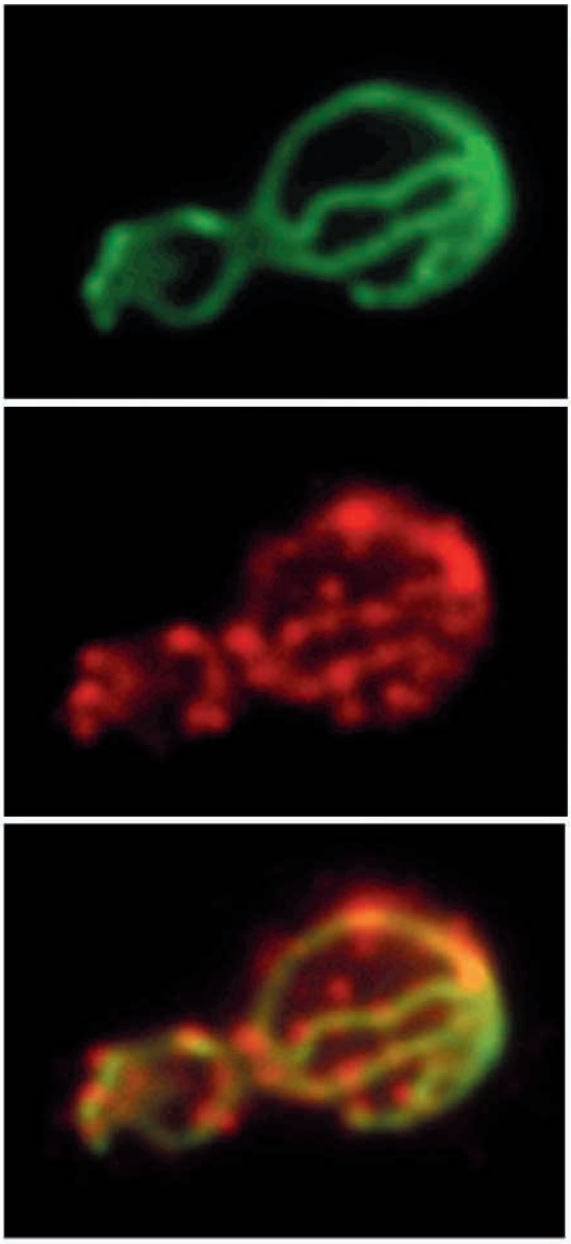

Fig.·4. Colocalization of Arp2p with mitochondria in yeast. Budding yeast cells expressing a fusion protein consisting of the mitochondrial signal sequence of citrate synthase 1 fused to GFP (CS1-GFP) were grown to mid-log phase, fixed with paraformaldehyde and stained for Arp2p using an antibody raised against a conserved peptide sequence found in Arp2p, but not in actin or any other actin-related protein (Moreau et al., 1996). Green, mitochondria visualized using CS1-GFP; red, Arp2p visualized by indirect immunofluorescence. Bar, 1·μm.

reversible, mitochondria could bind to actin cables and move along them in the presence of an applied force. The available evidence supports a role for actin polymerization as the force generator for mitochondrial movement. Taken together, mABP mediates reversible binding of mitochondria and their associated Arp2/3 complex to actin cables. Arp2/3 complex-driven actin nucleation would then initiate actin comet tail formation at the interface between mitochondria and actin cables. This would generate forces that drive mitochondrial movement along actin cables from mother cells to developing daughter cells.

Future perspectives

Early studies revealed a role for the Arp2/3 complex and actin polymerization in generating the forces for movement of bacterial pathogens in infected host cells. Recent findings indicate that endogenous particles, including endosomes in many cell types and mitochondria in budding yeast, may use the same force-generators for their movement. These observations raise additional questions. Do other organelles and particles use this mechanism of movement? What is the mechanism for targeting Arp2/3 complex to endosomes and mitochondria? What proteins control Arp2/3 complex activity on yeast mitochondria? The answers to some or all of these questions are the subject of ongoing exploration.

We thank members of the Pon laboratory for critical evaluation of the manuscript. This work was supported by research grants to L.P. from the National Institutes of Health (GM45735 and GM66307) and the American Cancer Society (RPG-97-163-04-CSM).

References

Bernardini, M. L., Mounier, J., d'Hauteville, H., Coquis-Rondon, M. and Sansonetti, P. J. (1989). Identification of *icsA*, a plasmid locus of *Shigella flexneri* that governs bacterial intra- and intercellular spread through interaction with F-actin. *Proc. Natl. Acad. Sci. USA* **86**, 3867-3871.

Blanchoin, L., Amann, K. J., Higgs, H. N., Marchand, J. B., Kaiser, D. A. and Pollard, T. D. (2000). Direct observation of dendritic actin filament networks created by Arp2/3 complex and WASP/ Scar proteins. *Nature* **404**, 1007-1011.

Boldogh, I. R., Yang, H.-C., Nowakowski, W. D., Karmon, S. L., Hays, L. G., Yates, J. R., III and Pon, L. A. (2001). Arp2/3 complex and actin dynamics are required for actin-based mitochondrial motility in yeast. *Proc. Natl. Acad. Sci. USA* **98**, 3162-3167.

Boldogh, I., Vojtov, N., Karmon S. and Pon, L. A. (1998). Interaction between mitochondria and the actin cytoskeleton in budding yeast requires two integral mitochondrial outer membrane proteins, Mmm1p and Mdm10p. *J. Cell Biol.* **141**, 1371-1381.

Cameron, L. A., Footer, M. J., Van Oudenaarden, A. and Theriot, J. (1999). Motility of ActA protein-coated microspheres driven by actin polymerization. *Proc. Natl. Acad. Sci. USA* **96**, 4908-4913.

Carlier, M.-F., Laurent, V., Santolini, J., Melki, R., Didry, D., Xia, G.-X., Hong, Y., Chua, N.-H. and Pantaloni, D. (1997). Actin depolymerizing factor (ADF/cofilin) enhances the rate of filament turnover: implication in actin-based motility. *J. Cell Biol.* **136**, 1307-1323.

Chakraborty, T., Ebel, F., Domann, E., Niebuhr, K., Gerstel, B., Pistor, S., Temm-Grove, C. J., Jockusch, B. M., Reinhard, M., Walter, U. and Wehland, J. (1995). A focal adhesion factor directly linking intracellularly motile *Listeria monocytogenes* and *Listeria ivanovii* to the actin-based cytoskeleton of mammalian cells. *EMBO J.* **14**, 1314-1321.

Chang, F. S., Stefan, C. J. and Blumer, K. J. (2003). A WASp homolog powers actin polymerization-dependent motility of endosomes *in vivo*. *Curr. Biol.*, in press.

Drubin, D. G., Jones, H. D. and Wertman, K. F. (1993). Actin structure and function: roles in mitochondrial organization and morphogenesis in budding yeast and identification of the phalloidin-binding site. *Mol. Biol. Cell* **4**, 1277-1294.

Duncan, M. C., Cope, M. J., Goode, B. L., Wendland, B. and Drubin, D.

G. (2001). Yeast Eps15-like endocytic protein, Pan1p, activates the Arp2/3 complex. *Nat. Cell Biol.* **3**, 687-690.

Egile, C., Loisel, T. P., Laurent, V., Li, R., Pantaloni, D., Sansonetti, P. J. and Carlier, M.-F. (1999). Activation of the CDC42 effector N-WASP by the *Shigella flexneri* IcsA protein promotes actin nucleation by Arp2/3 complex and bacterial actin-based motility. *J. Cell Biol.* **146**, 1319-1332.

Evangelista, M., Klebl, B. M., Tong, A. H., Webb, B. A., Leeuw, T., Leberer, E., Whiteway, M., Thomas, D. Y. and Boone, C. (2000). A role for myosin-I in actin assembly through interactions with Vrp1p, Bee1p, and the Arp2/3 complex. *J. Cell Biol.* **148**, 353-362.

Fujimoto, L. M., Roth, R., Heuser, J. E. and Schmid, S. L. (2000). Actin assembly plays a variable, but not obligatory role in receptor-mediated endocytosis in mammalian cells. *Traffic* **1**, 161-171.

Gaidarov, I., Santini, F., Warren, R. A. and Keen, J. H. (1999). Spatial control of coated-pit dynamics in living cells. *Nature Cell Biol.* **1**, 1-7.

Goldberg, M. A. (2001). Actin-based motility of intracellular microbial pathogens. *Microbiol. Mol. Biol. Rev.* **65**, 595-626.

Goode, B. L., Rodal, A. A., Barnes, G. and Drubin, D. G. (2001). Activation of the Arp2/3 complex by the actin filament binding protein Abp1p. *J. Cell Biol.* **153**, 627-634.

Gouin, E., Gantelet, H., Egile, C., Lasa, I., Ohayon, H., Villiers, V., Gounon, P., Sansonetti, P. J. and Cossart, P. (1999). A comparative study of the actin-based motilities of the pathogenic bacteria *Listeria monocytogenes*, *Shigella flexneri*, and *Rickettsia conorii*. *J. Cell Sci.* **112**, 1697-1708.

Heinzen, R. A., Hayes, S. F., Peacock, M. G. and Hackstadt, T. (1993). Directional actin polymerization associated with spotted fever group *Rickettsia* infection of Vero cells. *Infect. Immun.* **61**, 1926-1935.

Heinzen, R. A., Grieshaber, S. S., Van Kirk, L. S. and Devin, C. J. (1999). Dynamics of actin-based movement by *Rickettsia rickettsii* in Vero cells. *Infect. Immun.* **67**, 4201-4207.

Insall, R., Müller-Taubenberger, A., Machesky, L., Köhler, J., Simmeth, E., Atkinson, S. J., Weber, I. and Gerisch, G. (2001). Dynamics of the *Dictyostelium* Arp2/3 complex in endocytosis, cytokinesis, and chemotaxis. *Cell Motil. Cytoskel.* **50**, 115-128.

Jung, G., Remmert, K., Wu, X., Volosky, J. M. and Hammer, J. A., III. (2001). The *Dictystelium* CARMIL protein links capping protein and the Arp2/3 complex to type I myosins through their SH3 domains. *J. Cell Biol.* **153**, 1479-1497.

Kaksonen, M., Peng, B. and Rauvala, H. (2000). Association of cortactin with dynamic actin in lamellipodia and on endosomal vesicles. *J. Cell Sci.* **113**, 4421-4426.

Kelleher, J. F., Atkinson, S. J. and Pollard, T. D. (1995). Sequences, structural models, and cellular localization of the actin-related protein Arp2 and Arp3 from *Acanthamoeba*. *J. Cell Biol.* **131**, 385-397.

Kocks, C., Marchand, J. B., Gouin, E., d'Huateville, H., Sansonetti, P. J., Carlier, M. F. and Cossart, P. (1995). The unrelated surface proteins ActA of *Listeria monocytogenes* and IcsA of *Shigella flexneri* are sufficient to confer actin-based motility on *Listeria innocua* and *Eschericia coli*, respectively. *Mol. Microbiol.* **18**, 413-423.

Laine, R. O., Zeile, W., Kang, F., Purch, D. L. and Southwick, F. S. (1997). Vinculin proteolysis unmasks an ActA homolog for actin-based *Shigella* motility. *J. Cell Biol.* **138**, 1255-1264.

Lamaze, C., Fujimoto, L. M., Yin, L. H. and Schmid, S. L. (1997). The actin cytoskeleton is required for receptor-mediated endocytosis in mammalian cells. *J. Biol. Chem.* **272**, 20332-20335.

Lazzarino, D. A., Boldogh, I., Smith, M. G., Rosand, J. and Pon, L. A. (1994). Yeast mitochondria contain ATP-sensitive, reversible actin-binding activity. *Mol. Biol. Cell* **5**, 807-818.

Lechler, T., Shevchenko, A. and Li, R. (2000). Direct involvement of yeast type I myosins in Cdc42-dependent actin polymerization. *J. Cell Biol.* **148**, 363-373.

Lee, W. L., Bezanilla, M. and Pollard, T. D. (2000). Fission yeast myosin-I, Myo1p, stimulates actin assembly by Arp2/3 complex and shares functions with WASp. *J. Cell Biol.* **151**, 789-800.

Lett, M.-C., Sasakawa, C., Okada, N., Sakai, T., Makino, S., Yamada, M., Komatsu, K. and Yoshikawa, M. (1989). *virG*, a plasmid-coded virulence gene of *Shigella flexneri*: identification of the *virG* protein and determination of the complete coding sequence. *J. Bacteriol.* **171**, 353-359.

Loisel, T. P., Boujemaa, R., Pantaloni, D. and Carlier, M.-F. (1999). Reconstitution of actin-based motility of *Listeria* and *Shigella* using pure proteins. *Nature* **401**, 613-616.

Ma, L., Rohatgi, R. and Kirschner, M. W. (1998). The Arp2/3 complex mediates actin polymerization induced by the small GTP-binding protein Cdc42. *Proc. Natl. Acad. Sci. USA* **95**, 15362-15367.

Machesky, L. M., Atkinson, S. J., Ampe, C., Vandekerckhove, J. and Pollard, T. D. (1994). Purification of a cortical complex containing two unconventional actins from *Acanthamoeba* by affinity chromatography on profilin-agarose. *J. Cell Biol.* **127**, 107-115.

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). Scar, a WASp-related protein, activates nucleation of actin filaments by the Arp2/3 complex. *Proc. Natl. Acad. Sci. USA* **96**, 3739-3744.

May, R. C., Hall, M. E., Higgs, H. N., Pollard, T. D., Chakraborty, T., Wehland, J., Machesky, L. M. and Sechi, A. S. (1999). The Arp2/3 complex is essential for the actin-based motility of *Listeria monocytogenes*. *Curr. Biol.* **9**, 759-762.

Merrifield, C. J., Moss, S. E., Ballestrem, C., Imhof, B. A., Giese, G., Wunderlich, I. and Almers, W. (1999). Endocytic vesicles move at the tips of actin tails in cultured mast cells. *Nat. Cell Biol.* **1**, 71-74.

Moreau, V., Galan, J., Devilliers, G., Haguenauer-Tsapis, R. and Winsor, B. (1997). The yeast actin-related Arp2p is required for the internalization step of endocytosis. *Mol. Biol. Cell* **8**, 1361-1375.

Moreau, V., Madania, A., Martin, R. P. and Winsor, B. (1996). The *Saccharomyces cerevisiae* actin-related Arp2 is involved in the actin cytoskeleton. *J. Cell Biol.* **134**, 117-132.

Mullins, R. D., Heuser, J. A. and Pollard, T. D. (1998). 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* **95**, 6181-6186.

Mullins, R. D. and Pollard, T. D. (1999). Rho-family GTPases require the Arp2/3 complex to stimulate actin polymerization in *Acanthamoeba* extracts. *Curr. Biol.* **9**, 405-415.

Nunnari, J., Marshall, W. F., Straight, A., Murray, A., Sedat, J. W. and Walter, P. (1997). Mitochondrial transmission during mating in *Saccharomyces cerevisiae* is determined by mitochondrial fusion and fission and the intramitochondrial segregation of mitochondrial DNA. *Mol. Biol. Cell* **8**, 1233-1242.

Pistor, S., Chakraborty, T., Niebuhr, K., Domann, E. and Wehland, J. (1994). The ActA protein of *Listeria monocytogenes* acts as a nucleator inducing reorganization of the actin cytoskeleton. *EMBO J.* **13**, 758-763.

Robinson, R. C., Turbedsky, K., Kaiser, D. A., Marchand, J. B., Higgs, H. N., Choe, S. and Pollard, T. D. (2001). Crystal structure of Arp2/3 complex. *Science* **294**, 1679-1684.

Rohatgi, R., Ma, L., Miki, H., Lopez, M., Kirchhausen, T., Takenawa, T. and Kirschner, M. W. (1999). The interaction between N-WASP and the Arp2/3 complex links Cdc42-dependent signals to actin assembly. *Cell* **97**, 221-231.

Rosenblatt, J., Agnew, B. J., Abe, H., Bamburg, J. R. and Mitchison, T. J. (1997). Xenopus actin-depolymerizing factor (XAC) is responsible for the turnover of actin filaments in *Listeria monocytogenes* tails. *J. Cell Biol.* **136**, 1323-1332.

Simon, V. R., Swayne, T. C. and Pon, L. A. (1995). Actin-dependent mitochondrial motility in mitotic yeast and cell-free system: identification of a motor activity on the mitochondrial surface. *J. Cell Biol.* **130**, 345-354.

Simon, V. R., Karmon, S. L. and Pon, L. A. (1997). Mitochondrial inheritance: cell cycle and actin cable dependence of polarized mitochondrial movements in *Saccharomyces cerevisiae*. *Cell Motil. Cytoskel.* **37**, 199-210.

Smith, G. A., Portnoy, D. A. and Theriot, J. A. (1995). Asymmetric distribution of the *Listeria monocytogenes* ActA protein is required and sufficient to direct actin-based motility. *Mol. Microbiol.* **17**, 945-951.

Smith, G. A., Theriot, J. A. and Portnoy, D. A. (1996). The tandem repeat domain in the *Listeria monocytogenes* ActA protein controls the rate of actin-based motility, the percentage of moving bacteria, and the localization of vasodilator-stimulated phosphoprotein and profilin. *J. Cell Biol.* **135**, 647-660.

Snapper, S. B., Takeshima, F., Anton, I., Liu, C.-H., Thomas, S., Nguyen, D., Dudley, D., Fraser, H., Putich, D., Lopez-Ilasaca, M. et al. (2001). N-WASP deficiency reveals distinct pathways for cell surface projections and microbial actin-based motility. *Nat. Cell Biol.* **3**, 897-904.

Stevens, B. J. (1977). Variation in number and volume of the mitochondria in yeast according to growth conditions. A study based on serial sectioning and computergraphics reconstruction. *Biol. Cell* **28**, 37-56.

Suzuki, T., Miki, H., Takenawa, T. and Saskawa, C. (1998). Neural Wiskott-Aldrich syndrome protein is implicated in the actin-based motility of *Shigella flexneri*. *EMBO J.* **17**, 2767-2776.

Svitkina, T. M. and Borisy, G. G. (1999). Arp2/3 complex and actin depolymerizing factor/cofilin in dendritic organization and treadmilling of actin filament arrays in lamellipodia. *J. Cell Biol.* **145**, 1009-1026.

Taunton, J., Rowling, B. A., Coughlin, M. L., Wu, M., Moon, R. T., Mitchison, T. J. and Larabell, C. A. (2000). Actin-dependent propulsion of endosomes and lysosomes by recruitment of N-WASP. *J. Cell Biol.* **148**, 519-530.

Theriot, J. A., Rosenblatt, J., Portnoy, D. A., Goldschmidt-Clermont, P. J. and Mitchison, T. J. (1994). Involvement of profilin in the actin-based motility of *L. monocytogenes* in cells and in cell-free extracts. *Cell* **76**, 505-517.

Tilney, L. G. and Portnoy, D. A. (1989). Actin filaments and the growth, movement, and spread of the intracellular bacterial parasite *Listeria monocytogenes. J. Cell Biol.* **109**, 1597-1608.

Tilney, L. G., Connelly, P. S. and Portnoy, D. A. (1990). Actin filament nucleation by the bacterial pathogen, *Listeria monocytogenes. J. Cell Biol.* **111**, 2979-2988.

Trifaró, J.-M. and Vitale, M. L. (1993). Cytoskeleton dynamics during neurotransmitter release. *Trends Neurosci.* **16**, 466-472.

Uruno, T., Liu, J., Zhang, P., Fan, Y., Egile, C., Li, R., Mueller, S. C. and Zhan, X. (2001). Activation of Arp2/3 complex-mediated actin polymerization by cortactin. *Nat. Cell Biol.* **3**, 259-266.

Van Kirk, L. S., Hayes, S. F. and Heinzen, R. A. (2000). Ultrastructure of *Rickettsia rickettsii* actin tails and localization of cytoskeletal proteins. *Infect. Immun.* **68**, 4706-4713.

Volkmann, N., Amann, K. J., Stoilova-McPhie, S., Egile, C., Winter, D. C., Hazelwood, L., Heuser, J. E., Li, R., Pollard, T. D. and Hanein, D. (2001). Structure of Arp2/3 complex in its activated state and in actin filament branch junctions. *Science* **293**, 2456-2459.

Weaver, A. M., Karginov, A. V., Kinley, A. W., Weed, S. A., Li, Y., Parsons, J. T. and Cooper, J. A. (2001). Cortactin promotes and stabilizes Arp2/3-induced actin filaments network formation. *Curr. Biol.* **11**, 370-374.

Weed, S. A., Karginov, A. V., Schafer, D. A., Weaver, A. M., Kinley, A. W., Cooper, J. A. and Parsons, J. T. (2000). Cortactin localization to sites of actin assembly in lamellipodia requires interactions with F-actin and the Arp2/3 complex. *J. Cell Biol.* **151**, 29-40.

Welch, M. D., Iwamatsu, A. and Mitchison, T. J. (1997a). Actin polymerization is induced by the Arp2/3 protein complex at the surface of *Listeria monocytogenes*. *Nature* **385**, 265-269.

Welch, M. D., DePace, A. H., Verma, S., Iwamatsu, A. and Mitchison, T. J. (1997b). The human Arp2/3 complex is composed of evolutionarily conserved subunits and is localized to cellular regions of dynamic actin filament assembly. *J. Cell Biol.* **138**, 375-384.

Welch, M. D., Rosenblatt, J., Skoble, J., Portnoy, D. A. and Mitchison, T. J. (1998). Interaction of human Arp2/3 complex and the *Listeria monocytogenes* ActA protein in actin filament nucleation. *Science* **281**, 105-108.

Winter, D., Podtelejnikov, A. V., Mann, M. and Li, R. (1997). Purification and in vivo functional analysis of the yeast Arp2/3 complex. *Curr. Biol.* **7**, 519-529.

Winter, D. C., Choe, E. Y. and Li, R. (1999a). Genetic dissection of the budding yeast Arp2/3 complex: a comparison of the in vivo and structural roles of individual subunits. *Proc. Natl. Acad. Sci. USA* **96**, 7288-7293.

Winter, D., Lechler, T. and Li, R. (1999b). Activation of the yeast Arp2/3 complex by Bee1p, a WASP-family protein. *Curr. Biol.* **9**, 501-504.

Yang, H.-C., Palazzo, A., Swayne, T. C. and Pon, L. A. (1999). A retention mechanism for distribution of mitochondria during cell division in budding yeast. *Curr. Biol.* **9**, 1111-1114.

Yarar, D., To, W., Abo, A. and Welch, M. D. (1999). The Wiskott-Aldrich syndrome protein directs actin-based motility by stimulating actin nucleation with the Arp2/3 complex. *Curr. Biol.* **9**, 555-558.