The ARP2/3 complex: an actin nucleator comes of age

Erin D. Goley and Matthew D. Welch

Abstract | The cellular functions of the actin cytoskeleton require precise regulation of both the initiation of actin polymerization and the organization of the resulting filaments. The actin-related protein-2/3 (ARP2/3) complex is a central player in this regulation. A decade of study has begun to shed light on the molecular mechanisms by which this powerful machine controls the polymerization, organization and recycling of actin-filament networks, both *in vitro* and in the living cell.

Barbed end
(also called the + end). The more dynamic end of the actin filament, where growth and shrinkage are fast. In the actin monomer, the barbed end is on the side of the molecule opposite the nucleotide-binding cleft.

Pointed end
(also called the – end). The less dynamic end of the actin filament. In the actin monomer, the pointed end is on the same side of the molecule as the nucleotide-binding cleft.

Department of Molecular and Cell Biology, University of California, Berkeley, California 94720, USA. Correspondence to M.D.W. e-mail: welch@berkeley.edu
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The eukaryotic actin cytoskeleton has an important role in remarkably diverse processes, including cell migration, endocytosis, vesicle trafficking and cytokinesis, many of which are essential for the survival of the cell. The core constituent of the actin cytoskeleton is monomeric globular (G)-actin, a 43-kDa ATPase that can self-assemble into filamentous (F)-actin. Each asymmetric filament possesses a fast growing barbed end and a slower growing pointed end that are distinguishable by their structural characteristics and kinetic properties. ATP hydrolysis in the filament is tightly coupled to polymerization and regulates the kinetics of assembly and disassembly, as well as the association of interacting proteins. These biochemical properties are integral to the cellular activities of actin[1].

In cells, actin filaments function as force-generating polymer motors, structural scaffolds and tracks for motor proteins. The dynamic assembly and disassembly of filaments and the formation of larger scale filament structures are crucial aspects of actin's function, and are therefore under scrupulous control by over a hundred actin-binding proteins. These proteins bind directly to filaments or monomers and control actin structure and dynamics by: nucleating; capping; stabilizing; severing; depolymerizing; crosslinking; bundling; sequestering or delivering monomers; or by promoting monomer nucleotide exchange. The coordinated actions of specific subsets of actin-binding proteins regulate the dynamics of distinct arrays of actin filaments at specific times and places within the cell[1].

An important set of actin regulators initiate formation of new actin filaments by a process that is called nucleation (BOX 1). Spontaneous nucleation is a kinetic hurdle in the process of actin polymerization, and, therefore, factors that can accelerate or bypass this step are important for efficient actin assembly in the cell. So far, three classes of protein have been identified that initiate new filament polymerization: the actin-related protein-2/3 (ARP2/3) complex, the formins and spire (BOX 1). Formins and spire have been reviewed in detail recently[2,3] and are not discussed further. Instead, this review focuses on the ARP2/3 complex, which was the first of these molecules to be identified, and has since been shown to have a crucial role in the formation of branched-actin-filament networks during diverse processes ranging from cell motility to endocytosis. This review introduces the ARP2/3 complex, describes recent advances in understanding its molecular mechanism of action and details the cellular processes that use its activity. We conclude by discussing the ways in which the ARP2/3 complex is misregulated during disease.

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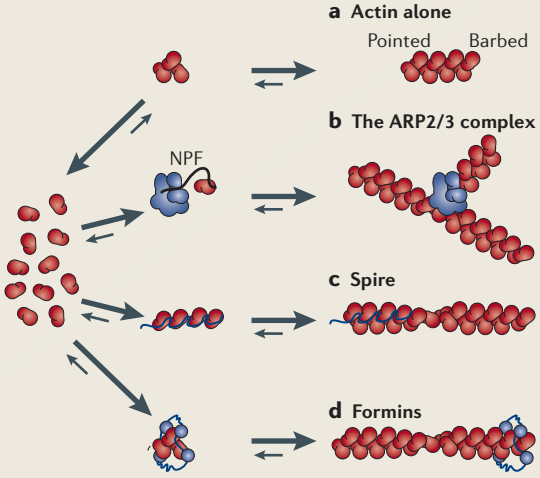
The intact ARP2/3 complex was first purified from *Acanthamoeba castellanii* based on its affinity for the actin-binding protein profilin[4], and was shown to consist of a stable assembly of seven polypeptides (FIG. 1a–c). Two of the subunits were actin-related proteins of the ARP2 and ARP3 subfamilies, giving the complex its name. These proteins had been previously identified by genetic and genomic approaches in *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*[5,6], *Drosophila melanogaster*[7] and *Caenorhabditis elegans*[8]. The remaining five subunits were originally named by size, but are now referred to as ARPC1 (actin-related protein complex-1), ARPC2, ARPC3, ARPC4 and ARPC5. ARPC1 (which is present in two isoforms in humans, ARPC1A and ARPC1B) is a WD-repeat-containing protein, whereas the other four ARPC subunits do not contain common sequence motifs. Since its isolation from *A. castellanii*, the entire complex has been purified from yeasts[9,10] and vertebrates[11,12]. Moreover, most eukaryotes for which genomes have been sequenced contain genes that are predicted to

Box 1 | **Paths to nucleation**

The spontaneous initiation of actin-filament assembly requires the formation of a trimeric nucleus in a process that is called nucleation (part **a**). Spontaneous nucleation is kinetically unfavourable and is the rate-limiting step in polymerization, because the actin dimer intermediate is very unstable[1].

So far, three main classes of protein have been identified that bypass the need for spontaneous nucleation and promote the initiation of new filament assembly. These factors, commonly referred to as nucleators, are the actin-related protein-2/3 (ARP2/3) complex, spire and formins. Each promotes nucleation by a distinct mechanism. The ARP2/3 complex is thought to mimic an actin dimer or trimer and to function as a template for the initiation of a new actin filament that branches off of an existing filament, generating y-branched actin networks (part **b**). The spire proteins, which are conserved among metazoan species, were recently discovered to nucleate actin assembly. Biochemical studies with *Drosophila melanogaster* spire indicate that its four tandem G-actin-binding Wiskott–Aldrich syndrome protein (WASP)-homology-2 (WH2) domains mediate longitudinal association of four actin subunits and function as a scaffold for polymerization into an unbranched filament[167] (part **c**). Spire might remain associated with the pointed end of the filament, as it can cap pointed ends and prevent their depolymerization *in vitro*[167].



The formins, which are conserved in most eukaryotes, also promote the nucleation of unbranched filaments[3]. Biochemical and structural studies with yeast and mammalian formins indicate that a dimer of formin-homology-2 (FH2) domains stabilizes an actin dimer or trimer to facilitate the nucleation event (part **d**). In contrast to spire and ARP2/3, formins remain associated with the growing barbed ends of filaments, and sequential binding and release interactions might allow formins to 'walk' with the polymerizing barbed end. The existence of multiple classes of nucleator gives the cell the flexibility to assemble distinct populations of actin filaments with particular geometries and polymerization characteristics in response to diverse signals. NPF; nucleation-promoting factor.

encode all seven subunits, with the apparent exception of some protistan parasites including the *Apicomplexa*[13] and *Leishmania major*[14].

The ARP2/3 complex possesses little biochemical activity on its own. However, when engaged by nucleation-promoting factor (NPF) proteins, it is activated to initiate the formation of a new (daughter) filament that emerges from an existing (mother) filament in a y-branch configuration with a regular 70° branch angle[15,16] (FIG. 1d). This coupling of nucleation and branching by the ARP2/3 complex is referred to as autocatalytic branching or dendritic nucleation, and is central to its functions *in vivo*.

Subunit organization and structure. Early attempts to gain insight into the structure of the ARP2/3 complex relied on genetics, biochemical reconstitution and chemical crosslinking *in vitro*[17–21]. These techniques led to a crude picture of the interactions between subunits that has held up well in light of more recent structural studies. The details of the ARP2/3 complex organization were revealed when a crystal structure of the bovine complex was solved at 2.0-Å resolution[22] (FIG. 1b). In this structure, ARP2 and ARP3 are structurally similar to actin, as predicted from their sequences[23]. However, their nucleotide-binding clefts are empty and open compared to ATP-bound actin, a result of the fact that the complex was purified in the absence of ATP. A nucleotide-bound structure has also been solved[24] (see below). Of the other subunits, ARPC2 and ARPC4 form the structural core of the complex, with the remaining subunits organized around them, consistent with information from genetic and biochemical reconstitution experiments[18,20,21]. ARPC1 is a seven-bladed β-propeller protein, whereas ARPC3 and ARPC5 are primarily α-helical and are the most peripheral of the subunits.

The ARP2/3 complex captured in the crystal is proposed to be in an inactive conformation because none of the binding partners that are required for activity are present. Moreover, ARP2 and ARP3 are splayed apart in the structure, which is inconsistent with the leading hypothesis that they function as an actin-like heterodimer to template the nucleation of the daughter filament. Therefore, activation of the complex is proposed to require a significant conformational change[22] (FIG. 1b,c). So far, there are no high-resolution structures of activation intermediates, so further structural studies are needed to define the transitions that occur during activation.

There is also only limited structural information available about how the ARP2/3 complex crosslinks actin filaments at a y-branch junction. Nevertheless, new data is emerging and two models have recently been proposed[25,26] (FIG. 1e). One is based on the mapping of conserved surface residues on the complex together with data from cryo-electron microscopy (EM), the crystal structure and biochemical studies[25]. The other is based on recent cryo-EM of activated ARP2/3-containing bulky tags that were used to map the positions of individual subunits in the branch point[26]. These two models differ in their details, but each proposes the following

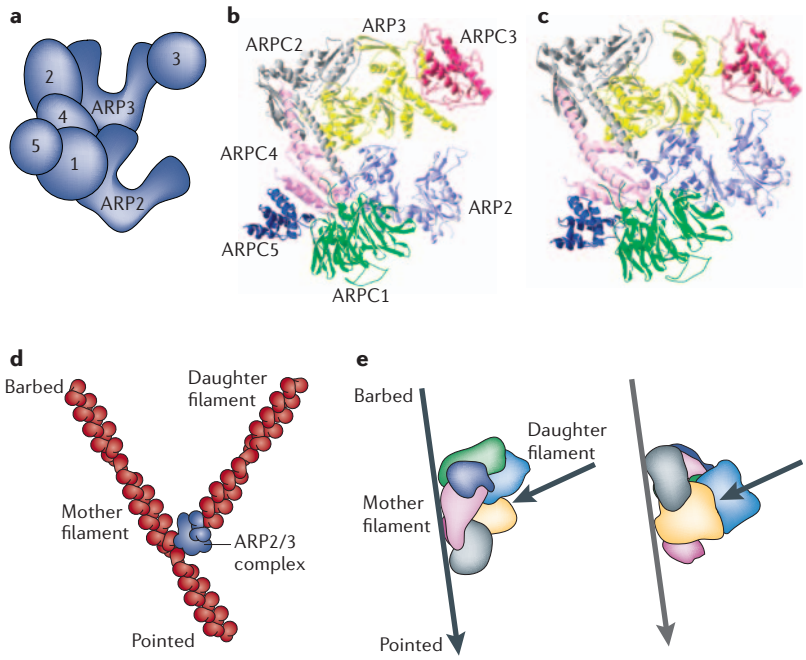


Figure 1 | **Structure and function of the ARP2/3 complex. a** | Cartoon representation of the subunit organization in the inactive actin-related protein-2/3 (ARP2/3) complex. ARP2, ARP3 and ARP complex-1 (ARPC1) through ARPC5 are shown (labelled as 1–5). **b** | Ribbon diagram of the crystal structure of the bovine ARP2/3 complex (Protein Data Bank (PDB) accession code 1A8K) with subunits labelled and displayed in different colours. Subdomain-1 and -2 of ARP2 were modelled on the corresponding subdomains of actin (PDB accession code 1ATN)[22]. **c** | Ribbon diagram of the predicted active conformation of the ARP2/3 complex. Structure was modelled after that proposed by Robinson *et al.*[22]. Subunits are coloured as in part **b**. **d** | Cartoon diagram of ARP2/3 complex binding to the side of the mother filament and the pointed end of the daughter filament in the y-branch. The two filaments are oriented at a ~70° angle. **e** | Two models for the orientation of the ARP2/3 complex at a y-branch junction. Subunits are coloured as in part **b**. Both models propose that ARP2 (light blue) and ARP3 (yellow) associate with the pointed end of the daughter filament, and ARPC2 (grey), ARPC4 (pink) and other subunits mediate contacts with the mother filament. In the Egile *et al.* model[26] (displayed on the right), the complex is rotated ~100° anticlockwise relative to the axis of the mother filament compared to the Beltzner and Pollard model[25] (displayed on the left).

common principles: ARP2 and ARP3 interact with the pointed end of the daughter filament, and ARPC2 and ARPC4 make substantial contacts with the mother filament (FIG. 1e). However, the resolution of EM is still too low to present a clear picture of these interactions at the atomic level. Future structural studies are required to incorporate activating factors such as NPFs, actin filaments and G-actin into the picture during ARP2/3 complex activation and branching.

Regulation of ARP2/3 complex activity by ATP

The mechanisms of the transition of the ARP2/3 complex from the inactive state to the active form that is found in a branch point, and the transition back again, have been the subject of intense study since the activities of the complex were first characterized. As with actin, nucleotide binding and hydrolysis by ARP2 and ARP3 influence the structure and function of the complex. ARP2 and ARP3 each bind to ATP with micromolar affinity[27–30], and mutations that lower the affinity of either ARP for ATP cause severe reductions in activity[29,30], indicating that nucleotide binding to both ARPs is important. The crystal structure of the ATP-bound complex[24] and fluorescence resonance energy transfer (FRET) experiments[29] showed that the binding of ATP to ARP3 closes its nucleotide-binding cleft and causes a global conformational change in the complex. However, the ATP-bound structure still does not resemble the predicted active conformation. Notably, subdomain-1 and -2 of ARP2 are flexible and disordered in the presence and absence of ATP[24]. The disordered structure of ARP2 could result from a constraint that was introduced by the method of crystallization, which involved soaking nucleotide-free crystals in ATP, rather than crystallization in the presence of ATP. On the other hand, the structure might reflect a physiological role for NPFs or actin in ATP binding. Consistent with this idea, binding of an NPF to the complex increases its affinity for nucleotide[27,28] and the nucleotide bound to the complex influences its affinity for NPF[27].

In addition to nucleotide binding, nucleotide hydrolysis also has a role in the function of the ARP2/3 complex. ATP hydrolysis on ARP2 is observed using the purified ARP2/3 complex *in vitro*, but only in the presence of actin and an NPF[31–33]. Curiously, hydrolysis has not been observed on ARP3, although its ATPase activity might require as yet unidentified cofactors. Nucleotide hydrolysis by ARP2 and/or ARP3 was initially thought to be essential for ARP2/3 activity, as nucleation and branching are inhibited by the non-hydrolysable ATP analogue AMP–PNP[27,28]. However, recently characterized Arp2 and Arp3 yeast mutants that are incapable of ATP hydrolysis are as active as the wild-type proteins in promoting actin assembly *in vitro*[33].

The role of hydrolysis by ARP2 is controversial, as it has been linked temporally to the nucleation event[32], or temporally and functionally to branch disassembly and the recycling of the ARP2/3 complex[31] (FIG. 2). The most recent evidence from hydrolysis-defective mutants in yeast indicates that both ideas are likely to be correct[33], as hydrolysis was found to be temporally linked to nucleation and functionally important for promoting branch disassembly[33]. How hydrolysis is coupled to branch disassembly remains to be determined, but it might involve the weakening of ARP–actin contacts, similar to the role of ATP hydrolysis and phosphate release in the actin filament.

Activation of the ARP2/3 complex by NPFs

Since the discovery of the first NPF nearly a decade ago, many more have been identified (FIG. 3). These proteins are classified into two main groups, class I and class II, based on the mechanism by which they activate the ARP2/3 complex and their effect on the y-branching reaction.

Importantly, the activities of NPFs are regulated by signal-transduction pathways that coordinate actin polymerization in space and time. The best studied of these pathways involve the activation of class I NPFs by the Rho-family GTPases CDC42 and Rac (BOX 2). Because the regulation of NPFs has been the focus of several recent reviews[34,35], this section focuses instead on the molecular mechanisms by which NPFs interact with and activate ARP2/3, and on the cellular processes that NPFs direct.

FRET
A technique for measuring changes in the distance and orientation between two fluorescent molecules that can be used to monitor protein–protein interactions, or protein conformational dynamics.

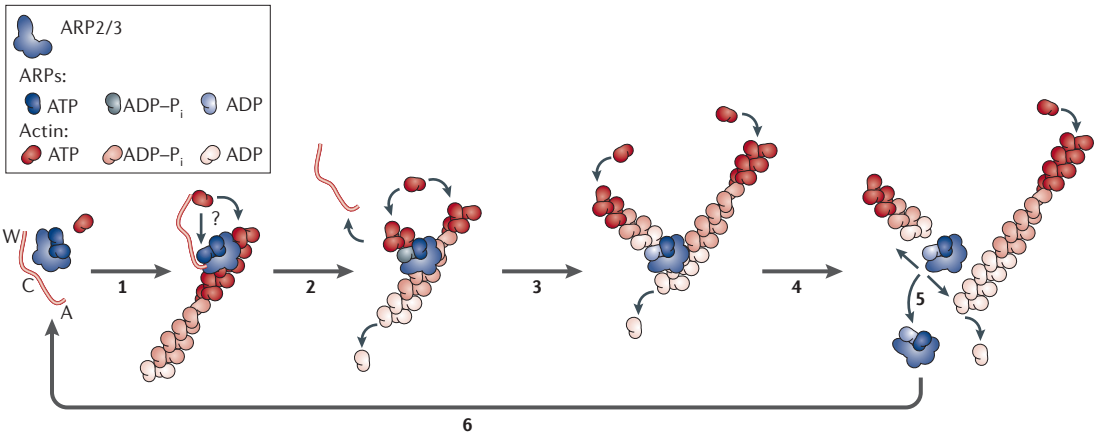


Figure 2 | **Model for activation and recycling of the ARP2/3 complex.** Actin-related protein-2/3 (ARP2/3) complex is shown in blue and actin in red. The nucleotide state of ARPs and actin is indicated by different shading (ARPs: ARP2/3–ATP, blue; ARP2/3–ADP–inorganic-phosphate (P_i), grey; ARP2/3–ADP, light grey. Actin: actin–ATP, red; actin–ADP–P_i, pink; actin–ADP, light pink). The ARP2/3 complex starts in an inactive, open conformation. (step 1) Binding of WCA (Wiskott–Aldrich syndrome protein (WASP)-homology-2, central, acidic) domain promotes a conformational change that primes the complex for activation, which occurs upon binding of the WCA–actin–ARP2/3 assembly to the mother filament, preferentially near the barbed end. WCA domain presents an ATP–actin monomer to the complex and/or possibly to the barbed end of the mother filament. (step 2) ATP is hydrolyzed on ARP2 concomitant with or shortly after nucleation of the daughter filament. The WCA dissociates, although the trigger for this is unknown. (step 3) Phosphate is released from ARP2. Mother and daughter filaments elongate and age by ATP hydrolysis and phosphate release. (step 4) Phosphate release from ARP2 and filament ageing weaken the interactions between ARP2/3 and the daughter and/or mother filament, (step 5) allowing branch disassembly and release of the ARP2/3 complex, presumably in an inactive, ADP-bound conformation. (step 6) Nucleotide exchange on ARP2 (and possibly on ARP3) occurs and the cycle begins again.

Class I NPFs. The first class I NPF to be identified was ActA[36], a protein that is found on the surface of the bacterial pathogen *Listeria monocytogenes* and is required for actin-based bacterial motility in the host cytoplasm. The characterization of ActA was quickly followed by the identification of two eukaryotic class I NPFs: Wiskott—Aldrich syndrome protein (WASP), and suppressor of cyclic AMP repressor (SCAR; also called WASP-family verprolin-homologous protein (WAVE))[18,37–40]. Other class I NPFs that were recently identified include fungal type I myosins[41,42], metazoan CARMIL (capping protein ARP2/3 and myosin-I linker)[43] and pathogen proteins such as RickA from *Rickettsia*[44,45]. The number and diversity of class I NPFs varies from one organism to another; *S. cerevisiae* and *S. pombe* each have one WASP homologue; *D. melanogaster* and *C. elegans* have both WASP and SCAR/WAVE; plants only have SCAR/WAVE; and mammals have WASP, the closely related neural (N)-WASP, and three SCAR/WAVE isoforms.

Class I NPFs are diverse in their overall domain organization (FIG. 3). They only possess a common WCA domain, which consists of a WASP-homology-2 (WH2 or W; also called verprolin-homology) domain that binds to G-actin in a manner that enables polymerization onto the barbed end of a growing filament[46] and a central (C; also called cofilin-homology or connector) and acidic (A; both regions together are known as CA) region that mediates binding to ARP2/3 (REFS 47,48) (note that CARMIL and type I myosins might lack some elements of WCA). The WCA domain is sufficient for the activation of the ARP2/3 complex *in vitro* to polymerize branched actin filaments.

The simplest model for the action of the WCA domain is that it delivers an actin monomer to the complex, forming a trimer of ARP2, ARP3 and actin that functions as the nucleus for the new filament. However, several lines of evidence indicate that the actual mechanism is more complex. For example, NPFs differ in their capability to activate ARP2/3, but NPF activity does not correlate directly with the affinities of NPFs for either the ARP2/3 complex or G-actin[48,49]. Moreover, kinetic modelling of ARP2/3-mediated nucleation indicates the existence of a distinct activation step that occurs subsequently to formation of the NPF–ARP2/3–actin assembly[49]. Last, tethering of a WH2 domain to the ARP2/3 complex is not sufficient to promote activation; the ARP2/3-binding CA region is also required *in trans*[29]. Therefore, the CA region seems to have a more complex function than simply delivering G-actin.

Recent evidence indicates that CA promotes a conformational change in the ARP2/3 complex that depends on conserved residues in the central region of an NPF. This structural rearrangement in the complex, which brings ARP2 and ARP3 closer together, has been observed by both FRET[29] and cryo-EM[50]. Consistent with the idea that NPF binding closes a gap between ARP2 and ARP3, WCA fragments have been chemically crosslinked to ARP2, ARP3 and the neighbouring ARPC1 and ARPC3 subunits[51–53]. Moreover, cryo-EM places the WCA domain near the interface of these subunits[50]. As well as modulating ARP2/3 conformation, the central region of the WCA domain has been shown to interact with an actin monomer in a manner that is mutually exclusive

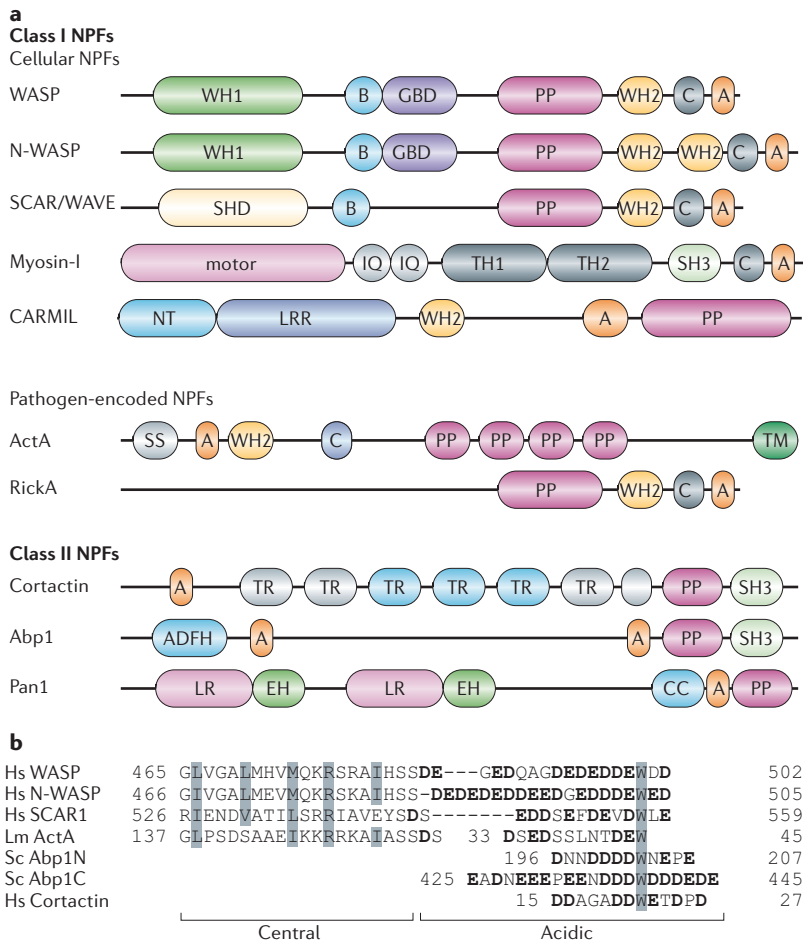


Figure 3 | **Domain organization of nucleation-promoting factors. a** | Domain organization of representative class I and class II nucleation-promoting factors (NPFs). For class I NPFs, activation of actin-related protein-2/3 (ARP2/3) requires binding of globular (G)-actin through the Wiskott–Aldrich syndrome protein (WASP)-homology-2 (WH2) region and binding of ARP2/3 complex through the central (C) and acidic (A) region. Note that CARMIL (capping protein ARP2/3 and myosin-I linker) lacks a discernible central region and myosin-I lacks a WH2 region. Activation of the ARP2/3 complex by class II NPFs requires binding to the ARP2/3 complex through the A region and to filamentous (F)-actin through the central tandem repeat (TR) of cortactin, the actin-depolymerizing-factor homology (ADFH) of actin-binding protein-1 (Abp1), or the coiled-coil (CC) of Pan1. Other regions of NPFs bind to signalling molecules and other cytoskeletal regulators. IQ is a calmodulin-binding motif. B, basic; EH, Eps15 homology; GBD, GTPase-binding domain; LRR, Leu-rich repeat; LR, long repeat; NT, N-terminal; PP, poly-proline; SCAR, suppressor of cyclic AMP repressor; SHD, SCAR-homology domain; SH3, Src-homology-3; SS, signal sequence; TH, tail homology; TM, transmembrane; WAVE, WASP-family verprolin-homologous protein. **b** | Alignment of the ARP2/3-binding central and acidic regions of human (Hs) NPFs, ActA from *Listeria monocytogenes* (Lm) and Abp1 from *Saccharomyces cerevisiae* (Sc). Grey boxes highlight conserved residues in the predicted amphipathic helix in the central region and the conserved tryptophan residue in the acidic region. Acidic residues are in bold. N, neural.

with its binding to the ARP2/3 complex, indicating that it undergoes a dynamic series of interactions with both G-actin and ARP2/3 (REF. 54).

Taken together, these data indicate a model for class I NPF function in which (FIG. 2) the acidic region mediates binding to ARP2/3, the central region initiates an activating conformational change in the complex and the WH2 and central regions present an actin monomer to the complex, facilitating the formation of a nucleus for the polymerization of the daughter filament. After the initiation of a y-branch, class I NPFs dissociate from the ARP2/3 complex[26] and might function catalytically in multiple rounds of ARP2/3 activation. The precise nature and timing of the dynamic interactions between the class I NPFs, G-actin and the ARP2/3 complex that result in actin nucleation still remain to be defined.

Class II NPFs. Class II NPFs include *S. cerevisiae* actin-binding protein-1 (Abp1)[55] and Pan1 (REF. 56), as well as metazoan cortactin[57–59]. Like class I NPFs, these proteins contain an ARP2/3-binding acidic region, but they lack a G-actin-binding region. Instead, they contain an F-actin-binding region that is required for the activation of ARP2/3 (FIG. 3). This is a functionally relevant distinction, as Abp1, Pan1 and cortactin are far less potent activators of the ARP2/3 complex *in vitro* than class I NPFs.

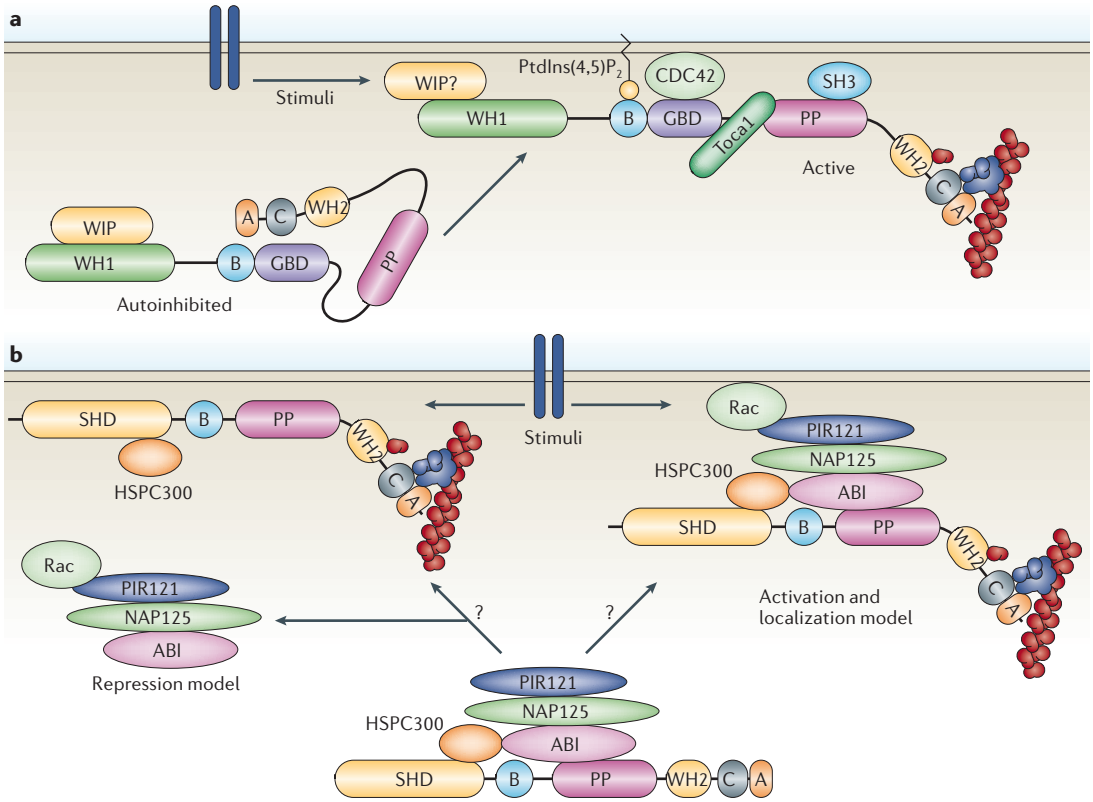
The mechanism of ARP2/3 activation by class II NPFs is not entirely clear. Cortactin lacks a central region and fails to promote an activating conformational change in the ARP2/3 complex[29], perhaps explaining its weak NPF activity. The mechanism by which class II NPFs activate ARP2/3 might involve a capability to promote the association of ARP2/3 with F-actin[55,58,59], which itself is an activator of the complex (see below). Cortactin and N-WASP can bind simultaneously to the ARP2/3 complex[52]; N-WASP is released after y-branching, whereas cortactin remains associated with the complex at the branch point[26] and inhibits branch dissociation[59]. Therefore, rather than serving as primary activators of the ARP2/3 complex, the more important function of class II NPFs might be to stabilize the y-branched organization of actin networks that are generated by the ARP2/3 complex and class I NPFs. It remains to be determined how the activities of class I and class II NPFs are coordinated to regulate the dynamics of actin networks in the cell.

Actin branching and debranching

Preformed actin filaments are also activators of ARP2/3-mediated nucleation *in vitro*[37,60]. Kinetic models indicate that actin polymerization by the ARP2/3 complex is autocatalytic, with the rate of the reaction increasing as more polymer is generated[61]. This conclusion is corroborated by other functional evidence, for example, anti-ARP2/3 antibodies that prevent binding to filaments diminish nucleation activity[62]. These observations indicate that filament nucleation and y-branching are tightly coupled, perhaps inseparable, activities.

Exactly how the nucleation and branching activities are linked together has been a matter of debate, and two competing models have been suggested. The first one proposes that branching occurs from the sides of existing filaments, and the second proposes that it occurs from the barbed ends of filaments. The most compelling experimental evidence supports the side-branching model, as branching from the sides of filaments can be directly observed with purified proteins *in vitro* using fluorescence microscopy[16,63,64]. Other

Box 2 | **Signalling to WASP and SCAR/WAVE**



The capability of nucleation-promoting factors (NPFs) to activate the actin-related protein-2/3 (ARP2/3) complex is regulated by signal-transduction pathways that involve Rho-family GTPases and lipid second messengers. The mechanism of regulation has been well characterized for two class I NPFs, Wiskott–Aldrich syndrome protein (WASP and neural (N)-WASP) and SCAR (suppressor of cyclic AMP repressor; also known as WAVE (WASP-family verprolin-homologous protein)), and differs between the two protein families[35]. Under resting conditions, WASP and N-WASP exist in an autoinhibited conformation in which intramolecular interactions between the GTPase-binding domain (GBD) and the central (C) region obscure the regions that are required for ARP2/3 activation (part **a**). WASP-interacting protein (WIP) or related proteins interact with WASP and N-WASP through the WASP-homology-1 (WH1) domains and modulate activation. Autoinhibition is regulated by the binding of the lipid second messenger phosphatidylinositol-(4,5)-bisphosphate (PtdIns(4,5)P_2) to the basic (B) region, the binding of GTP-loaded CDC42 to the GBD region, and the binding of Src-homology-3 (SH3)-domain-containing proteins such as Nck, Grb2 and Toca1 to the poly-proline (PP) region. These factors function both individually and in combination to relieve autoinhibition and to promote ARP2/3 activation.

Regulation of SCAR/WAVE proteins is less well mechanistically understood (part **b**). Nevertheless, it is clear that under resting conditions these proteins form a complex with four other proteins: PIR121 (p53-inducible mRNA), NAP125 (Nck-associated protein), ABI (Abl-interactor), and HSPC300 (haematopoietic stem-cell progenitor). ABI and HSPC300 directly bind to SCAR/WAVE, and ABI links PIR121 and NAP125 to the NPF. Upon stimulation, GTP-bound Rac binds directly to PIR121 and leads to SCAR/WAVE activation. Two models have been proposed to explain the activation of actin nucleation. In the repression model, association of PIR121, NAP125 and ABI with SCAR/WAVE inhibits SCAR/WAVE activity and Rac binding dissociates these factors to activate actin nucleation. In the activation and localization model, binding of activated Rac causes the recruitment of the entire SCAR/WAVE complex to the membrane, where it can then function to promote nucleation. A, acidic; SHD, SCAR-homology domain.

experimental results are more equivocal. For example, some studies conclude that capping barbed ends has no effect on ARP2/3 activation and branching, in support of the side-branching model[65], whereas others conclude that capping inhibits branching, in support of the barbed-end-branching model[66,67]. Support for the barbed-end-branching model is also derived from EM data, which shows a correlation between mother-filament and daughter-filament lengths at the branch point[61].

An attractive compromise between these models (FIG. 2) is based on observations that side branching is biased to newer ATP-containing portions near the barbed ends of mother filaments compared to older ADP-containing portions towards the pointed ends[16,64]. Further support for this biased-side-branching mechanism comes from kinetic modelling, which indicates that this model gives the best fit to kinetic data[68]. Biased side branching would ensure that new filament ends are generated proximal to sites that require actin-mediated

force generation in cells. Future experiments will address how the branching reaction occurs and what contributes to the autocatalytic nature of this reaction.

Mechanisms of debranching. Release of the mother or daughter filament from the ARP2/3 complex, known as debranching, is crucial for recycling actin networks in the cell. *In vitro*, the half-life of a y-branch ranges from 8 to 27 minutes[31,33,69]. This half-life is probably much shorter in cells, as actin filaments turn over in less than a minute in regions of dynamic actin disassembly[70].

Several factors have been reported to contribute to debranching. Nucleotide hydrolysis and release of inorganic phosphate (P_i) by actin subunits in the mother filament have been proposed to affect branching frequency[64,69], however, these data did not distinguish between effects on branch initiation or dissociation. ATP hydrolysis and P_i release from actin subunits in the daughter filament have also been reported to reduce the affinity of the ARP2/3 complex for pointed ends and to promote dissociation[69]. These results are inconsistent with observations showing that locking actin in the ADP–P_i state has no effect on debranching[31], and that ATP hydrolysis and P_i release from actin occur much more rapidly than debranching. Resolution of these issues might require the development of more physiological and quantitative debranching assays.

Despite this confusion, it is becoming clear that ATP hydrolysis and P_i release from ARP2 has an important role in debranching[31,33]. Although the precise timing of ARP2 ATP hydrolysis in relation to debranching has been a matter of debate[31,32] (see above), a recent study using a mutant ARP2 that cannot hydrolyze ATP showed that hydrolysis is temporally linked to nucleation but functionally important for the facilitation of debranching[33]. This intriguing result indicates that nucleotide hydrolysis on ARP2 might initiate the debranching clock, but does not directly trigger the branch dissociation. Actin-binding proteins, similarly to nucleotide, can regulate y-branch lifetime. For example, debranching can be accelerated by actin-depolymerizing factor (ADF; also known as cofilin)[69], and can be inhibited by the class II NPF cortactin[59]. These and other regulatory factors are likely to function in the cell to ensure that the ARP2/3 complex and actin are recycled and that the actin network is remodelled as required.

Cellular functions of the ARP2/3 complex

Coordinated nucleation and branching by the ARP2/3 complex has an important role in several cellular processes.

Evidence from genetics. Genetic studies have shown that the ARP2/3 complex is essential for viability of both unicellular and multicellular organisms. In *S. cerevisiae* and *S. pombe*, inactivating or deleting genes that encode subunits of the ARP2/3 complex causes severe growth defects or lethality[5,9,10,19,71]. In *D. melanogaster*, the disruption of ARP2/3 function causes lethality before adulthood, with defects in cytoplasmic organization in the blastoderm, axon development, ring canal expansion and eye morphology[72,73]. RNA interference (RNAi)-mediated knockdown of ARP2/3 subunits in *C. elegans* results in lethality and affects ventral enclosure in the developing worm[74]. Although there are no published reports of ARP2/3-knockout mammals, the inactivation of ARPC3 in human HeLa cells by RNAi is lethal[75]. Furthermore, inactivation or knockout of individual NPFs in mice results in defects ranging from embryonic or post-natal mortality to more mild tissue-specific irregularities[76–81].

The function of the ARP2/3 complex is clearly important for some organisms, however, it is not essential for all eukaryotes. In *Arabidopsis thaliana*, mutations in subunits of the ARP2/3 complex cause defects in the shape of epidermal cells but do not affect the viability of the plant[82–84]. Also, apicomplexan parasites completely lack the ARP2/3 complex[13] and *L. major* encodes only some of the subunits[14]. Nevertheless, the severity of the phenotypes that are induced when the function of the ARP2/3 complex is perturbed in diverse species reflects its role in fundamental and conserved cellular processes (see following sections).

Cell migration and adhesion. Crawling cell migration is a basic behaviour of many cell types, ranging from unicellular amoeba that migrate to forage for food, to cells in multicellular organisms that migrate during development, wound healing and immune response. Efficient migration involves dynamic actin assembly adjacent to the plasma membrane in structures known as lamellipodia, pseudopodia and filopodia, which are protrusions that drive the advance of the leading edge of the cell[1].

The ARP2/3 complex is localized to lamellipodia and pseudopodia, usually within several micrometers of the leading edge[23,85–87], but not to filopodia. Moreover, the ARP2/3 complex has been observed by immuno-EM to organize actin into y-branched networks in lamellipodia, similar to its activity *in vitro*[15,88]. The notion that the ARP2/3 complex has an important role in lamellipodial protrusion is supported by findings showing that this process is inhibited when ARP2/3 activity is reduced in various cell types using RNAi[89,90], inhibitory antibodies[62] or dominant-negative fragments of NPFs[18]. There is a conflicting report that silencing ARP3 expression by RNAi does not inhibit lamellipodial protrusion[91], but in this case RNAi also failed to completely inhibit *L. monocytogenes* motility, a process that is known to depend on ARP2/3 activity[40,92].

Some of these discrepancies might be due to a differential role for the ARP2/3 complex in different cell types, as ARP2/3 activity was also found to be dispensable for leading-edge protrusion during neuronal growth cone translocation[93]. Moreover, the failure to form detectable lamellipodia that contain ARP2/3 does not necessarily inhibit leading-edge protrusion or whole-cell migration[94]. With regard to filopodia, it has been proposed that their bundled filament cores originate from ARP2/3-generated y-branched-filament networks in lamellipodia[95,96]. Nevertheless, a recent study concludes that ARP2/3 is not required for filopodia formation[90]. These observations highlight the potential importance of other actin-polymerization pathways.

Ring canal
Intercellular bridges that connect the developing *D. melanogaster* oocyte to the nurse cells and serve as conduits for the transfer of cytoplasmic components.

Lamellipodia
Flat, sheet-like cellular protrusions that contain a network of actin filaments that mediate the protrusion of the leading edge of a migrating cell.

Pseudopodia
Large cellular protrusions that contain a network of actin filaments that mediate the protrusion of the leading edge of an amoeboid cell or a phagocyte during crawling migration.

Filopodia
Thin, finger-like structures with a bundled core of actin filaments that form at the leading edge of migrating animal cells.

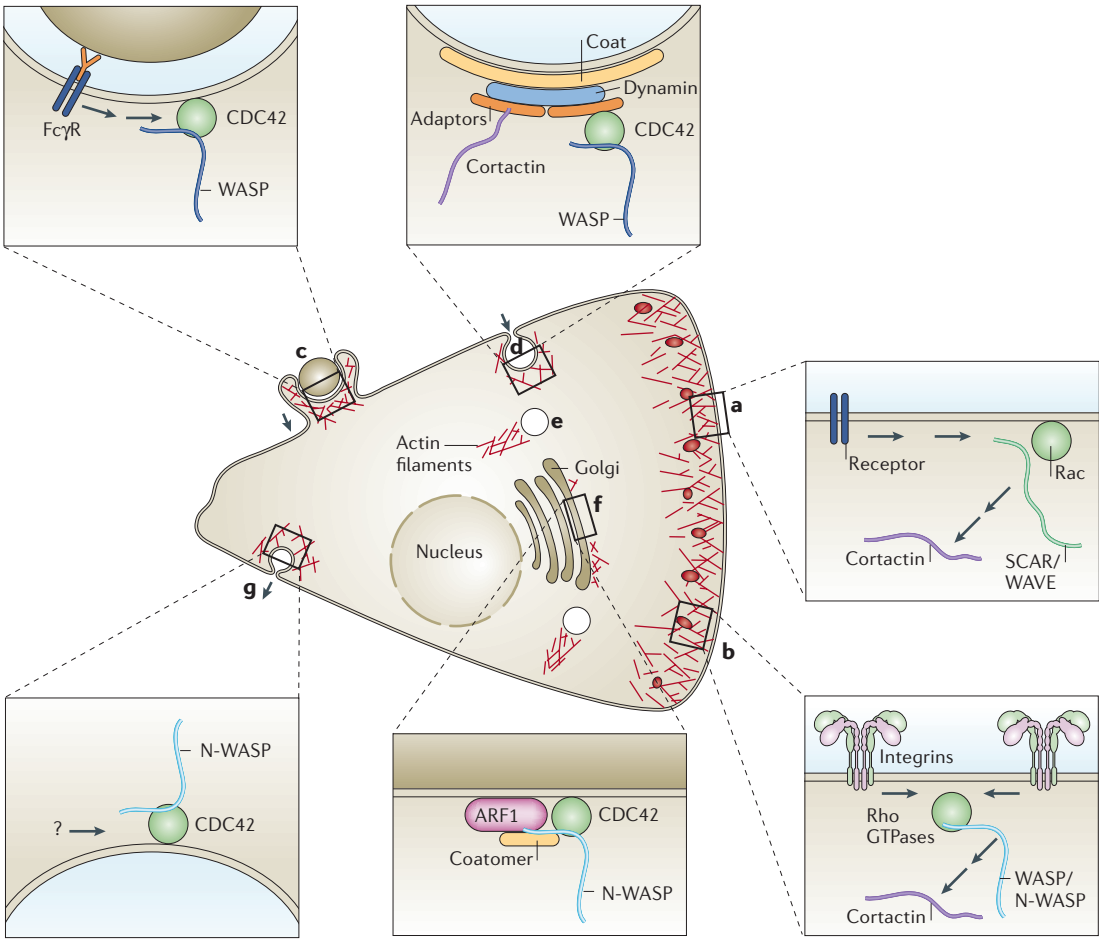


Figure 4 | **Cellular functions of the ARP2/3 complex.** The actin-related protein-2/3 (ARP2/3) complex organizes actin filaments (red lines) into branched networks that are capable of generating protrusive force and resisting mechanical deformation. Its nucleation and branching activities are implicated in (**a**) lamellipodial protrusion, (**b**) adhesion and podosome formation, (**c**) phagocytosis, (**d**) endocytosis, (**e**) vesicle and organelle motility, (**f**) trafficking within and from the Golgi apparatus, and (**g**) exocytosis. Insets illustrate the nucleation-promoting factors (NPFs) that have been demonstrated to be involved in each process (Wiskott–Aldrich syndrome protein (WASP), blue; neural (N)-WASP, light blue; suppressor of cyclic AMP repressor (SCAR; also known as WASP-family verprolin-homologous protein (WAVE)), green; cortactin, purple), and a subset of the molecules involved in recruiting and/or activating the NPFs at the sites of actin polymerization. During lamellipodia protrusion (**a**), adhesion (**b**), and phagocytosis (**c**), engagement of transmembrane proteins (integrins and receptors) causes activation of Rho-family GTPases (CDC42 and Rac) and NPFs, leading to ARP2/3-mediated actin polymerization. At sites of endocytosis (**d**) and internal membranes (**e**,**f**), coat proteins (coatamer as well as others), Rho-family GTPases, and other molecules (for example, dynamin and ARF1) activate NPFs and the ARP2/3 complex. FcγR, Fcγ receptor.

The NPFs that are responsible for activating the ARP2/3 complex in lamellipodia and pseudopodia are the SCAR/WAVE proteins (FIG. 4). In mammalian cells there are three SCAR/WAVE isoforms that exhibit tissue-specific expression patterns[97,98], all of which localize to the leading edge of migrating cells[99–101]. By examining fibroblasts and other cells types that are depleted of these proteins by mutations or RNAi, it has become clear that SCAR2/WAVE2 (REFS 80,81) and SCAR3/WAVE3 (REF. 102) are crucial for lamellipodia formation and directed cell migration, whereas SCAR1/WAVE1 is important for dorsal ruffle formation and stabilization of lamellipodial protrusions[103,104]. The importance of SCAR/WAVE proteins is not unique to mammals, as the loss of these proteins in *D. melanogaster* S2 cells also prevents formation of lamella[89]. The function of SCAR/WAVE proteins at the leading edge is thought to be controlled by the small GTPase Rac, which has a key role in lamellipodia formation[105] (BOX 2). Future work is needed to understand how the SCAR/WAVE–ARP2/3 pathway cooperates with other pathways to mediate actin polymerization during cell migration.

Actin remodelling in the lamellipodium is coupled to the formation of adhesive contacts that link the actin cytoskeleton to the extracellular matrix or to neighbouring cells. Emerging evidence indicates that the ARP2/3 complex might be transiently associated with nascent focal contacts through its direct binding to the integrin-associated protein vinculin, perhaps functioning to link adhesion with membrane protrusion[106]. Similarly, it was

recently observed that E-cadherin interacts with the ARP2/3 complex to promote local actin assembly and lamellipodial protrusion during the formation of early cell–cell adhesive contacts[107], an effect that is reduced upon maturation of adhesion sites[108]. This provides an explanation for the transition of these sites from dynamic contact sites to quiescent junctions that are required for a stable interaction between cells.

Moving membranes and their cargoes. Actin polymerization has also been linked to the generation of forces that remodel or transport membranes during trafficking events. Phagocytosis, or engulfment of large (greater than 0.5 μm in diameter) particles such as bacteria, requires actin polymerization to drive the extension of the plasma membrane around the particle surface (FIG. 4). The ARP2/3 complex localizes to both Fcγ receptor-mediated and complement receptor-mediated phagosomes and is essential for actin polymerization in the phagocytic cup as well as subsequent particle engulfment[109]. This function is likely to be of primary importance in professional phagocytes of the immune system, which help to clear infectious agents. The only NPF so far implicated in phagocytosis is WASP, which is specifically expressed in haematopoietic cells in mammals. WASP localizes to Fcγ-receptor-induced phagocytic cups[110,111], and cells from patients with Wiskott–Aldrich syndrome (WAS) that have null alleles for WASP show a reduced capacity for phagocytosis and defects in actin accumulation at phagocytic structures[112].

Actin is also involved in the internalization of small cargoes by endocytosis (FIG. 4). In *S. cerevisiae*, the ARP2/3 complex and NPFs including Pan1, myosin-5, Las17 (the WASP homologue) and Abp1 are recruited to endocytic sites prior to, or concomitant with, actin polymerization[113,114]. Likewise, in mammalian cells, N-WASP, cortactin and the ARP2/3 complex localize to sites of clathrin-mediated endocytosis[115,116]. Disrupting the activity of the ARP2/3 complex and its activators in yeasts or mammalian cells causes defects in endocytosis[117–120].

The precise role of ARP2/3-mediated actin polymerization in endocytosis is not yet clear. Actin polymerization might assist in invaginating the membrane, pinching off vesicles, and/or driving vesicles away from the plasma membrane[121]. The possibility that actin polymerization drives vesicles away from the plasma membrane is supported by the observations that endosomes, phagosomes, macropinosomes and lysosomes can rocket through the cytoplasm or through cell-free extracts by the polymerization of a closely associated comet tail of actin filaments[122–125]. Rocketing motility of endosomes involves the activity of the NPFs WASP or N-WASP, which localize to the surface of motile vesicles[124] and are functionally important for actin assembly[122,126].

ARP2/3-mediated actin polymerization has also been implicated in other membrane trafficking events (FIG. 4). Actin and actin-binding proteins associate with the Golgi apparatus and have a role in both anterograde and retrograde transport[127]. Initiation of actin polymerization at Golgi membranes is regulated by the recruitment of the ARP2/3 complex, N-WASP and its upstream regulator CDC42 (REFS 128–131). The precise role of actin in transport to and from the Golgi remains to be shown, but similarly to its roles in endocytosis, it might function in vesicle scission or motility. The ARP2/3 complex might also have a role in exocytosis. Although the details of its involvement are unclear, the ARP2/3 complex has been localized to secretory granules in PC12 neuroendocrine cells, and N-WASP and CDC42 have been shown to promote exocytosis[132]. Understanding the biophysical role of ARP2/3 in membrane remodelling and how this process is controlled in cells are important areas for future investigation.

The ARP2/3 complex and disease

Given the range of important cellular functions that are attributed to the ARP2/3 complex, it is not surprising that its malfunction is associated with disease.

Pathogens abuse the ARP2/3 complex's power. Numerous microbial pathogens manipulate the ARP2/3 complex to their advantage during infection (FIG. 5). Microbes differ considerably in the mechanism by which they access and activate the ARP2/3 complex, but they can generally be grouped into two classes: those that activate the ARP2/3 complex from the outside of the cell, and those that activate the complex intracellularly.

Pathogens that activate ARP2/3 from the outside have evolved strategies to mimic or tap into receptor-mediated-signalling events that lead to actin polymerization at the plasma membrane. Two related examples of this are the diarrhoeagenic agents enteropathogenic and enterohaemorrhagic *Escherichia coli* (EPEC and EHEC), which form actin-rich pedestals that mediate bacterial attachment to and motility across intestinal epithelial cells[133]. EPEC uses a type III secretion system to introduce the translocated intimin receptor (Tir) into the host cell's plasma membrane. Tir in turn recruits host factors including the NPF N-WASP, leading to activation of the ARP2/3 complex[76,134,135]. In a slight variation on this strategy, EHEC uses the bacterial effector protein EspFU in combination with Tir to recruit host N-WASP and the ARP2/3 complex[136]. Vaccinia virus, a relative of the causative agent of smallpox, forms pedestals upon exit from host cells in a process that is thought to promote cell–cell spread[137]. Similar to EPEC and EHEC, the vaccinia A36R protein mimics receptor-tyrosine-kinase signalling, leading to activation of host N-WASP and the ARP2/3 complex[138]. Another example is *Salmonella enterica*, which induces actin polymerization in non-phagocytic cells to promote bacterial internalization. Like *E. coli*, *S. enterica* uses type III secretion to deliver bacterial effectors into the host cell cytoplasm[139]. However, the *S. enterica* effectors activate CDC42 and Rac, which in turn activate WASP or N-WASP and SCAR/WAVE, leading to ARP2/3-mediated membrane ruffling that gathers up and internalizes bacteria[140–142].

In contrast to the example above, pathogens that reside within the cytoplasm have direct access to the ARP2/3 complex and NPFs. Many of these pathogens have evolved the capability to recruit and activate

Fcγ receptor
A family of receptors found on the surface of phagocytic cells. They bind to the constant (Fc) region of immunoglobulins and mediate the phagocytosis of pathogens.

Complement receptor
A family of receptors found on the surface of phagocytic cells. They bind to complement proteins and mediate the phagocytosis of pathogens.

Clathrin-mediated endocytosis
The uptake of material into the cell by a mechanism that involves the assembly of a clathrin protein into a cage-like structure on the cytoplasmic surface of the membrane.

Type III secretion system
A needle-like complex of proteins used by many Gram-negative bacterial pathogens to inject virulence factors (called effectors) directly into the cytoplasm of a host cell.

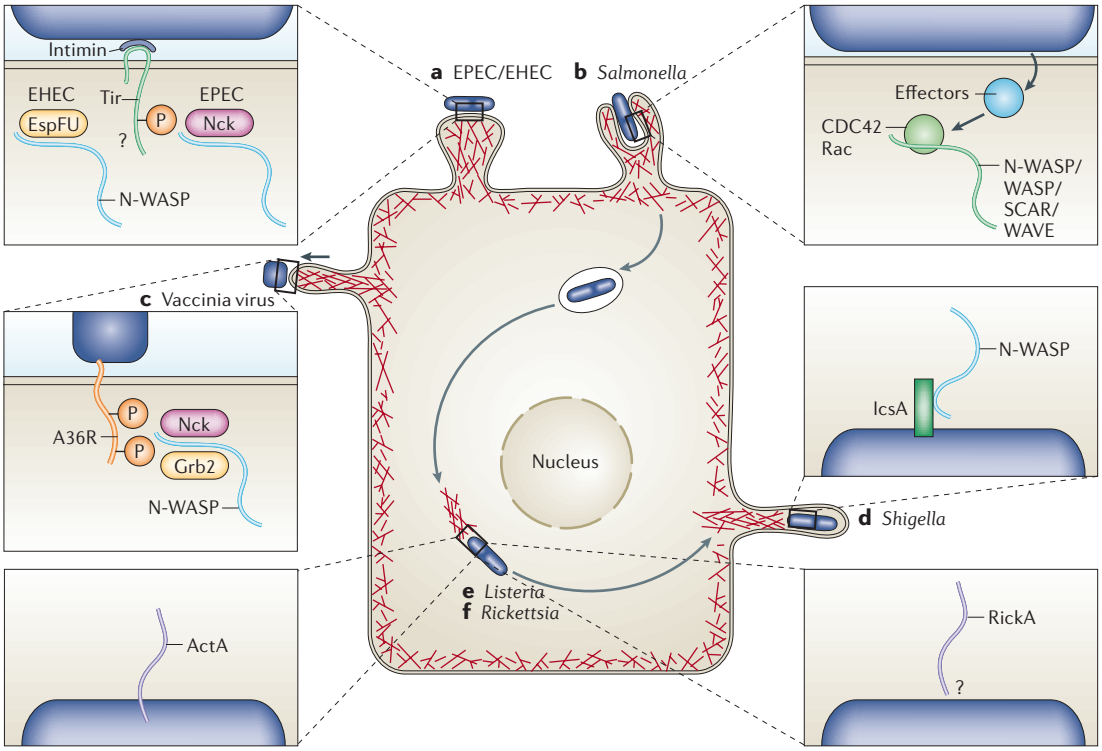


Figure 5 | **Pathogens use ARP2/3 complex activities during infection.** Several viral and bacterial pathogens have adapted mechanisms that tap into actin-related protein-2/3 (ARP2/3)-mediated actin nucleation and branching. Some activate the ARP2/3 complex from outside the cell to promote actin polymerization at the plasma membrane, facilitating attachment (**a**, enteropathogenic and enterohaemorrhagic *Escherichia coli* (EPEC and EHEC)), cell–cell spread (**c**, vaccinia virus), or phagocytosis (**b**, *Salmonella*). Others (**d**, *Shigella*; **e**, *Listeria*; **f**, *Rickettsia*) initiate actin assembly at their surface after entry into the cytoplasm by recruitment and activation of the ARP2/3 complex to form a comet tail of filaments that mediates their motility and cell–cell spread. Insets illustrate the nucleation-promoting factors (NPFs) that have been demonstrated to be involved in each process (neural Wiskott–Aldrich syndrome protein (N-WASP), light blue; suppressor of cyclic AMP repressor (SCAR; also known as WASP-family verprolin-homologous protein (WAVE)), green; ActA and RickA, light purple), and the molecules involved in recruiting and activating NPFs at the sites of actin polymerization. EPEC and EHEC (**a**), as well as vaccinia (**c**) insert proteins (Tir (which binds to bacterial intimin) and A36R) into the plasma membrane, thereby recruiting adaptor proteins (Grb2 and Nck), that bind and activate N-WASP. *Salmonella* (**b**) injects effectors into the cytoplasm, thereby activating Rac and CDC42, leading to NPF activation and actin polymerization. The *Shigella* (**d**) protein IcsA recruits N-WASP to the bacterial surface, whereas *Listeria* (**e**) and *Rickettsia* (**f**) produce their own NPFs (ActA and RickA) that initiate ARP2/3-mediated actin polymerization.

ARP2/3 at their surface, leading to actin polymerization and actin-based motility that promotes bacterial cell–cell spread (FIG. 5). This type of motility was first described for *L. monocytogenes* and *Shigella flexneri*, and was later observed with phylogenetically diverse bacteria including *Rickettsia* species, *Burkholderia pseudomallei* and *Mycobacterium marinum*[133]. In the case of *L. monocytogenes* and *Rickettsia* species, the bacteria encode their own NPFs (ActA and RickA, respectively) that directly activate the ARP2/3 complex[36,44,45]. By contrast, *S. flexneri* expresses a protein called IcsA that recruits host N-WASP[143], and *M. marinum* also recruits host WASP or N-WASP by an unknown mechanism[144]. The precise mechanisms used by *B. pseudomallei* to polymerize actin are not yet understood[145]. The actin-based motility of *L. monocytogenes* and *S. flexneri* can now be reconstituted with purified components *in vitro*[146], serving as a beautiful model for understanding the biochemical and biophysical properties of force-generating actin networks.

The ARP2/3 complex and human disease. In addition to abuse by invading microorganisms, the improper function of the ARP2/3 complex and its regulators can lead to disease. One important example is WAS, a rare recessive X-linked genetic disorder that involves defects in blood-cell function[147] and leads to susceptibility to infection, eczema and internal haemorrhages. The gene that is mutated in WAS encodes WASP, an NPF that is specifically expressed in haematopoietic cells[148]. In T cells, mutations in WASP cause specific defects in the function of the actin-rich immunological synapse that forms the interface between the T cell and the antigen-presenting cell[149,150], leading to defects in T-cell receptor signalling, interleukin-2 production and T-cell proliferation[147]. Recent work indicates that SCAR/WAVE proteins also have a prominent role in mediating actin dynamics at the immunological synapse[151,152].

In platelets, mutations in WASP cause cytoskeletal defects that result in low platelet number and volume[147]. Moreover, mutations in WASP cause defects

Antigen-presenting cell
A cell that displays on its surface a foreign antigen in association with a major histocompatibility complex (MHC) protein. Antigen presentation can lead to T-cell activation.

in actin-rich podosome formation in macrophages[153] and osteoclasts[154], phagocytic efficiency in macrophages[112] and chemotaxis in macrophages and dendritic cells[155–157]. The severity of the disease that results when the function of this single NPF is compromised emphasizes the importance of the precise regulation of the ARP2/3 complex in the immune response, and highlights the potential relevance of these factors in understanding and treating diseases of immunity and inflammation.

ARP2/3 complex dysfunction might also be associated with cancer metastasis, which relies on the capability of cancer cells to migrate away from primary tumours and invade healthy tissues[158]. The expression of ARP2/3 mRNAs and their protein levels[159–161], together with N-WASP[162], WAVE2 (REF. 161) and other factors that are functionally associated with cell motility[158], are upregulated in some tumour tissues and invasive cells. Cancer-cell invasion into tissues also requires the formation of actin-rich structures such as podosomes and invadopodia that have adhesive and protrusive activities and promote the degradation of the extracellular matrix. The activation of the ARP2/3 complex is required for podosome formation[153,154,163], and the complex has been colocalized with WASP[163], N-WASP[164] and cortactin[165] in the F-actin-rich podosome core (FIG. 4). Likewise, the ARP2/3 complex and N-WASP localize to invadopodia and are required for their formation[166]. The ARP2/3 complex and its activators are therefore likely to be crucial participants in the process of tumour-cell invasion and metastasis, and might represent targets for therapeutic intervention.

Concluding remarks

Ten years of research into the function and regulation of the ARP2/3 complex has produced abundant information about this fascinating molecular machine. Nevertheless, many questions remain to be answered. One important area for exploration relates to how the activity of ARP2/3 is regulated in the cell. For example, we know little about how the activities of distinct NPFs are coordinated to mediate actin polymerization and organization during different cellular behaviours. Moreover, we need to determine whether there are undiscovered NPFs that link the ARP2/3 complex to unique signalling pathways, subcellular locales and actin-dependent events. A second important area for exploration relates to the precise mechanism of actin nucleation and organization by the ARP2/3 complex. What is missing is a detailed molecular map of the complex when it is engaged with an NPF, mother filament, and daughter filament. Last, we are beginning to characterize the role of the ARP2/3 complex in pathogenesis, immunity, inflammation and cancer progression, and the information we gain from basic studies could be relevant to translational research that is aimed at diagnosing and treating disease. With the basic paradigms of ARP2/3 complex regulation and function defined, we are poised to begin exploring the complex web of interactions that allow it to function precisely in the complicated cellular world.

1. Pollard, T. D. & Borisy, G. G. Cellular motility driven by assembly and disassembly of actin filaments. *Cell* **112**, 453–465 (2003).
2. Baum, B. & Kunda, P. Actin nucleation: spire-actin nucleator in a class of its own. *Curr. Biol.* **15**, R305–R308 (2005).
3. Kovar, D. R. Molecular details of formin-mediated actin assembly. *Curr. Opin. Cell Biol.* **18**, 11–17 (2006).
4. Machesky, L. M., Atkinson, S. J., Ampe, C., Vandekerckhove, J. & Pollard, T. D. Purification of a cortical complex containing two unconventional actins from *Acanthamoeba* by affinity chromatography on profilin-agarose. *J. Cell Biol.* **127**, 107–115 (1994).
Initial purification and characterization of the ARP2/3 complex.
5. Lees-Miller, J. P., Henry, G. & Helfman, D. M. Identification of *act2*, an essential gene in the fission yeast *Schizosaccharomyces pombe* that encodes a protein related to actin. *Proc. Natl Acad. Sci. USA* **89**, 80–83 (1992).
6. Schwob, E. & Martin, R. P. New yeast actin-like gene required late in the cell cycle. *Nature* **355**, 179–182 (1992).
7. Fyrberg, C. & Fyrberg, E. A *Drosophila* homologue of the *Schizosaccharomyces pombe act2* gene. *Biochem. Genet.* **31**, 329–341 (1993).
8. Waterston, R. *et al.* A survey of expressed genes in *Caenorhabditis elegans*. *Nature Genet.* **1**, 114–123 (1992).
9. Winter, D., Podtelejnikov, A. V., Mann, M. & Li, R. The complex containing actin-related proteins Arp2 and Arp3 is required for the motility and integrity of yeast actin patches. *Curr. Biol.* **7**, 519–529 (1997).
The *S. cerevisiae* Arp2/3 complex is characterized *in vitro* and *in vivo* and is shown to be important for the function of cortical actin patches.
10. Morrell, J. L., Morphew, M. & Gould, K. L. A mutant of Arp2p causes partial disassembly of the Arp2/3 complex and loss of cortical actin function in fission yeast. *Mol. Biol. Cell* **10**, 4201–4215 (1999).
11. Welch, M. D., Iwamatsu, A. & Mitchison, T. J. Actin polymerization is induced by the Arp2/3 protein complex at the surface of *Listeria monocytogenes*. *Nature* **385**, 265–269 (1997).
The Arp2/3 complex is biochemically isolated as a host factor that promotes actin nucleation at the surface of the bacterial pathogen *L. monocytogenes*.
12. Ma, L., Rohatgi, R. & Kirschner, M. W. The Arp2/3 complex mediates actin polymerization induced by the small GTP-binding protein Cdc42. *Proc. Natl Acad. Sci. USA* **95**, 15362–15367 (1998).
13. Gordon, J. L. & Sibley, L. D. Comparative genome analysis reveals a conserved family of actin-like proteins in apicomplexan parasites. *BMC Genomics* **6**, 179 (2005).
14. Berriman, M. *et al.* The genome of the African trypanosome *Trypanosoma brucei*. *Science* **309**, 416–422 (2005).
15. Mullins, R. D., Heuser, J. A. & Pollard, T. D. 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 (1998).
Shows that the ARP2/3 complex binds to the sides and pointed ends of actin filaments and mediates filament branching.
16. Amann, K. J. & Pollard, T. D. Direct real-time observation of actin filament branching mediated by Arp2/3 complex using total internal reflection fluorescence microscopy. *Proc. Natl Acad. Sci. USA* **98**, 15009–15013 (2001).
Initiation of ARP2/3-mediated daughter-filament formation from the sides of mother filaments is observed in real time.
17. Mullins, R. D., Stafford, W. F. & Pollard, T. P. Structure, subunit topology, and actin-binding activity of the Arp2/3 complex from *Acanthamoeba*. *J. Cell Biol.* **136**, 331–343 (1997).
18. Machesky, L. M. & Insall, R. H. Scar1 and the related Wiskott–Aldrich syndrome protein, WASP, regulate the actin cytoskeleton through the Arp2/3 complex. *Curr. Biol.* **8**, 1347–1356 (1998).
WASP and SCAR1 are shown to interact physically with the ARP2/3 complex and to regulate the actin cytoskeleton.
19. Winter, D. C., Choe, E. Y. & Li, R. 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 (1999).
20. Gournier, H., Goley, E. D., Niederstrasser, H., Trinh, T. & Welch, M. D. Reconstitution of human Arp2/3 complex reveals critical roles of individual subunits in complex structure and activity. *Mol. Cell* **8**, 1041–1052 (2001).
21. Zhao, X., Yang, Z., Qian, M. & Zhu, X. Interactions among subunits of human Arp2/3 complex: p20–Arc as the hub. *Biochem. Biophys. Res. Commun.* **280**, 513–517 (2001).
22. Robinson, R. C. *et al.* Crystal structure of Arp2/3 complex. *Science* **294**, 1679–1684 (2001).
The crystal structure of the inactive ARP2/3 complex shows molecular details of its organization and provides insights into its mechanism of action.
23. Kelleher, J. F., Atkinson, S. J. & Pollard, T. D. Sequences, structural models, and cellular localization of the actin-related proteins Arp2 and Arp3 from *Acanthamoeba*. *J. Cell Biol.* **131**, 385–397 (1995).
24. Nolen, B. J., Littlefield, R. S. & Pollard, T. D. Crystal structures of actin-related protein 2/3 complex with bound ATP or ADP. *Proc. Natl Acad. Sci. USA* **101**, 15627–15632 (2004).
25. Beltzner, C. C. & Pollard, T. D. Identification of functionally important residues of Arp2/3 complex by analysis of homology models from diverse species. *J. Mol. Biol.* **336**, 551–565 (2004).
26. Egile, C. *et al.* Mechanism of filament nucleation and branch stability revealed by the structure of the Arp2/3 complex at actin branch junctions. *PLoS Biol* **3**, e383 (2005).
The most recent cryo-EM structure of the activated ARP2/3 complex in which the subunits of the complex are localized in a branch point by addition of bulky tags.

27. Dayel, M. J., Holleran, E. A. & Mullins, R. D. Arp2/3 complex requires hydrolyzable ATP for nucleation of new actin filaments. *Proc. Natl Acad. Sci. USA* **98**, 14871–14876 (2001).
28. Le Clainche, C., Didry, D., Carlier, M. F. & Pantaloni, D. Activation of Arp2/3 complex by Wiskott–Aldrich syndrome protein is linked to enhanced binding of ATP to Arp2. *J. Biol. Chem.* **276**, 46689–46692 (2001).
29. Goley, E. D., Rodenbusch, S. E., Martin, A. C. & Welch, M. D. Critical conformational changes in the Arp2/3 complex are induced by nucleotide and nucleation promoting factor. *Mol. Cell* **16**, 269–279 (2004).
FRET studies show that conformational changes induced by activating factors are important for the activation of the ARP2/3 complex.
30. Martin, A. C. *et al.* Effects of Arp2 and Arp3 nucleotide-binding pocket mutations on Arp2/3 complex function. *J. Cell Biol.* **168**, 315–328 (2005).
31. Le Clainche, C., Pantaloni, D. & Carlier, M. F. ATP hydrolysis on actin-related protein 2/3 complex causes debranching of dendritic actin arrays. *Proc. Natl Acad. Sci. USA* **100**, 6337–6342 (2003).
ATP hydrolysis on ARP2 is found to be temporally and functionally linked to actin branch dissociation.
32. Dayel, M. J. & Mullins, R. D. Activation of Arp2/3 complex: addition of the first subunit of the new filament by a WASP protein triggers rapid ATP hydrolysis on Arp2. *PLoS Biol.* **2**, E91 (2004).
The timing of and requirements for ATP hydrolysis on ARP2 are defined.
33. Martin, A. C., Welch, M. D. & Drubin, D. G. Arp2/3 ATP hydrolysis-catalyzed branch dissociation is critical for endocytic force generation. *Nature Cell Biol.* **8**, 826–833 (2006).
***In vivo* and *in vitro* characterization of hydrolysis defective mutants in yeast Arp2 and Arp3 supports a functional role for ATP hydrolysis on Arp2 in debranching and remodelling of actin networks in yeast.**
34. Bompard, G. & Caron, E. Regulation of WASP/WAVE proteins: making a long story short. *J. Cell Biol.* **166**, 957–962 (2004).
35. Stradal, T. E. & Scita, G. Protein complexes regulating Arp2/3-mediated actin assembly. *Curr. Opin. Cell Biol.* **18**, 4–10 (2006).
36. Welch, M. D., Rosenblatt, J., Skoble, J., Portnoy, D. & Mitchison, T. J. Interaction of human Arp2/3 complex and the *Listeria monocytogenes* ActA protein in actin filament nucleation. *Science* **281**, 105–108 (1998).
ActA is identified as the first activator for ARP2/3.
37. Machesky, L. M. *et al.* Scar, a WASp-related protein, activates nucleation of actin filaments by the Arp2/3 complex. *Proc. Natl Acad. Sci. USA* **96**, 3739–3744 (1999).
Shows that SCAR/WAVE proteins and pre-formed actin filaments are activators of the ARP2/3 complex.
38. Rohatgi, R. *et al.* The interaction between N-WASP and the Arp2/3 complex links Cdc42-dependent signals to actin assembly. *Cell* **97**, 221–231 (1999).
Shows that CDC42 and phosphatidylinositol bisphosphate activate N-WASP, which in turn activates the ARP2/3 complex.
39. Winter, D., Lechler, T. & Li, R. Activation of the yeast Arp2/3 complex by Bee1p, a WASP-family protein. *Curr. Biol.* **9**, 501–504 (1999).
40. Yarar, D., To, W., Abo, A. & Welch, M. D. The Wiskott–Aldrich syndrome protein directs actin-based motility by stimulating actin nucleation with the Arp2/3 complex. *Curr. Biol.* **9**, 555–558 (1999).
41. Lee, W. L., Bezanilla, M. & Pollard, T. D. Fission yeast myosin-I, Myo1p, stimulates actin assembly by Arp2/3 complex and shares functions with WASp. *J. Cell Biol.* **151**, 789–800 (2000).
42. Lechler, T., Jonsdottir, G. A., Klee, S. K., Pellman, D. & Li, R. A two-tiered mechanism by which Cdc42 controls the localization and activation of an Arp2/3-activating motor complex in yeast. *J. Cell Biol.* **155**, 261–270 (2001).
43. Jung, G., Remmert, K., Wu, X., Volosky, J. M. & Hammer, J. A. 3rd. The *Dictyostelium* CARMIL protein links capping protein and the Arp2/3 complex to type I myosins through their SH3 domains. *J. Cell Biol.* **153**, 1479–1497 (2001).
44. Gouin, E. *et al.* The RickA protein of *Rickettsia conorii* activates the Arp2/3 complex. *Nature* **427**, 457–461 (2004).
45. Jeng, R. L. *et al.* A *Rickettsia* WASP-like protein activates the Arp2/3 complex and mediates actin-based motility. *Cell Microbiol.* **6**, 761–769 (2004).
46. Chereau, D. *et al.* Actin-bound structures of Wiskott–Aldrich syndrome protein (WASP)-homology domain 2 and the implications for filament assembly. *Proc. Natl Acad. Sci. USA* **102**, 16644–16649 (2005).
47. Marchand, J. B., Kaiser, D. A., Pollard, T. D. & Higgs, H. N. Interaction of WASP/Scar proteins with actin and vertebrate Arp2/3 complex. *Nature Cell Biol.* **3**, 76–82 (2001).
48. Panchal, S. C., Kaiser, D. A., Torres, E., Pollard, T. D. & Rosen, M. K. A conserved amphipathic helix in WASP/Scar proteins is essential for activation of Arp2/3 complex. *Nature Struct. Mol. Biol.* **10**, 591–598 (2003).
Identifies conserved residues in the central region of the WCA domain that contribute to ARP2/3 activation and autoinhibition of N-WASP.
49. Zalevsky, J., Lempert, L., Kranitz, H. & Mullins, R. D. Different WASP family proteins stimulate different Arp2/3 complex-dependent actin-nucleating activities. *Curr. Biol.* **11**, 1903–1913 (2001).
50. Rodal, A. A. *et al.* Conformational changes in the Arp2/3 complex leading to actin nucleation. *Nature Struct. Mol. Biol.* **12**, 26–31 (2005).
Cryo-EM is used to visualize conformational changes that are required for the activation of the ARP2/3 complex.
51. Zalevsky, J., Grigorova, I. & Mullins, R. D. Activation of the Arp2/3 complex by the *Listeria* ActA protein. ActA binds two actin monomers and three subunits of the Arp2/3 complex. *J. Biol. Chem.* **276**, 3468–3475 (2001).
52. Weaver, A. M. *et al.* Interaction of cortactin and N-WASp with Arp2/3 complex. *Curr. Biol.* **12**, 1270–1278 (2002).
53. Kreishman-Deitrick, M. *et al.* NMR analyses of the activation of the Arp2/3 complex by neuronal Wiskott–Aldrich syndrome protein. *Biochemistry* **44**, 15247–15256 (2005).
54. Kelly, A. E., Kranitz, H., Dotsch, V. & Mullins, R. D. Actin binding to the central domain of WASP/Scar proteins plays a critical role in the activation of the Arp2/3 complex. *J. Biol. Chem.* **281**, 10589–10597 (2006).
55. Goode, B. L., Rodal, A. A., Barnes, G. & Drubin, D. G. Activation of the Arp2/3 complex by the actin filament binding protein Abp1p. *J. Cell Biol.* **153**, 627–634 (2001).
56. Duncan, M. C., Cope, M. J., Goode, B. L., Wendland, B. & Drubin, D. G. Yeast Eps15-like endocytic protein, Pan1p, activates the Arp2/3 complex. *Nature Cell Biol.* **3**, 687–690 (2001).
57. Weed, S. A. *et al.* 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 (2000).
58. Uruno, T. *et al.* Activation of Arp2/3 complex-mediated actin polymerization by cortactin. *Nature Cell Biol.* **3**, 259–266 (2001).
59. Weaver, A. M. *et al.* Cortactin promotes and stabilizes Arp2/3-induced actin filament network formation. *Curr. Biol.* **11**, 370–374 (2001).
Shows that the class II NPF cortactin functions to stabilize ARP2/3-mediated branches.
60. Higgs, H. N., Blanchoin, L. & Pollard, T. D. Influence of the C terminus of Wiskott–Aldrich syndrome protein (WASp) and the Arp2/3 complex on actin polymerization. *Biochemistry* **38**, 15212–15222 (1999).
61. Pantaloni, D., Boujemaa, R., Didry, D., Gounon, P. & Carlier, M. F. The Arp2/3 complex branches filament barbed ends: functional antagonism with capping proteins. *Nature Cell Biol.* **2**, 385–391 (2000).
62. Bailly, M. *et al.* The F-actin side binding activity of the Arp2/3 complex is essential for actin nucleation and lamellipod extension. *Curr. Biol.* **11**, 620–625 (2001).
63. Fujiwara, I., Suetsugu, S., Uemura, S., Takenawa, T. & Ishiwata, S. Visualization and force measurement of branching by Arp2/3 complex and N-WASP in actin filament. *Biochem. Biophys. Res. Commun.* **293**, 1550–1555 (2002).
64. Ichetovkin, I., Grant, W. & Condeelis, J. Cofilin produces newly polymerized actin filaments that are preferred for dendritic nucleation by the Arp2/3 complex. *Curr. Biol.* **12**, 79–84 (2002).
Shows that ATP–actin-containing mother filaments support more ARP2/3-mediated branching than ADP-containing or ADP–P_i-containing mother filaments.
65. Amann, K. J. & Pollard, T. D. The Arp2/3 complex nucleates actin filament branches from the sides of pre-existing filaments. *Nature Cell Biol.* **3**, 306–310 (2001).
66. Boujemaa-Paterski, R. *et al. Listeria* protein ActA mimics WASp family proteins: it activates filament barbed end branching by Arp2/3 complex. *Biochemistry* **40**, 11390–11404 (2001).
67. Falet, H. *et al.* Importance of free actin filament barbed ends for Arp2/3 complex function in platelets and fibroblasts. *Proc. Natl Acad. Sci. USA* **99**, 16782–16787 (2002).
68. Carlsson, A. E., Wear, M. A. & Cooper, J. A. End versus side branching by Arp2/3 complex. *Biophys. J.* **86**, 1074–1081 (2004).
69. Blanchoin, L., Pollard, T. D. & Mullins, R. D. Interactions of ADF/cofilin, Arp2/3 complex, capping protein and profilin in remodeling of branched actin filament networks. *Curr. Biol.* **10**, 1273–1282 (2000).
70. Theriot, J. A. & Mitchison, T. J. Actin microfilament dynamics in locomoting cells. *Nature* **352**, 126–131 (1991).
71. Balasubramanian, M. K., Feoktistova, A., McCollum, D. & Gould, K. L. Fission yeast Sop2p: a novel and evolutionarily conserved protein that interacts with Arp3p and modulates profilin function. *EMBO J.* **15**, 6426–6437 (1996).
72. Hudson, A. M. & Cooley, L. A subset of dynamic actin rearrangements in *Drosophila* requires the Arp2/3 complex. *J. Cell Biol.* **156**, 677–687 (2002).
73. Zallen, J. A. *et al.* SCAR is a primary regulator of Arp2/3-dependent morphological events in *Drosophila*. *J. Cell Biol.* **156**, 689–701 (2002).
74. Sawa, M. *et al.* Essential role of the *C. elegans* Arp2/3 complex in cell migration during ventral enclosure. *J. Cell Sci.* **116**, 1505–1518 (2003).
75. Harborth, J., Elbashir, S. M., Bechert, K., Tuschl, T. & Weber, K. Identification of essential genes in cultured mammalian cells using small interfering RNAs. *J. Cell Sci.* **114**, 4557–4565 (2001).
76. Lommel, S. *et al.* Actin pedestal formation by enteropathogenic *Escherichia coli* and intracellular motility of *Shigella flexneri* are abolished in N-WASP-defective cells. *EMBO Rep.* **2**, 850–857 (2001).
77. Snapper, S. B. *et al.* N-WASP deficiency reveals distinct pathways for cell surface projections and microbial actin-based motility. *Nature Cell Biol.* **3**, 897–904 (2001).
References 76 and 77 describe the effects of N-WASP deficiency on murine development and on actin-dependent events in mouse fibroblasts.
78. Dahl, J. P. *et al.* Characterization of the WAVE1 knock-out mouse: implications for CNS development. *J. Neurosci.* **23**, 3343–3352 (2003).
79. Soderling, S. H. *et al.* Loss of WAVE-1 causes sensorimotor retardation and reduced learning and memory in mice. *Proc. Natl Acad. Sci. USA* **100**, 1723–1728 (2003).
80. Yamazaki, D. *et al.* WAVE2 is required for directed cell migration and cardiovascular development. *Nature* **424**, 452–456 (2003).
81. Yan, C. *et al.* WAVE2 deficiency reveals distinct roles in embryogenesis and Rac-mediated actin-based motility. *EMBO J.* **22**, 3602–3612 (2003).
References 80 and 81 describe the effects of WAVE2 deficiency on murine development and on cell migration.
82. Le, J., El-Assal Sel, D., Basu, D., Saad, M. E. & Szymanski, D. B. Requirements for *Arabidopsis* ATARP2 and ATARP3 during epidermal development. *Curr. Biol.* **13**, 1341–1347 (2003).
83. Mathur, J., Mathur, N., Kernebeck, B. & Hulskamp, M. Mutations in actin-related proteins 2 and 3 affect cell shape development in *Arabidopsis*. *Plant Cell* **15**, 1632–1645 (2003).
84. Mathur, J. *et al. Arabidopsis* CROOKED encodes for the smallest subunit of the ARP2/3 complex and controls cell shape by region specific fine F-actin formation. *Development* **130**, 3137–3146 (2003).
85. Machesky, L. M. *et al.* Mammalian actin-related protein 2/3 complex localizes to regions of lamellipodial protrusion and is composed of evolutionarily conserved proteins. *Biochem. J.* **328**, 105–112 (1997).
86. Welch, M. D., DePace, A. H., Verma, S., Iwamatsu, A. & Mitchison, T. J. 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 (1997).
87. Weiner, O. D. *et al.* Spatial control of actin polymerization during neutrophil chemotaxis. *Nature Cell Biol.* **1**, 75–81 (1999).

88. Svitkina, T. M. & Borisy, G. G. Arp2/3 complex and actin depolymerizing factor/cofilin in dendritic organization and treadmilling of actin filament array in lamellipodia. *J. Cell Biol.* **145**, 1009–1026 (1999).
EM study that shows that filaments in lamellipodia are organized into branched networks with the ARP2/3 complex localized to branch points.
89. Rogers, S. L., Wiedemann, U., Stuurman, N. & Vale, R. D. Molecular requirements for actin-based lamella formation in *Drosophila* S2 cells. *J. Cell Biol.* **162**, 1079–1088 (2003).
90. Steffen, A. *et al.* Filopodia formation in the absence of functional WAVE- and Arp2/3-complexes. *Mol. Biol. Cell* **17**, 2581–2591 (2006).
91. Di Nardo, A. *et al.* Arp2/3 complex-deficient mouse fibroblasts are viable and have normal leading-edge actin structure and function. *Proc. Natl Acad. Sci. USA* **102**, 16263–16268 (2005).
92. May, R. C. *et al.* The Arp2/3 complex is essential for the actin-based motility of *Listeria monocytogenes*. *Curr. Biol.* **9**, 759–762 (1999).
93. Strasser, G. A., Rahim, N. A., VanderWaal, K. E., Gertler, F. B. & Lanier, L. M. Arp2/3 is a negative regulator of growth cone translocation. *Neuron* **43**, 81–94 (2004).
94. Gupton, S. L. *et al.* Cell migration without a lamellipodium: translation of actin dynamics into cell movement mediated by tropomyosin. *J. Cell Biol.* **168**, 619–631 (2005).
95. Svitkina, T. M. *et al.* Mechanism of filopodia initiation by reorganization of a dendritic network. *J. Cell Biol.* **160**, 409–421 (2003).
96. Biyasheva, A., Svitkina, T., Kunda, P., Baum, B. & Borisy, G. Cascade pathway of filopodia formation downstream of SCAR. *J. Cell Sci.* **117**, 837–748 (2004).
97. Suetsugu, S., Miki, H. & Takenawa, T. Identification of two human WAVE/SCAR homologues as general actin regulatory molecules which associate with the Arp2/3 complex. *Biochem. Biophys. Res. Commun.* **260**, 296–302 (1999).
98. Sossey-Alaoui, K., Head, K., Nowak, N. & Cowell, J. K. Genomic organization and expression profile of the human and mouse WAVE gene family. *Mamm. Genome* **14**, 314–322 (2003).
99. Miki, H., Suetsugu, S. & Takenawa, T. WAVE, a novel WASP-family protein involved in actin reorganization induced by Rac. *EMBO J.* **17**, 6932–6941 (1998).
100. Nozumi, M., Nakagawa, H., Miki, H., Takenawa, T. & Miyamoto, S. Differential localization of WAVE isoforms in filopodia and lamellipodia of the neuronal growth cone. *J. Cell Sci.* **116**, 239–246 (2003).
101. Stovold, C. F., Millard, T. H. & Machesky, L. M. Inclusion of Scar/WAVE3 in a similar complex to Scar/WAVE1 and 2. *BMC Cell Biol.* **6**, 11 (2005).
102. Sossey-Alaoui, K., Ranalli, T. A., Li, X., Bakin, A. V. & Cowell, J. K. WAVE3 promotes cell motility and invasion through the regulation of MMP-1, MMP-3, and MMP-9 expression. *Exp. Cell Res.* **308**, 135–145 (2005).
103. Suetsugu, S., Yamazaki, D., Kurisu, S. & Takenawa, T. Differential roles of WAVE1 and WAVE2 in dorsal and peripheral ruffle formation for fibroblast cell migration. *Dev. Cell* **5**, 595–609 (2003).
Shows differential roles for WAVE1 and WAVE2 in the formation of actin-rich structures during cell migration using WAVE1- or WAVE2-deficient mouse embryonic fibroblasts.
104. Yamazaki, D., Fujiwara, T., Suetsugu, S. & Takenawa, T. A novel function of WAVE in lamellipodia: WAVE1 is required for stabilization of lamellipodial protrusions during cell spreading. *Genes Cells* **10**, 381–392 (2005).
105. Ridley, A. J., Paterson, H. F., Johnston, C. L., Diekman, D. & Hall, A. The small GTP-binding protein rac regulates growth factor-induced membrane ruffling. *Cell* **70**, 401–410 (1992).
106. DeMali, K. A., Barlow, C. A. & Burridge, K. Recruitment of the Arp2/3 complex to vinculin: coupling membrane protrusion to matrix adhesion. *J. Cell Biol.* **159**, 881–891 (2002).
107. Kovacs, E. M., Goodwin, M., Ali, R. G., Paterson, A. D. & Yap, A. S. Cadherin-directed actin assembly: E-cadherin physically associates with the Arp2/3 complex to direct actin assembly in nascent adhesive contacts. *Curr. Biol.* **12**, 379–382 (2002).
108. Drees, F., Pokutta, S., Yamada, S., Nelson, W. J. & Weis, W. I. α-catenin is a molecular switch that binds E-cadherin–β-catenin and regulates actin-filament assembly. *Cell* **123**, 903–915 (2005).
109. May, R. C., Caron, E., Hall, A. & Machesky, L. M. Involvement of the Arp2/3 complex in phagocytosis mediated by FcγR or CR3. *Nature Cell Biol.* **2**, 246–248 (2000).
Shows that the ARP2/3 complex is functionally important for actin polymerization during phagocytosis.
110. Castellano, F., Le Clainche, C., Patin, D., Carlier, M. F. & Chavrier, P. A WASp–VASP complex regulates actin polymerization at the plasma membrane. *EMBO J.* **20**, 5603–5614 (2001).
111. Coppolino, M. G. *et al.* Evidence for a molecular complex consisting of Fyb/SLAP, SLP-76, Nck, VASP and WASP that links the actin cytoskeleton to Fcγ receptor signalling during phagocytosis. *J. Cell Sci.* **114**, 4307–4318 (2001).
112. Lorenzi, R., Brickell, P. M., Katz, D. R., Kinnon, C. & Thrasher, A. J. Wiskott–Aldrich syndrome protein is necessary for efficient IgG-mediated phagocytosis. *Blood* **95**, 2943–2946 (2000).
113. Kaksonen, M., Sun, Y. & Drubin, D. G. A pathway for association of receptors, adaptors, and actin during endocytic internalization. *Cell* **115**, 475–487 (2003).
Uses live-cell microscopy to temporally define the assembly of actin cytoskeletal proteins, including the ARP2/3 complex and NPFs, during endocytosis in yeast, providing insights into how the actin cytoskeleton and endocytosis are coordinated.
114. Kaksonen, M., Toret, C. P. & Drubin, D. G. A modular design for the clathrin- and actin-mediated endocytosis machinery. *Cell* **123**, 305–320 (2005).
115. Merrifield, C. J., Qualmann, B., Kessels, M. M. & Almers, W. Neural Wiskott–Aldrich syndrome protein (N-WASP) and the Arp2/3 complex are recruited to sites of clathrin-mediated endocytosis in cultured fibroblasts. *Eur. J. Cell Biol.* **83**, 13–18 (2004).
116. Merrifield, C. J., Perrais, D. & Zenisek, D. Coupling between clathrin-coated-pit invagination, cortactin recruitment, and membrane scission observed in live cells. *Cell* **121**, 593–606 (2005).
117. Moreau, V., Galan, J. M., Devilliers, G., Haguenauer-Tsapis, R. & Winsor, B. The yeast actin-related protein Arp2p is required for the internalization step of endocytosis. *Mol. Biol. Cell* **8**, 1361–1375 (1997).
118. Schaerer-Brodbeck, C. & Riezman, H. Functional interactions between the p35 subunit of the Arp2/3 complex and calmodulin in yeast. *Mol. Biol. Cell* **11**, 1113–1127 (2000).
119. Jonsdottir, G. A. & Li, R. Dynamics of yeast Myosin I: evidence for a possible role in scission of endocytic vesicles. *Curr. Biol.* **14**, 1604–1609 (2004).
120. Benesch, S. *et al.* N-WASP deficiency impairs EGF internalization and actin assembly at clathrin-coated pits. *J. Cell Sci.* **118**, 3103–3115 (2005).
121. Kaksonen, M., Toret, C. P. & Drubin, D. G. Harnessing actin dynamics for clathrin-mediated endocytosis. *Nature Rev. Mol. Cell Biol.* **7**, 404–414 (2006).
122. Rozelle, A. L. *et al.* Phosphatidylinositol4,5-bisphosphate induces actin-based movement of raft-enriched vesicles through WASP–Arp2/3. *Curr. Biol.* **10**, 311–320 (2000).
123. Schafer, D. A., D'Souza-Schorey, C. & Cooper, J. A. Actin assembly at membranes controlled by ARF6. *Traffic* **1**, 896–907 (2000).
124. Taunton, J. *et al.* Actin-dependent propulsion of endosomes and lysosomes by recruitment of N-WASP. *J. Cell Biol.* **148**, 519–530 (2000).
125. Zhang, F., Southwick, F. S. & Purich, D. L. Actin-based phagosome motility. *Cell Motil. Cytoskeleton* **53**, 81–88 (2002).
126. Benesch, S. *et al.* Phosphatidylinositol4,5-biphosphate (PIP2)-induced vesicle movement depends on N-WASP and involves Nck, WIP, and Grb2. *J. Biol. Chem.* **277**, 37771–37776 (2002).
127. Stamnes, M. Regulating the actin cytoskeleton during vesicular transport. *Curr. Opin. Cell Biol.* **14**, 428–433 (2002).
128. Fucini, R. V., Chen, J. L., Sharma, C., Kessels, M. M. & Stamnes, M. Golgi vesicle proteins are linked to the assembly of an actin complex defined by mAbp1. *Mol. Biol. Cell* **13**, 621–631 (2002).
129. Luna, A. *et al.* Regulation of protein transport from the Golgi complex to the endoplasmic reticulum by CDC42 and N-WASP. *Mol. Biol. Cell* **13**, 866–879 (2002).
130. Chen, J. L., Lacomis, L., Erdjument-Bromage, H., Tempst, P. & Stamnes, M. Cytosol-derived proteins are sufficient for Arp2/3 recruitment and ARF/coatomer-dependent actin polymerization on Golgi membranes. *FEBS Lett.* **566**, 281–286 (2004).
131. Matas, O. B., Martinez-Menarguez, J. A. & Egea, G. Association of Cdc42/N-WASP/Arp2/3 signaling pathway with Golgi membranes. *Traffic* **5**, 838–846 (2004).
132. Gasman, S., Chasserot-Golaz, S., Malacombe, M., Way, M. & Bader, M. F. Regulated exocytosis in neuroendocrine cells: a role for subplasmalemmal Cdc42/N-WASP-induced actin filaments. *Mol. Biol. Cell* **15**, 520–531 (2004).
133. Stevens, J. M., Galyov, E. E. & Stevens, M. P. Actin-dependent movement of bacterial pathogens. *Nature Rev. Microbiol.* **4**, 91–101 (2006).
134. Gruenheid, S. *et al.* Enteropathogenic *E. coli* Tir binds Nck to initiate actin pedestal formation in host cells. *Nature Cell Biol.* **3**, 856–859 (2001).
135. Lommel, S., Benesch, S., Rohde, M., Wehland, J. & Rottner, K. Enterohaemorrhagic and enteropathogenic *Escherichia coli* use different mechanisms for actin pedestal formation that converge on N-WASP. *Cell Microbiol.* **6**, 243–254 (2004).
136. Campellone, K. G., Robbins, D. & Leong, J. M. EspFU is a translocated EHEC effector that interacts with Tir and N-WASP and promotes Nck-independent actin assembly. *Dev. Cell* **7**, 217–228 (2004).
137. Munter, S., Way, M. & Frischknecht, F. Signaling during pathogen infection. *Sci. STKE* 16 May 2006 (doi:10.1126/stke.3352006re5).
138. Frischknecht, F. *et al.* Actin-based motility of vaccinia virus mimics receptor tyrosine kinase signalling. *Nature* **401**, 926–929 (1999).
139. Patel, J. C. & Galan, J. E. Manipulation of the host actin cytoskeleton by *Salmonella* — all in the name of entry. *Curr. Opin. Microbiol.* **8**, 10–15 (2005).
140. Stender, S. *et al.* Identification of SopE2 from *Salmonella typhimurium*, a conserved guanine nucleotide exchange factor for Cdc42 of the host cell. *Mol. Microbiol.* **36**, 1206–1221 (2000).
141. Criss, A. K. & Casanova, J. E. Coordinate regulation of *Salmonella enterica* Serovar Typhimurium invasion of epithelial cells by the Arp2/3 complex and Rho GTPases. *Infect. Immun.* **71**, 2885–2891 (2003).
142. Unsworth, K. E., Way, M., McNiven, M., Machesky, L. & Holden, D. W. Analysis of the mechanisms of *Salmonella*-induced actin assembly during invasion of host cells and intracellular replication. *Cell Microbiol.* **6**, 1041–1055 (2004).
143. Egile, C. *et al.* 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 (1999).
144. Stamm, L. M. *et al.* Role of the WASP family proteins for *Mycobacterium marinum* actin tail formation. *Proc. Natl Acad. Sci. USA* **102**, 14837–14842 (2005).
145. Stevens, M. P. *et al.* Identification of a bacterial factor required for actin-based motility of *Burkholderia pseudomallei*. *Mol. Microbiol.* **56**, 40–53 (2005).
146. Loisel, T. P., Boujemaa, R., Pantaloni, D. & Carlier, M. F. Reconstitution of actin-based motility of *Listeria* and *Shigella* using pure proteins. *Nature* **401**, 613–616 (1999).
Describes the minimal set of cytoskeletal components sufficient to support actin-based motility of bacterial pathogens *in vitro*.
147. Thrasher, A. J. WASp in immune-system organization and function. *Nature Rev. Immunol.* **2**, 635–646 (2002).
148. Derry, J. M., Ochs, H. D. & Francke, U. Isolation of a novel gene mutated in Wiskott–Aldrich syndrome. *Cell* **79**, 922 (1994).
149. Dupre, L. *et al.* Wiskott–Aldrich syndrome protein regulates lipid raft dynamics during immunological synapse formation. *Immunity* **17**, 157–166 (2002).
150. Badour, K. *et al.* The Wiskott–Aldrich syndrome protein acts downstream of CD2 and the CD2AP and PSTPIP1 adaptors to promote formation of the immunological synapse. *Immunity* **18**, 141–154 (2003).
151. Nolz, J. C. *et al.* The WAVE2 complex regulates actin cytoskeletal reorganization and CRAC-mediated calcium entry during T cell activation. *Curr. Biol.* **16**, 24–34 (2006).
152. Zipfel, P. A. *et al.* Role for the Abi/Wave protein complex in T cell receptor-mediated proliferation and cytoskeletal remodeling. *Curr. Biol.* **16**, 35–46 (2006).

153. Linder, S. *et al.* The polarization defect of Wiskott–Aldrich syndrome macrophages is linked to dislocalization of the Arp2/3 complex. *J. Immunol.* **165**, 221–225 (2000).
154. Calle, Y. *et al.* WASp deficiency in mice results in failure to form osteoclast sealing zones and defects in bone resorption. *Blood* **103**, 3552–3361 (2004).
155. Zicha, D. *et al.* Chemotaxis of macrophages is abolished in the Wiskott–Aldrich syndrome. *Br. J. Haematol.* **101**, 659–665 (1998).
156. Burns, S., Thrasher, A. J., Blundell, M. P., Machesky, L. & Jones, G. E. Configuration of human dendritic cell cytoskeleton by Rho GTPases, the WAS protein, and differentiation. *Blood* **98**, 1142–1149 (2001).
157. de Noronha, S. *et al.* Impaired dendritic-cell homing *in vivo* in the absence of Wiskott–Aldrich syndrome protein. *Blood* **105**, 1590–1597 (2005).
158. Wang, W. *et al.* Tumor cells caught in the act of invading: their strategy for enhanced cell motility. *Trends Cell Biol.* **15**, 138–145 (2005).
159. Otsubo, T. *et al.* Involvement of Arp2/3 complex in the process of colorectal carcinogenesis. *Mod. Pathol.* **17**, 461–467 (2004).
160. Wang, W. *et al.* Identification and testing of a gene expression signature of invasive carcinoma cells within primary mammary tumors. *Cancer Res.* **64**, 8585–8594 (2004).
161. Semba, S. *et al.* Coexpression of actin-related protein 2 and Wiskott–Aldrich syndrome family verproline-homologous protein 2 in adenocarcinoma of the lung. *Clin. Cancer Res.* **12**, 2449–2454 (2006).
162. Yanagawa, R. *et al.* Genome-wide screening of genes showing altered expression in liver metastases of human colorectal cancers by cDNA microarray. *Neoplasia* **3**, 395–401 (2001).
163. Linder, S., Nelson, D., Weiss, M. & Aepfelbacher, M. Wiskott–Aldrich syndrome protein regulates podosomes in primary human macrophages. *Proc. Natl Acad. Sci. USA* **96**, 9648–9653 (1999).
164. Mizutani, K., Miki, H., He, H., Maruta, H. & Takenawa, T. Essential role of neural Wiskott–Aldrich syndrome protein in podosome formation and degradation of extracellular matrix in *src*-transformed fibroblasts. *Cancer Res.* **62**, 669–674 (2002).
165. Hiura, K., Lim, S. S., Little, S. P., Lin, S. & Sato, M. Differentiation dependent expression of tensin and cortactin in chicken osteoclasts. *Cell Motil. Cytoskeleton* **30**, 272–284 (1995).
166. Yamaguchi, H. *et al.* Molecular mechanisms of invadopodium formation: the role of the N-WASP–Arp2/3 complex pathway and cofilin. *J. Cell Biol.* **168**, 441–452 (2005).
167. Quinlan, M. E., Heuser, J. E., Kerkhoff, E. & Mullins, R. D. *Drosophila* Spire is an actin nucleation factor. *Nature* **433**, 382–388 (2005).

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Competing interests statement
The authors declare no competing financial interests.

DATABASES

The following terms in this article are linked online to:
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Abp1 | ActA | ARP2 | ARP3 | ARPC1A | ARPC1B | ARPC2 | ARPC3 | ARPC4 | ARPC5 | CDC42 | cortactin | N-WASP | Pan1 | WASP | WAVE1 | WAVE2 | WAVE3

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