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Review

The Arp2/3 complex: a central regulator of the actin cytoskeleton

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Abstract. In recent years the Arp2/3 complex has emerged as a central regulator of actin dynamics, assembling and cross-linking actin filaments to produce a diverse array of cellular structures. Here I discuss our current state of knowledge about this actin-remodelling machine. The predicted structure of the Arp2/3 complex can be directly correlated with its ability to nucleate, cap and cross-link actin filaments. A growing family of Arp2/3 complex activators such as the WASP family, type I myosins, and the newly identified activators cortactin and Abp1p tightly regulate this activity within the cell. Localised activation of the Arp2/3 complex produces structures such as lamellipodia or actin patches via a process termed dendritic nucleation. Furthermore, several pathogenic microorganisms have evolved strategies to 'hijack' the Arp2/3 complex to their own advantage. Finally, I discuss some of the questions which remain unanswered about this fascinating complex.

Key words. Arp2/3 complex; actin cytoskeleton; WASP; dendritic nucleation; *Listeria*.

Actin dynamics

The actin cytoskeleton underpins almost every aspect of cellular life, from pinching off the contractile ring during cell division to triggering membrane blebbing during apoptosis. Actin has been studied for more than 50 years, since the pioneering work of Straub and Feuer in the 1940s [1]. For almost the same length of time it has been known that actin can switch between a filamentous polymer (F-actin) and a monomeric, globular (G-actin) form, a conversion that is central to the dynamic nature of the cytoskeleton. Each actin monomer binds a molecule of ATP that is rapidly hydrolysed following polymerisation. This creates polarity in an actin filament, such that the 'newest' (barbed) end contains ATP-bound monomers, the neighbouring portion of the filament is composed of monomers containing ADP and unreleased-phosphate (ADP-Pi) and the 'oldest', or pointed, end contains ADP-bound monomers from which phosphate has been released (fig. 1). The association and dissociation constants are such that ATP-bound monomers preferentially add to the barbed end, whereas at the pointed end monomers are primarily lost from the filament. Thus, filament growth is driven in the direction of the barbed end.

Actin polymerisation can proceed spontaneously, but elongation (the addition of monomers onto the end of a preexisting filament) is energetically more favourable than nucleation (the polymerisation of monomers to form a filament de novo). To overcome this kinetic barrier to nucleation, evolution has produced a remarkable nucleating machine – the Arp2/3 complex.

The Arp2/3 complex: structure and biochemistry

In 1994 a search for binding partners of the actin-binding protein profilin yielded a seven-component complex that localised to the cortex of the protozoan *Acanthamoeba* and contained two unconventional actins, now known as actin-related proteins, or Arps [2]. Subsequent phyloge-

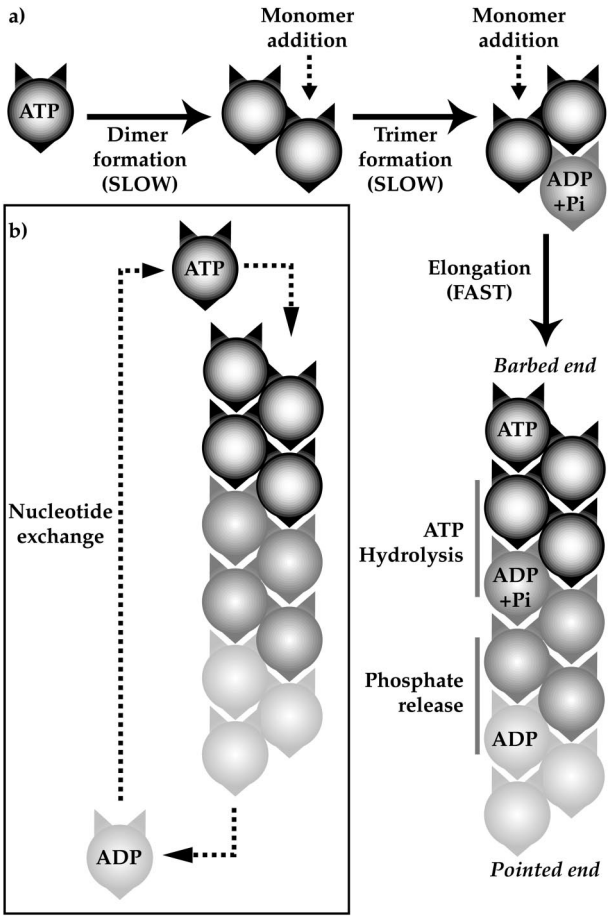

Figure 1. The polymerisation of actin filaments. (*a*) The association of individual actin monomers is slow, but assembly onto the end of a preexisting filament (elongation) is rapid. Shortly after polymerisation, ATP is hydrolysed, increasing the likelihood of filament disassembly. (*b*) The actin filament is thus polar; monomer addition occurs primarily at the barbed end, and monomer loss occurs mainly from the pointed end.

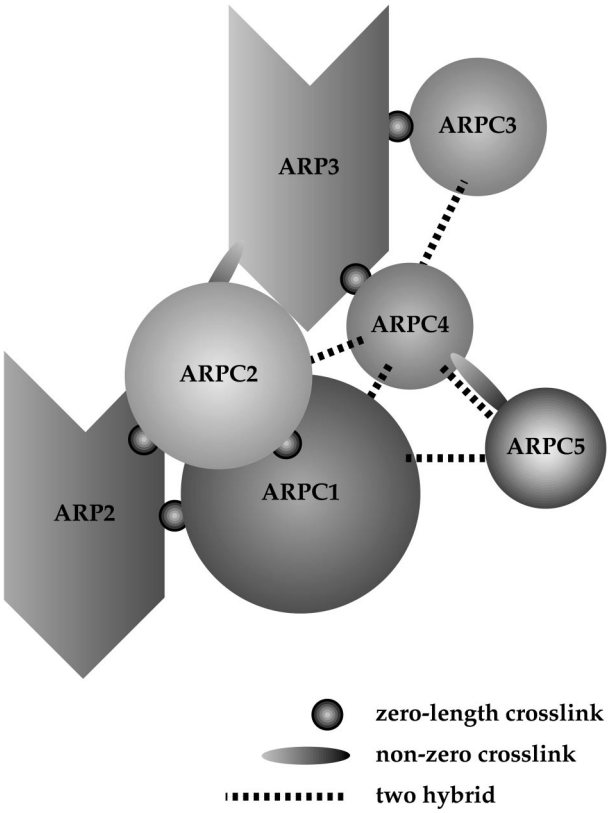

Figure 2. Structure of the Arp2/3 complex. Combining the results of chemical cross-linking and yeast two-hybrid experiments produces this model. It represents the Arp2/3 complex in the 'off' state (i.e. in the absence of activators such as WASP). The two Arps are distant from one another and do not present an efficient nucleus for actin polymerisation.

netic analysis revealed the larger (47 kDa) Arp to have homology to Arp3 genes in other species, whilst the smaller (44 kDa) Arp corresponded to Arp2 [3]. This led to the entire complex being named the Arp2/3 complex [4]. Ironically, the function of profilin-binding by the Arp2/3 complex is still unclear, but 7 years of intensive investigation have revealed this complex to be one of the most important players in remodelling the actin cytoskeleton.

The Arp2/3 complex is evolutionarily conserved from yeast to humans and in all cases appears to be present as a seven-subunit complex [5, 6] (fig. 2). It is abundant, particularly in motile cells like neutrophils or amoebae where the cellular concentration of Arp2/3 complex can be up to 10 μM [7]. Aside from Arp2 and Arp3, the remaining five components of the complex are novel, although the largest of the five contains a WD40 motif present in a variety of other proteins [2]. Arp2 and Arp3 are highly conserved between species (for example, 72 and 68% identity between *Dictyostelium* and human Arp2 and Arp3, respectively [Insall et al., unpublished]). Similarity is lower for the smaller subunits, with the exception of ARPC4, which shows 60–70% identity between species, presumably reflecting a critical (although as-yet unknown) function for this subunit.

An aside on nomenclature

In recent years our ability to categorise and name new proteins has been exceeded by the rate at which they are identified. This can lead to considerable confusion, particularly for proteins that have been conserved during evolution but may have alternative names in different species. This is clearly the case for cytoskeletal proteins, and for the Arp2/3 complex in particular. A unified nomenclature has recently been proposed for the Arp2/3 complex, in which the seven component proteins are designated Arp2, Arp3, ARPC1, ARPC2, ARPC3, ARPC4 and ARPC5 (table 1) with ARPC standing for Arp2/3 complex component [7]. This system is designed to pre-

Table 1. Nomenclature for the Arp2/3 complex.

Name used in text	NCBI name (human)	Molecular weight in different species (kDa)					Accession code (Human genes only)
		Saccharomyces cerevisiae	Schizosaccha-romyces pombe	Acanthamoeba castellanii	Dictyostelium discoideum	Homo sapiens*	
Arp2	ACTR2	44	44	44	44	45	NM_005722
Arp3	ACTR3	50	47	47	47	47	NM_005721
Arp3β	ARP3BETA					48	NM_020445
ARPC1A	ARPC1A	40	42+	40	41	42	NM_006409
ARPC1B	ARPC1B					41	NM_005720
ARPC2	ARPC2	35	35	35	34	34	NM_005731
ARPC3	ARPC3	18	21	19	21	21	NM_005719
ARPC4	ARPC4	19	19	18	20	20	NM_005718
N/A	FLJ14048 fis					17	AK024110
ARPC5	ARPC5	15	15	14	16	16	NM_005717

The most common nomenclature for the five novel subunits of the ARP2/3 complex has been to append the acronym ARC to the molecular weight of each subunit, either as a prefix or suffix. Thus, human ARPC1 has been described as p41-ARC, ARC41 and arc-p41. The use of the ARPC nomenclature avoids confusion arising from molecular weight variation between species and isoforms.
+ In *S. pombe* ARPC1 is named Sop2, for suppressor of profilin.
* There appear to be isoforms of several of the Arp2/3 complex subunits in humans: Arp3; two isoforms, Arp3 and Arp3β. Arp3β may also be alternatively spliced [10]; ARPC1; two isoforms. The subunit previously termed p41-ARC is now listed as ARPC1B, and that previously known as Sop2Hs (for Sop2, *Homo sapiens* homologue) is now ARPC1A; ARPC4; ARPC4, plus an unnamed putative isoform that is slightly smaller; Arp2-like and Arp3-like genes also appear to exist [8], although these have yet to be annotated on the database.

vent confusion arising from the previous system of molecular weight terminology, which varied between species. For example, ARPC3 in humans is a 21-kDa protein, previously termed p21-arc (Arp2/3 complex component), but its homologue in *Saccaromyces cerevisiae* is only 18 kDa in size and was thus called p18-arc. Similarly, database records for proteins that regulate or interact with the Arp2/3 complex are undergoing name revisions. In this review the nomenclature used will predominantly be that currently assigned to the human gene sequences. An exception will be made for Arp2 and Arp3, whose genes are named *ACTR2* and *ACTR3,* respectively (for actin-related). These will continue to be described as Arp2 and Arp3, since ACTR could be confused with conventional actin (*ACT* genes) and/or the bacterial product ActA. By convention, Arp2 and Arp3 have not been written in upper case, despite being abbreviations (for actin related protein).

Isoforms

It now appears that several subunits of the Arp2/3 complex may exist as multiple isoforms [8]. In humans, there appear to be two isoforms of ARPC1, termed ARPC1A (previously termed Sop2Hs) and ARPC1B (p40-ARC) [6]. Since the purified human Arp2/3 complex has a doublet band corresponding to ARPC1 on SDS-polyacrylamide gel electrophoresis (PAGE) [6], it is possible that either subunit can be used as a component of the Arp2/3 complex, generating two pools of the complex (one containing ARPC1A and one containing ARPC1B). Arp2/3 complex from *S. cerevisiae* is stable to the loss of this subunit [9], suggesting that it occupies a peripheral position in the complex. In human cells, this may mean that the two ARPC1 subunits can freely exchange within the complex. Alternatively, since ARPC1A has not yet been identified as a component of the Arp2/3 complex in human cells, perhaps it performs a totally different role to that of ARPC1B and never integrates into the Arp2/3 complex. In this case, the doublet band seen on SDS-PAGE may reflect posttranslational modification or partial degradation of ARPC1 during isolation of the complex.

A putative ARPC4 isoform is present in the human genomic database, and publication of the draft human genome sequence has revealed a possible Arp2 isoform and several Arp3-like genes [8]. An isoform of Arp3 has been identified in neuronal tissue, and this gene might also produce multiple products by alternative splicing [10]. Whether all of these genes are functional, and whether their products function as part of the Arp2/3 complex, remains to be discovered.

How does the Arp2/3 complex nucleate actin filaments?

When added to a solution of ATP-G-actin, the Arp2/3 complex stimulates an increase in actin polymerisation [11]. Although this activity is weak when the purified complex is added alone, stimulating the complex by simultaneously adding an activator such as WASF1 (also

known as Scar/WAVE 1 – discussed below) accelerates its ability to nucleate actin filaments 50-fold. How is the complex able to enhance actin polymerisation in this way?

The Arp2/3 complex mimics the barbed end of an actin dimer

Forming an actin filament from monomeric actin alone requires the initial association of G-actin molecules to form dimers and trimers (fig. 1). Kinetic modelling has demonstrated that the initial formation of dimers and trimers is energetically unfavourable [12–13], and thus presents a barrier to the nucleation of actin filaments. In other words, the formation of new filaments is unlikely as compared with the elongation of existing filaments.

Although crystallographic data have yet to be obtained for any of the subunits in the Arp2/3 complex, the similarity of Arp2 and Arp3 to actin has allowed these subunits to be modelled by homology [3]. In this model those molecular interfaces of Arp2 and Arp3 that resemble the barbed end of an actin monomer are projected outwards from the complex, an orientation that is likely to be critical for the nucleating activity of the complex (discussed below). Chemical cross-linking [13] and an analysis of intermolecular interactions via the yeast-two-hybrid system [14, 15] have shed light on the way in which the seven subunits are packed within the complex, as has the purification of Arp2/3 complex from yeast lacking individual subunits [16]. A 'best-fit' model, produced by combining these data, is shown in figure 2. Verification of this model awaits an experimentally determined high-resolution structure for the complex, although its large size makes this unlikely to be achieved in the near future.

The proposed structure of Arp2 and Arp3 [3] suggests a model to explain how the Arp2/3 complex may overcome the thermodynamic barrier to actin nucleation. Their similarity to actin indicates that both Arp subunits will have one molecular interface that resembles the barbed end of an actin molecule and that they could form a 'side-to-side' Arp2/Arp3 heterodimer, since residues which stabilise similar interactions in actin are also conserved in the two Arps [3]. Thus, a favourable orientation could produce a complex in which the two Arps display a molecular interface that is structurally very similar to that presented by the barbed end of an actin dimer (fig. 2). Actin monomers could add onto this 'pseudo barbed end' within the Arp2/3 complex. This would be equivalent to the formation of an actin trimer from an actin monomer and an actin dimer, a process that bypasses the kinetically unfavourable dimerisation step in actin polymerisation.

Interestingly, chemical crosslinking does not reveal a close interaction between the two Arps in the complex [13]. This might suggest one mechanism by which the Arp2/3 complex could be turned on and off. In the inactive state the two Arps are some distance from each other and do not present a 'barbed end dimer' interface. But upon activation, a conformational change within the complex brings the two proteins into close proximity, and the appropriate template for actin monomer addition is formed (fig. 3). As yet, no experimental evidence exists to either favour or disprove such a model.

Dendritic nucleation

A major advance in our understanding of the behaviour of the Arp2/3 complex was provided in 1998. Using an in vitro assay to measure actin polymerisation,

Mullins et al. demonstrated that the complex can bind to the pointed, but not the barbed, end of actin filaments and that it can stimulate the polymerisation of actin via nu-

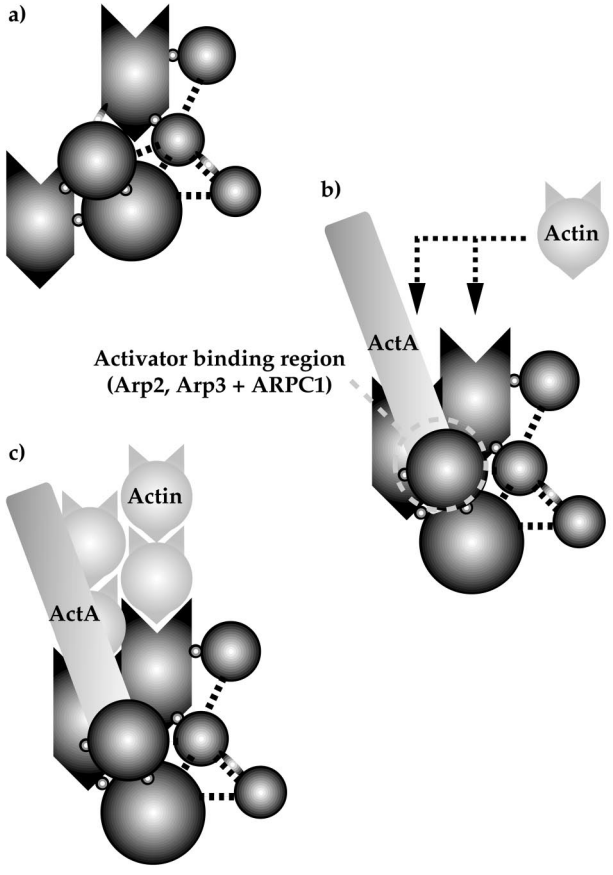

Figure 3. Activation of the Arp2/3 complex. (*a*) the Arp2/3 complex is at rest ('off'). The conformation is the same as that in fig. 2. (*b*) The Arp2/3 complex is bound by an activator, here the bacterial protein ActA. This changes the conformation of the complex, bringing Arp2 and Arp3 closer together. The close association of the two Arps generates an interface that is structurally similar to the barbed end of an actin dimer. ATP-G-actin molecules are able to add on to this interface as if they were adding to an actin filament. (*c*) Elongation then proceeds, with actin monomers adding onto the barbed end of the growing actin filament.

cleation, rather than severing or elongating preexisting actin filaments [11]. In addition, the Arp2/3 complex was shown to bind to the side of actin filaments [11], a process that further activates the complex (discussed below) and leads to the formation of F-actin side branches. By cross-linking actin filaments at an extraordinarily consistent angle of 70°, the Arp2/3 complex produces an orthogonal, 'Y-branched' network of F-actin (fig. 4) [17] identical to that observed at the leading edge of motile cells [18]. Direct observation of this process in vitro shows that branching occurs at the same time as nucleation and can occur from ATP-, ADP-Pi and ADP actin filaments, although ATP and ADP-Pi filaments appear to provide a 'firmer grip' for side branches than do ADP filaments [19, 20]. Chemical cross-linking implicates Arp3, ARPC2 and one of the smaller subunits (ARPC3, ARPC4 or ARPC5) in the side binding of actin filaments [13, 21], and an anti-ARPC2 antibody can block side binding by the Arp2/3 complex [22]. This binding site must be able to 'read' the polarity of the existing actin filament so that the growth of side filaments is in the same direction as, but at 70° to, the direction of growth of the 'mother' filament. Together, these data have been used to construct a model of actin polymerisation that is now known as dendritic nucleation, since the resultant actin network is composed of numerous branched filaments [11]. In this model, the complex binds to the side of an actin filament in an orientation that projects the barbed end of Arp2 and Arp3 outwards at 70° to the direction of the filament. Actin monomers add onto the Arp subunits, producing a new F-actin side branch (fig. 4).

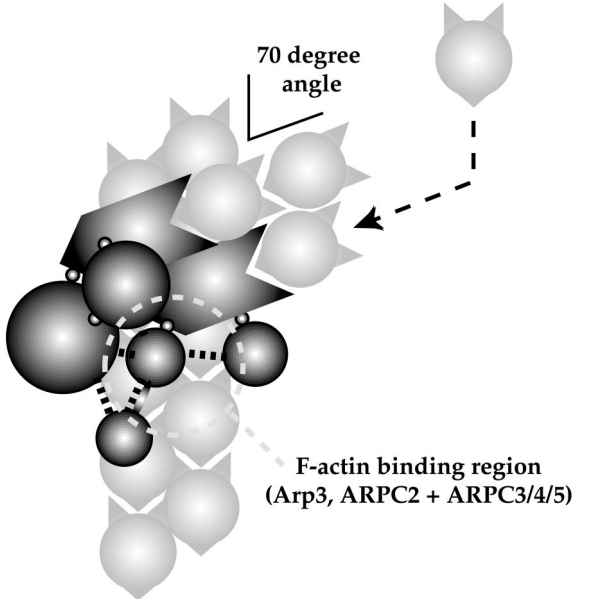

Figure 4. Side branching. The Arp2/3 complex can bind to the side of preexisting actin filaments. Here, an activated Arp2/3 complex binds to an actin filament and nucleates a new filament. This 'daughter' filament is produced by the same mechanism as in fig. 3 and is always produced at an angle of 70° to the mother filament. Note that the activator protein (ActA or WASP, for example) has been omitted from the diagram in the interest of clarity. It is not known whether activators remain bound to the Arp2/3 complex during side binding, although some activators (e.g. WASL) contain F-actin binding sites that may serve to enhance junction stability.

Experiments by Pantaloni et al. [23] have suggested a slightly different model to that of Mullins et al. [11]. In this work, the rate of Arp2/3-dependent polymerisation was proportional to the availability of actin filament barbed ends, rather than the total length of filament in the mix. Pantaloni et al. have thus proposed a barbed-end nucleation model in which the Arp2/3 complex integrates into the growing end of an actin filament, producing a new side-branched actin filament as well as continuing the growth of the original filament [23]. However, Amann and Pollard have reinterpreted these data and used fluorescence imaging of nucleation to show that the Arp2/3 complex can nucleate actin from the side of preexisting filaments, suggesting that the dendritic model is more likely to be correct [20].

The dendritic model accounts well for the observed network of actin filaments at the leading edge. However, it is clearly unable to account for other arrangements of F-actin, such as the parallel arrays seen in filopodia or stress fibres (fig. 5e). In addition, Y-branched networks formed by the Arp2/3 complex must be able to disassemble to maintain a continual supply of actin monomers and allow restructuring of the cytoskeleton. The key to both issues probably lies with debranching. If the side-branching activity of the Arp2/3 complex could be 'turned off', Y-branched networks of filaments would be converted into a pool of short actin filaments. These might then act as 'seeds', elongating and being bundled by other actin-binding proteins (such as fimbrin or α-actinin) to produce parallel arrangements of F-actin for filopodia or stress fibres. Similarly, if debranching occurred together with filament severing, the dendritic network could be rapidly depolymerised. The recent development of an antibody that blocks side binding (but not filament nucleation) by the Arp2/3 complex [22] is likely to prove useful in probing branching and debranching behaviour.

The mechanism of debranching is currently unclear, although it appears that ATP hydrolysis within the actin filament may act as an intrinsic 'timer' in F-actin networks. Not only are ADP-actin filaments more likely to depolymerise than ATP-filaments, but ADP-actin filaments bind to the Arp2/3 complex with lower affinity than ATP-actin filaments [19]. Thus branches in 'older' areas of the actin network will be far less stable than those that are newly formed. The half-life for debranching is approximately 500 s in vitro, but this can be reduced to only 30 s by the activity of actophorin [24]. Actophorin is a member of the ADF/cofilin family of actin-depolymerising proteins that bind to F-actin, increasing the rate of phosphate release

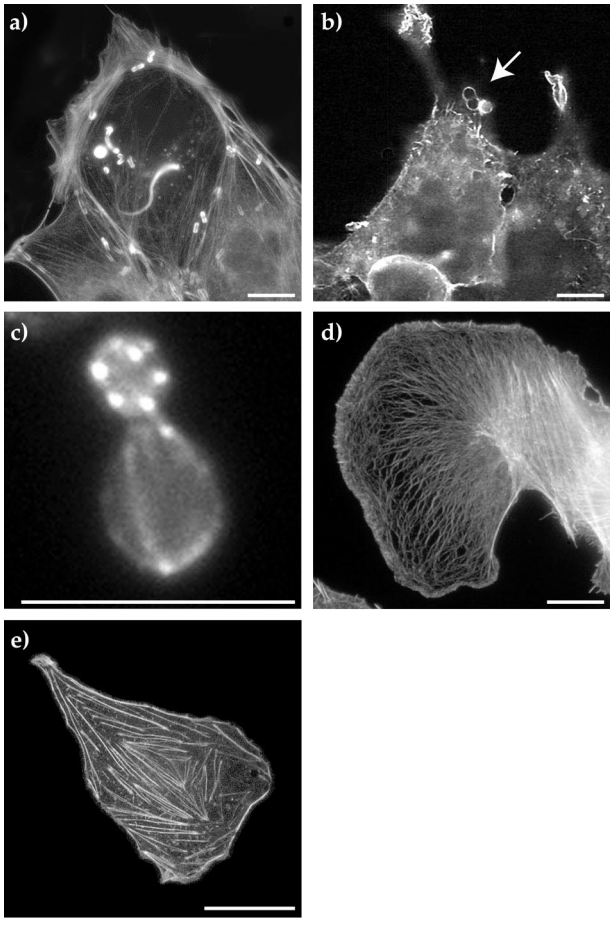

Figure 5. Cellular structures produced by the Arp2/3 complex. All images are of fixed cells stained with fluorescent phalloidin to reveal actin filaments. All scale bars correspond to 10 μm. (*a*) The Arp2/3 complex controls actin nucleation by pathogenic bacteria. A large comet tail has been produced by a motile *Listeria* inside this Ptk2 fibroblast. Other *Listeria* within the cell have started to assemble actin filaments at their surface, but have yet to produce a complete tail. (*b*) The Arp2/3 complex mediates actin nucleation at phagosomes in mammalian cells (arrowed). (*c*) Actin patches in yeast (shown here in a dividing *S. cerevisiae* and thus concentrated in the bud) are motile structures that contain the Arp2/3 complex. Actin patches are abnormal or missing in Arp2/3 mutants. (*d*) Lamellipodia, sheetlike protrusions at the leading edge of a cell (here a B16F1 mouse melanoma cell), are composed of a dendritic array of actin filaments which is produced by the activity of the Arp2/3 complex. (*e*) In contrast to all of the above, stress fibres do not contain the Arp2/3 complex, although the complex appears to have a role in producing and maintaining these structures. Images (*c*), (*d*) and (*e*) were kindly provided by Terry Lechler, Barbara Behrendt and Helen Warwick.

and altering the 'twist' of the actin filament helix [25]. Presumably one or both effects reduce the affinity of the Arp2/3 complex for actin. An intriguing third possibility is that actophorin directly manipulates the Arp2/3 complex. Direct binding between actophorin and the Arp2/3 complex has been reported and mapped to a binding site involving ARPC1, Arp2 and possibly one of the smaller subunits [24, 26]. Perhaps this binding alters the conformation of the Arp2/3 complex, reducing its affinity for actin.

Activating the Arp2/3 complex

Although the Arp2/3 complex is intrinsically a weak nucleator of actin polymerisation [11], its activity can be dramatically increased by a variety of regulatory molecules (fig. 6 and table 2a). The first such activator to be identified was the bacterial protein ActA [27], but this list now extends to a variety of cellular proteins such as members of the WASP (Wiskott-Aldrich syndrome protein) family [14, 28–31], cortactin [32, 33], Abp1p [34] and type I myosins in yeast [35, 36]. Despite their diversity, each activator interacts with the Arp2/3 complex through the same conserved motif, termed the acidic (A) domain because of the density of aspartic and glutamic acid residues [14].

Endogenous activators of the Arp2/3 complex

The first such interaction to be investigated was that which occurs between the Arp2/3 complex and the A-domain of a protein then known as Scar1 (for the human homologue of a suppressor of cyclic AMP receptor mutation in *Dictyostelium discoideum* [37]) and now termed WASF1 (for WASP-family member 1). WASF1 binds to the 21-kDa subunit of the Arp2/3 complex, ARPC3, increasing the nucleating activity of the complex 50-fold [14, 28]. Similar A-domains in the related proteins WASP and WASL (previously termed N-WASP) mediate the interaction of these proteins with the Arp2/3 complex in the same way (fig. 6) [14, 29, 31].

Yeast possess a WASP homologue, termed Bee1p/Las17p, that activates the Arp2/3 complex [30, 38]. This protein also contains an A-domain (fig. 6), but in contrast to vertebrate WASP, it apparently interacts with the Arp2/3 complex via the ARPC1 subunit [35] rather than ARPC3. Deletion of the A-domain of Bee1 produces a far milder perturbation of the actin cytoskeleton than does deletion of the entire gene [30]. This is because in addition to directly binding the Arp2/3 complex, Las17p indirectly recruits the Arp2/3 complex via type I myosins (fig. 9) [35, 36]. Fungal type I myosins, Myo3p and Myo5p in *Saccharomyces cerevisiae* and Myo1p in *Schizosaccharomyces pombe*, contain an A-domain in their C-terminus that interacts with the Arp2/3 complex through the ARPC1 and ARPC4 subunits (fig. 6) [35, 36, 39]. Thus, Las17p and type I myosins in yeast are partially redundant in their ability to activate actin polymerisation through the Arp2/3 complex (fig. 9). Type I myosins in non-fungi do not contain A-domains, and are predicted not to interact directly with the Arp2/3 complex in this way.

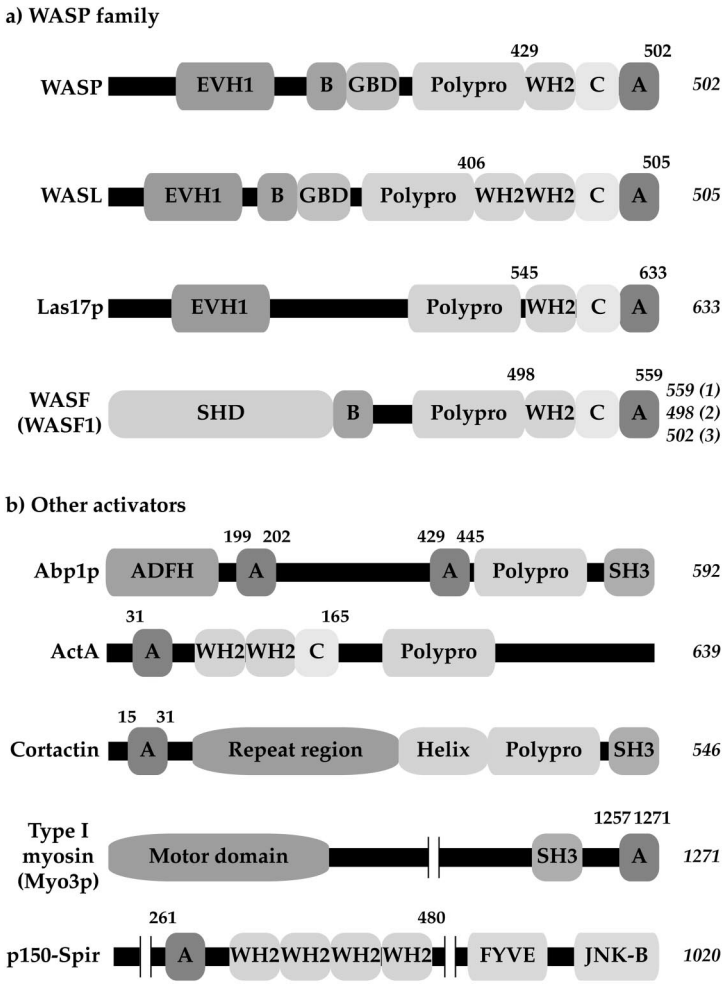

Figure 6. Arp2/3-binding molecules. (*a*) The WASP family. (*b*) Other proteins known to activate the Arp2/3 complex. Note that p150-Spir has not been shown to bind or activate the Arp2/3 complex, although it possesses an A-domain and WH2 domains, and perturbs the actin cytoskeleton when overexpressed. Where multiple forms of a protein exist (e.g. WASF), the particular isoform depicted is given in brackets (WASF1). The total length of the molecule (in amino acid residues) is given in italics on the right. Domains are abbreviated as follows; EVH1, EVL/VASP homology 1; B, Basic; GBD, GTPase binding domain, also known as CRIB; Polypro, proline-rich sequence; WH2, WASP homology 2; C, central, or cofilin homology; A, acidic; SHD, Scar homology domain; ADFH, actin depolymerising factor homology; FYVE, zinc-finger motif, named after the first four proteins in which it was identified; JNK-B, JNK-binding; SH3, Src-homology 3. The numbers above each molecule indicate the amino acid positions of regions involved in activating actin polymerisation (i.e. the WH2, C- and A-domains). Note that Myo3p and p150-Spir are very large proteins – regions without discernible functional domains have been omitted and are indicated by a gap in the molecule.

The actin-binding protein cortactin localises to lamellipodia [40] and is implicated in the progression of several cancers [41]. Cortactin contains an acidic domain that binds to the Arp2/3 complex (fig. 6) [42]. Originally, this binding was reported not to activate the complex [42], but more recent data suggest that cortactin-binding does activate the Arp2/3 complex, although to a weaker degree than, and possibly by a different mechanism to, WASP family proteins (discussed below) [32, 33]. This alternative mechanism also appears to be employed by the yeast protein Abp1p (actin-binding protein 1), which contains two A-domains and activates the Arp2/3 complex [34].

The p150-Spir protein contains both an acidic domain and four WH2 (WASP homology 2) motifs, suggesting that it may be a distant member of the WASP family of proteins (fig. 6). Like WASP, p150-Spir is able to bind G-actin [43], and overexpression induces actin clusters in fibroblasts [44]. Whether p150-Spir directly activates the Arp2/3 complex is, however, still unknown. TRAP (thrombospondin-related anonymous protein), a protein found in *Toxoplasma*, also contains a putative acidic domain [39], although it has not yet been implicated in actin remodelling.

WASP-family proteins must bind G-actin to efficiently activate the Arp2/3 complex

A critical feature for activation of the Arp2/3 complex by WASP family proteins appears to be the simultaneous re-

Table 2. A: Activators of the Arp 2/3 complex.

Name used in text	Full name	Alternative names	Description	Accession code (human sequence, unless otherwise stated)
WASP	Wiskott-Aldrich syndrome protein		type member of the WASP family, haemopoeitic only	NM_000377
WASL	WASP-like	N-WASP (neural WASP)	highly homologous to WASP, but ubiquitous expression	NM_003941
Las17p	local-anaesthetic-sensitive mutant 17	Bee1p	yeast WASP homologue	D78487 (*S. cerevisiae*)
WASF1	WASP-family protein number 1	SCAR1 (suppressor of cyclic AMP receptor mutation), WAVE1 (WASP family verprolin-homologous protein)	similarity to WASP in C-terminus, but a divergent N-terminal domain.	NM_003931
WASF2	WASP-family protein number 2	SCAR2, WAVE2		NM_006990
WASF3*	WASP-family protein number 3	SCAR3, WAVE3		NM_006646
ActA			bacterial activator of the Arp 2/3 complex	AF103807 (*L. monocytogenes*)
Abp1p	actin-binding protein 1		yeast activator of the Arp 2/3 complex	NP-010012 (*S. cerevisae*)
Cortactin	cortactin	oncogene EMS1	activates the Arp2/3 complex, apparently by a novel mechanism	NM_005231
Myo1p	myosin 1, a type I myosin		binds and activates the Arp 2/3 complex	CAB46766 (*S. pombe*)
Myo3p+	myosin 3, a type I myosin		bind to Las17p and the Arp 2/3 complex	S76960 (*S. cerevisiae*)
Myo5p+	myosin 5, a type I myosin			NP_013827 (*S. cerevisiae*)

* WASF3 has not yet been demonstrated to activate the Arp 2/3 complex.
+ Type I myosins in *S. cerevisiae* have not yet been shown to activate the Arp 2/3 complex in vitro, although they are necessary to activate actin nucleation in permeabilised cells [36].

cruitment of actin monomers. WASP-family proteins all contain a motif adjacent to the A-domain termed the WH2 or V (verprolin homology) domain (fig. 6). This domain, first identified in the yeast protein verprolin [45], binds G-actin, and without it WASP family proteins no longer activate the Arp2/3 complex [28, 29]. WASP and the three WASF proteins have one WH2 domain, whilst WASL contains two such domains in a tandem array, a feature that has been invoked to explain the stronger activation of the Arp2/3 complex by this protein [46].

The necessity for G-actin binding has led to a proposal that the WH2 domain of WASP-family proteins could 'shuttle' actin monomers towards the Arp2/3 complex, assisting filament nucleation (fig. 7b) [47]. This activity may be enhanced by the ability of some Arp2/3 activators, as well as the Arp2/3 complex itself, to bind profilin, which may also supply actin monomers to the complex (fig. 7c) [48]. However, molecular interactions mediated by the C-terminal region of WASP-family proteins are likely to prove more complex than this model. In a recent study [49] it was demonstrated that the C-terminus of WASP contains two low-affinity G-actin-binding sites, one in the WH2 domain and a second contained within the A-domain and neighbouring C (for cofilin-homology, central or connecting) domain. These synergise to bind a single actin monomer with sufficiently high affinity to compete with the actin-sequestering proteins profilin and thymosin-β4. The C-domain is also required to aid binding between the A-domain and the Arp2/3 complex. Most puzzling of all is the lack of correlation between the ability of the different WASP mutants to stimulate actin nucleation and their affinities for G-actin and/or the Arp2/3 complex. Similarly, the observation that dimerising the C-terminal domain of WASP increased its ability to stimulate actin nucleation 100-fold [49] is intriguing. These data suggest that activating the Arp2/3 complex via WASP-family proteins requires more than simply bringing together G-actin and the Arp2/3 complex.

Table 2. B: Binding partners for Arp 2/3-activators.

Molecule	Full name	Activator target(s)	Description
Abl	abelson tyrosine kinase	WASF1	effect of binding unknown
Bzz1p*		Las17p	forms complex with Myo3p/5p, Las17p and Vrp1p
Cdc42	originally identified as a cell division cycle gene	WASL, WASP	small GTPase, activates WASP and WASL
CIP4	Cdc42-interacting protein 4	WASP	redistributes WASP to microtubules
GRB2	growth-factor receptor-bound factor 2	WASP, WASL	Activates WASL
IRSp53	53/58-kDa insulin receptor substrate	WASF2	activates WASF2. Also binds WASF1 weakly
Nck		WASP, WASL	effect of binding is unknown. also binds to WIP (WASPIP)
PKA	protein kinase, cAMP-dependent	WASF1	effect of binding unknown
PtdIns 4,5P_2	Phosphatidylinositol 4,5 bisphosphate	WASP, WASL	phospholipid. Co-activates WASP/WASL together with Cdc42 and other activators
PSTPIP1	proline, serine, threonine phosphatase interacting protein number 1	WASP	effect of binding on WASP activity is unknown
Src-family kinases		WASP	effect of binding is unknown
Syndapin	synaptic, dynamin-associated protein	WASL	two isoforms, both bind WASL; overexpression of Syndapin II induces filopodia in an Arp2/3-dependent manner
WIP (WASPIP) *Vertebrate*	WASP-interacting protein	WASP	forms complex with Las17p and type I myosins in yeast
Vrp1p *Yeast*	verprolin	Las17p	
WISH	WASL-interacting SH3 protein	WASL	activates WASL-induced nucleation; also able to activate the Arp2/3 complex; independent of WASL

* Bzz1p has yet to be published, although the sequence is deposited in the database.

Actin filaments also activate the Arp2/3 complex

During dendritic nucleation, the Arp2/3 complex receives a dual activation signal. Initial activation by binding partners such as WASP-family proteins is then enhanced by a second stimulus from actin filaments. Although activators like WASF1 stimulate the ability of the Arp2/3 complex to induce actin polymerisation, there is still a lag before the maximum rate of polymerisation is attained. Preincubation of the Arp2/3 complex and WASF1 with actin filaments eliminates this lag [28, 50], demonstrating that the Arp2/3 complex is activated by its own 'product', F-actin. This secondary signal means that the Arp2/3 complex is more likely to trigger actin polymerisation from the side of a preexisting filament than de novo (fig. 4), a feature that leads to the highly branched dendritic network of F-actin seen in lamellipodia [18]. In addition, the activating protein WASL is also able to bind to actin filaments via its N-terminal domain [47], which presumably enhances the stability of the junction from which the new actin filament is nucleated.

Activating the Arp2/3 complex without a WH2

Cortactin and Abp1p both activate the Arp2/3 complex, but neither protein contains a WH2 (G-actin binding) domain, distinguishing them from WASP family proteins (fig. 6). Cortactin contains a series of tandem repeats that bind to actin filaments [42, 51], a feature that is critical for Arp2/3 activation [32, 33]. Similarly, the ADFH (actin depolymerising factor homology) domain of Abp1p binds to F-actin and is essential for Arp2/3 activation [34]. By stabilising the association of the Arp2/3 complex with preexisting actin filaments, cortactin and Abp1p might maintain the complex in an active, 'branching-competent' conformation.

This suggests two alternative processes by which dendritic nucleation may be achieved (fig. 8). In the first (WASP-family mediated), a WASP-family protein converts the Arp2/3 complex into an active conformation (fig. 8b). When this 'primed' complex binds to the side of a preexisting actin filament, it becomes fully activated and nucleates a new filament (fig. 8c). In the alternative (cortactin or Abp1p-mediated) process, the presence of cortactin/Abp1p provides a stabilising 'arm' that in-

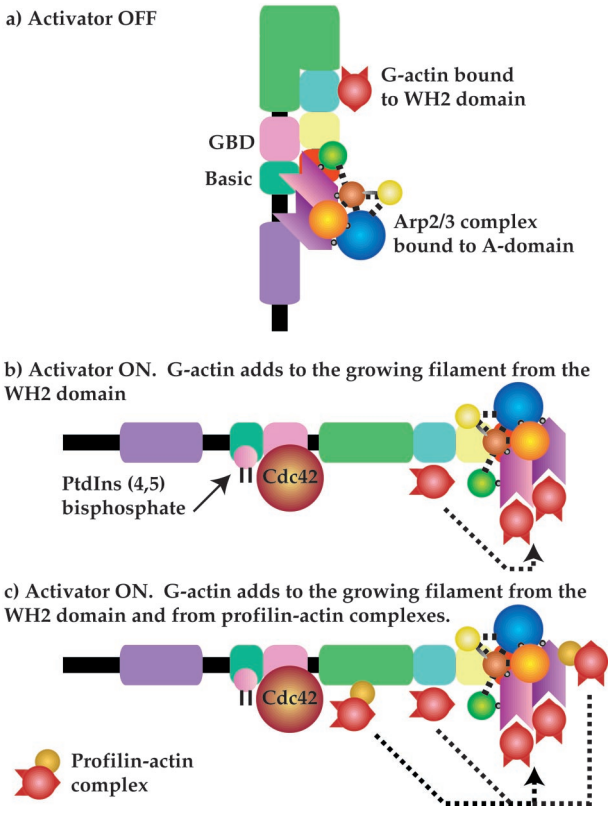

Figure 7. Signal integration by WASP family proteins. (*a*) At rest, WASP family proteins are autoinhibited by an interaction between the A-domain and the GBD/basic domain. Although G-actin and the Arp2/3 complex are bound, they are in an inappropriate orientation to nucleate an actin filament. (*b*) Signals can destabilise the autoinhibited conformation of WASP family proteins, opening the molecule and activating it. Cdc42 and PtdIns 4,5P_2 are two such signalling molecules, which bind to the GBD and the Basic domain, respectively. Once open, WASP-family proteins position the Arp2/3 complex and G-actin in a favourable orientation for actin nucleation. Actin monomers are 'shuttled' onto the growing filament from the WH2 domain. (*c*) Filament growth may be enhanced by the addition of further G-actin molecules, which are bound to profilin and are thus recruited to the nucleation 'machine' via the interaction of profilin with WASP and/or profilin with the Arp2/3 complex.

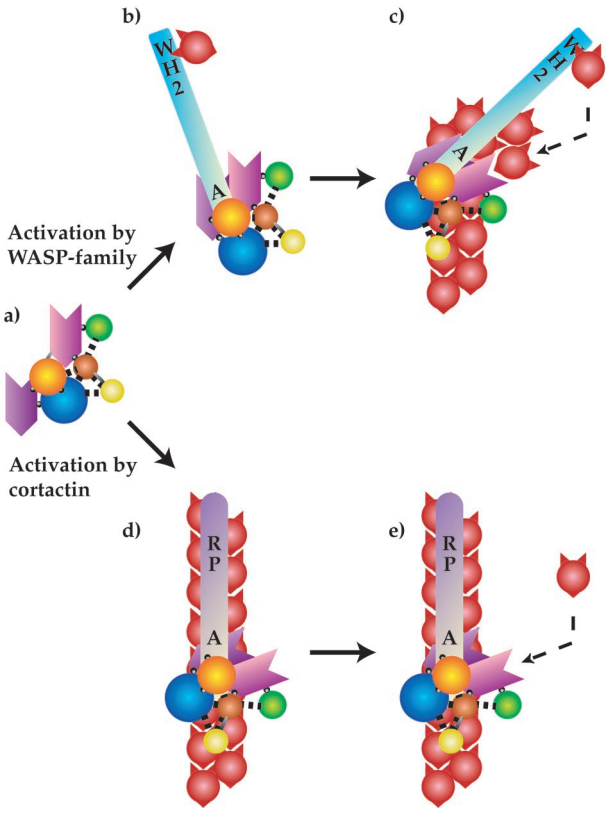

Figure 8. Different activators trigger dendritic nucleation via alternative strategies. (*a*) The inactive Arp2/3 complex. (*b*) Binding of a WASP-family activator activates the complex. The activator also binds to one or more G-actin molecules via WH2 domains. (*c*) The active Arp2/3 complex binds to the side of a pre-existing actin filament. G-actin is supplied to the Arp2/3 complex from the WH2 domain of the activator, triggering nucleation of an actin filament side branch. Cortactin, another activator of the Arp2/3 complex, lacks the ability to bind G-actin. Instead it stabilises the association of the Arp2/3 complex with actin filaments (*d*) via a repeat region (RP) that binds to F-actin. Abp1p is believed to activate the Arp2/3 complex in a similar manner, in this case binding to F-actin via an ADFH domain. The 'junctional complex' of F-actin, the Arp2/3 complex and cortactin/Abp1p is long-lived enough to allow actin monomers to add onto the Arp2/3 complex from the cytoplasm (*e*). Here, these are shown as free actin monomers, but in reality G-actin would probably be supplied from profilin-actin complexes.

creases the stability of the interaction between the Arp2/3 complex and F-actin (fig. 8d). The time of association is now sufficiently long that the Arp2/3 complex, having been activated by side binding to F-actin, can trigger the nucleation of a new actin filament (fig. 8e). This is likely to be a less efficient process than WASP-family-mediated activation, explaining why cortactin and Abp1p are weaker activators of the Arp2/3 complex than WASP-family proteins [32–34].

Myo3p and Myo5p in *S. cerevisiae* bind only weakly to G-actin, but are able to form a complex with verprolin and Las17p, both of which contain actin-binding WH2 domains [35, 36]. Thus, a large actin-nucleating complex containing two WH2 domains (in Las17p and verprolin) and two A-domains (in the type I myosin and Las17p) can be formed (fig. 9a). However, the equivalent type I myosin in *Schyosaccharomyces pombe*, Myo1p, is apparently able to stimulate actin polymerisation alone [39]. Either this molecule contains a 'cryptic' actin monomer binding site, or it is able to activate the Arp2/3 complex without binding to G-actin. Interestingly, the motor activity of type I myosins is essential for the activation of actin polymerisation by these molecules in *S. cerevisiae* [36], suggesting that the mode of activation of the Arp2/3 complex by type I myosins is likely to be more complex than initially envisaged.

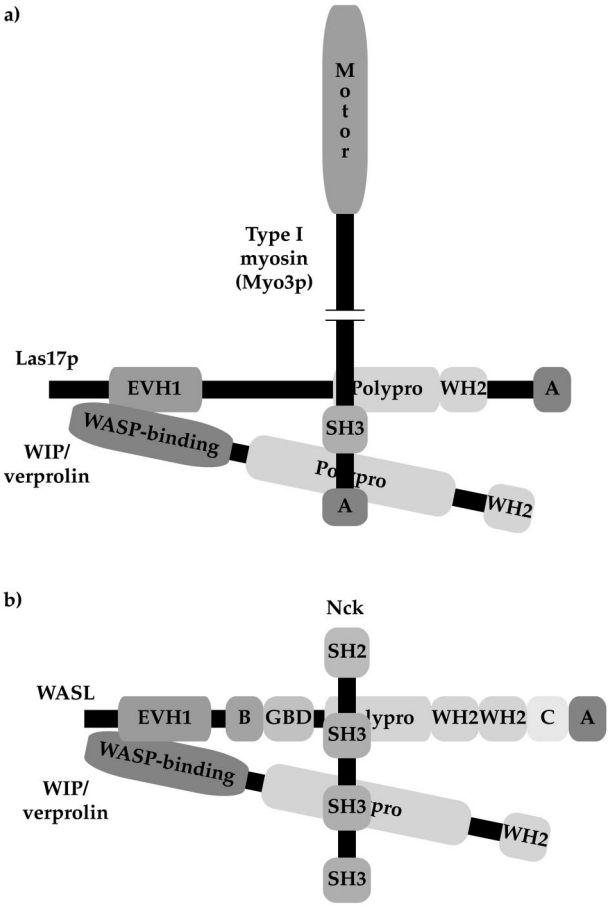

Figure 9. Two examples of actin-nucleating complexes found in cells. (*a*) In yeast, a complex is formed between Las17p, verprolin (WIP) and a type I myosin. This complex therefore contains two Arp2/3-binding A-domains, and two WH2 (actin monomer-binding) domains. (*b*) During the movement of *Vaccinia* virus in mammalian cells, a complex is formed between Nck, WIP and WASL. This complex stimulates actin polymerisation, forming a comet tail that propels the virus through the cytoplasm.

Listeria produce molecules which can activate the Arp2/3 complex

Another activator of the Arp2/3 complex has been evolved by the pathogenic bacteria *Listeria monocytogenes*. Following invasion into mammalian cells, *Listeria* recruit host cell components to nucleate actin filaments at the bacterial surface. These then form an actin 'comet tail' at one pole, and polymerisation within the tail propels the bacterium through the cytoplasm, increasing the spread of infection. The only bacterial molecule required for this movement is ActA [52], a protein that can also induce actin tail assembly and motion when expressed in nonmotile bacteria [53] or coated onto polystyrene beads [54]. In 1997 Welch et al. showed that ActA induces actin assembly via the Arp2/3 complex [4] and subsequently that ActA stimulates the nucleating activity of the complex [27]. The Arp2/3 complex is essential for *Listeria* motility [55], and in 1999 a landmark paper from Loisel et al. identified the 'minimal ingredients' for this process; actin, Arp2/3 complex, capping protein (which caps the barbed end of F-actin) and ADF (actin depolymerising factor) [56].

The N-terminal region of ActA is essential for actin-tail formation and contains a short sequence of basic residues that are required to recruit the Arp2/3 complex to the bacterial surface [57] and are critical for activation of the Arp2/3 complex by ActA [26, 58]. A similar motif is found in the C-domain of WASP family proteins [55], a region that is critical for activation of the Arp2/3 complex by these proteins. The extreme N-terminus of ActA contains a stretch of acidic amino acids very similar to the A-domain of WASP family proteins (fig. 6) [58]. Either or both of these domains in ActA may thus interact with the Arp2/3 complex in the same way as endogenous activators of the complex, providing a powerful tool with which to probe the mechanism of activation.

Two recent papers have investigated ActA-induced stimulation of the Arp2/3 complex. Skoble et al. proposed a synergy between three sites on ActA that together bind and activate the Arp2/3 complex [58]. These three sites correspond to the motifs similar to the A-domain and C-domain of WASP described above, plus a putative actin-binding domain. In contrast, Zalevsky et al. suggest that Arp2/3 binding is primarily mediated by only the C-domain motif [26]. This group showed that ActA binds two G-actin molecules, like WASL, and identified a second actin-binding motif in ActA adjacent to the first. As with the WASP family, it appears that ActA must bind both the Arp2/3 complex and actin monomers in order to activate actin polymerisation [26].

ActA, WASL and WASF1 compete for Arp2/3 complex binding, suggesting that all three activators bind and stimulate the complex through the same site. This site was mapped by chemical cross-linking to a junction between the Arp2, Arp3 and ARPC1 subunits (fig. 3), and overlaps with the profilin binding site [26]. This produces an orientation in which the activator is perfectly placed to stabilise an interaction between the 'barbed' end of Arp2 and the pointed end of an actin monomer as it adds onto the complex to initiate a new filament.

It is interesting that this work did not identify an interaction between any of the three activators and the ARPC3 subunit, which has been shown to interact with the A-domain of WASF1 in the yeast two-hybrid system [14]. Similarly, the interaction between Las17p and the Arp2/3 complex does not rely on the ARPC3 subunit [30, 35]. Perhaps the interaction between ARPC3 and activators is transient or of low affinity when this subunit is present as part of the Arp2/3 complex, and alternative binding sites elsewhere in the complex are able to substitute for the ARPC3-activator interaction.

Regulating the activators

As with any signalling pathway, accurate 'on/off' switches are essential to regulate actin polymerisation. Activators such as WASP are able to control the activity of the Arp2/3 complex, increasing its actin-nucleating activity 50-fold or more [28], but what regulates the behaviour of the activators themselves? A bewildering array of molecules are now known to interact with activators of the Arp2/3 complex (table 2b). Thus, activators behave as molecular 'processors', translating multiple inputs from diverse signal pathways into a single output in the form of actin polymerisation.

The first binding partner of an Arp2/3-activator to be identified was the small GTPase Cdc42. Cdc42 was shown to interact with WASP [59] before the latter was identified as an Arp2/3 activator, and the structural basis for this interaction was subsequently revealed by nuclear magnetic resonance [60]. Cdc42 interacts with both WASP and WASL through the conserved GBD [GTPase binding domain, also called a CRIB (Cdc42/Rac interaction/binding) domain] [60]. Similarly, the acidic phospholipid phosphatidylinositol 4,5 bisphosphate (PtdIns 4,5P_2) interacts with both WASL and WASP [61, 62]. Although originally proposed to occur through a putative plekstrin homology domain [61], the lack of conservation within this region [63] led to the demonstration that the PtdIns 4,5P_2 interaction site is a basic region adjacent to the GBD [64].

Cdc42 and PtdIns 4,5P_2 synergise to trigger Arp2/3-dependent actin polymerisation in cell extracts [65, 66]. This observation can now be understood in terms of signalling through the WASP family. At rest, WASP and WASL are maintained in an inactive state by autoinhibition (fig. 7a). The C-terminal region, containing the acidic and WH2 domains necessary for stimulating actin polymerisation, is 'hidden' by an interaction with the GBD domain [29, 67, 68] and, at least for WASL, the adjacent basic domain [64, 69]. In this 'closed' conformation, the activator can still bind to the Arp2/3 complex but does not activate its nucleating activity. Binding of either PtdIns 4,5P_2 or Cdc42 alone partially destabilises the closed conformation, but simultaneous binding of both molecules is required to fully open the molecule, releasing the block on the Arp2/3 complex and triggering actin polymerisation (fig. 7b) [29, 69].

Although Cdc42 and PtdIns 4,5P_2 are the best-understood regulators of the WASP family, many other molecules also bind to WASP-family proteins. WIP (WASP-interacting protein, also called WASPIP) is a proline-rich protein that binds to WASP [70], an interaction that is apparently critical for the in vivo function of WASP [71]. The equivalent Las17p-binding protein in yeast is verprolin [72], and human WIP is able to substitute for verprolin in *S. cerevisiae* [73]. In yeast the loss of verprolin perturbs the actin cytoskeleton and polarity and inhibits endocytosis [45, 74], and WIP mutants that lack the WH2 motif are unable to rescue this phenotype, suggesting that G-actin binding is essential for the function of WIP [73]. More recently, WIP has been shown to inhibit the ability of Cdc42 to trigger WASL-induced actin polymerisation [75]. Other activators of WASL can bypass this inhibition, suggesting that WIP may act to modify upstream signals that converge on WASL.

WIP also binds to the adaptor protein Nck [76], which is itself able to bind to WASP and WASL [77, 78]. Thus, a large complex consisting of Nck, WASP/WASL and WIP, together with their other binding partners, can be assembled (fig. 9b). Such a complex has been shown to function in the actin-based motility of *Vaccinia* virus (discussed below) [79, 80]. Since Nck activates WASL [81], the final 'output' (Arp2/3-induced actin polymerisation) of such a complex must result from a balance between the different stimulatory (e.g. Nck) and inhibitory (e.g. WIP) molecules that compose the complex.

GRB2, like Nck, is an adaptor protein that uses its SH3 domains to bind to WASP and WASL in vitro [82–84]. This binding stimulates the ability of WASL to activate the Arp2/3 complex, an effect that is enhanced by the simultaneous binding of Cdc42 to WASL [84]. GRB2 also binds to WISH (WASP interacting SH3 protein), a newly identified protein which activates WASL [85]. WISH is also able to activate the Arp2/3 complex via an unknown mechanism in the absence of WASL [85].

The brain-specific protein syndapin I contains an SH3 domain that binds in vitro to proteins involved in synaptic vesicle recycling, such as dynamin and synaptojanin, and also to WASL [86]. Syndapin II, an isoform of syndapin I that is more widely expressed, also binds to WASL and triggers filopodia formation when overexpressed [87]. Since this effect is Arp2/3 dependent, it appears likely that syndapins are also able to activate WASL.

A large number of other molecules have also been shown to utilise SH3 domains to bind to WASP and/or WASL. These include phospholipase C, the p85 subunit from phosphoinositide 3-kinase, PSTPIP (proline, serine, threonine phosphatase interacting protein), the Src-family kinases FYN and FGR, and the Tec family kinases BTK, TEC and ITK [83, 88, 89], although the biological relevance of these interactions remains unclear. One purpose may be to control tyrosine phosphorylation of WASP, an event that has been observed during diverse signalling events but is again of unknown significance [90–92]. Since WASP phosphorylation is enhanced by Cdc42 [91], perhaps phosphorylation serves to modulate signalling through WASP, for example by up- or downregulating its sensitivity to upstream signals.

An SH3-mediated interaction also occurs between CIP4 and WASP. CIP4 (Cdc42 interacting protein 4) binds in vitro to active Cdc42 and to the polyproline domain in WASP. When overexpressed in cos cells, CIP4 redistrib-

utes WASP to microtubules, which may mediate its delivery to sites of actin polymerisation in the cell [93]. Interestingly, the yeast protein Bzz1p is similar to CIP4, contains SH3 domains and interacts with Las17p [T. Lechler, personal communication]. It is not known whether Bzz1p provides a connection between microtubules and Las17p in a manner similar to mammalian CIP4.

Much less is known about the activation of WASF proteins. There appear to be three WASF proteins (WASF1, -2 and -3) which are closely related to each other, although differences in the behaviour of the three isoforms are emerging. WASF1 and WASF2 interact with the Arp2/3 complex, and WASF3 is predicted to interact in the same way since all three proteins contain a similar C-terminal domain to that found in WASP/WASL. WASFs also contain a polyproline region, but their N-terminal regions are homologous only to each other and not to WASP or WASL. Both WASP and WASL are inactive as full-length proteins, because the C-terminus responsible for activating the Arp2/3 complex is hidden by bonds within the molecule (discussed above). However, full-length WASF is just as active as its isolated (constitutively active) C-terminus [28], suggesting that it is not autoinhibited in the same way as WASP/WASL. In cells it is unlikely that WASF is constitutively active, since this would seriously disrupt the actin cytoskeleton. Thus, there must be regulators of WASF in the cell that are not present during in vitro assays for actin polymerisation. Binding partners and/or posttranslational modification are two possibilities.

Whereas WASP and WASL act downstream of the GTPase Cdc42, WASF appears to transduce signals from the related GTPase Rac, although the two molecules do not directly interact [94]. For at least one of the three known isoforms of WASF (WASF2), the Rac connection may be mediated by IRSp53 (53-kDa insulin receptor substrate) [95]. IRSp53 binds to active (GTP-bound) Rac and Cdc42 in vitro, and also interacts via its SH3 domain with the polyproline region of WASF2 [95]. Although WASF2 is constitutively active in actin polymerisation assays, binding to IRSp53 increases its activity further [95]. IRSp53 has also been shown to localise to F-actin in response to Cdc42 signalling [96] (note that slight size variability has resulted in IRSp53 also being termed IRS-58). Whether the two observations are connected is currently unclear.

In addition to IRSp53, WASF is also able to bind other SH3-domain-containing proteins. A search for protein kinase A (PKA)-anchoring proteins identified WASF1 as a molecule able to interact both with PKA and with the ABL tyrosine kinase [97]. Interestingly, only WASF1 could bind PKA, whereas all three WASF isoforms could bind to ABL. On the basis of these data, Westphal et al. suggested that WASF may function as a scaffold upon which multiple signalling components can assemble [97]. Little is currently known about how other activators of the Arp2/3 complex, such as type I myosins, Abp1p and cortactin, may themselves be switched on. This is primarily due to the very recent discovery of these proteins, and we are unlikely to remain ignorant for long. A significant challenge for the future will be to investigate signalling to the Arp2/3 complex in vivo.

The special case of profilin

Profilin is a small protein of about 14 kDa that binds ATP-G-actin with an affinity of 0.1 μM (its affinity for ADP-G-actin is fivefold lower) [7]. It has been proposed to 'shuttle' actin monomers onto the barbed end of actin filaments, since G-actin complexed with profilin adds to actin filaments at almost the same rate as free G-actin [98, 99]. Profilin has numerous binding partners, one of which is the Arp2/3 complex, an interaction that led to the initial discovery of the complex in *Acanthamoeba* [2]. Subsequently, profilin was shown to bind to the Arp2 subunit of the complex [3, 17]. This binding is of relatively low affinity [13, 17, 26, 100], but the abundance of profilin in cells means that a substantial fraction of the available Arp2/3 complex may be bound to profilin at any moment [17]. In fission yeast, Arp3 and ARPC1 (known as Sop2p in this organism, for Suppressor of Profilin) interact genetically with profilin, such that the lethality caused by a temperature-sensitive profilin mutation can be partially rescued by additionally mutating Arp3 [100] or ARPC1 [101], indicating some functional link between profilin and the Arp2/3 complex.

In higher organisms profilin has been shown to enhance Cdc42-induced actin polymerisation in cell extracts [102], a process that is Arp2/3-dependent [65, 103]. The mechanism of this enhancement is multifactorial and is not fully understood. Profilin binds to WASL [48] and may assist in activating it, whereupon the G-actin bound to profilin could contribute to actin nucleation by the Arp2/3 complex (fig. 7c). However, G-actin complexed to profilin is less able to support Arp2/3-induced nucleation than free G-actin, possibly because profilin competes with the WH2 domain of WASP-family proteins for actin binding [102]. Since the G-actin binding ability of the WH2 domain appears critical for the function of WASP family members, this competition would be likely to reduce, rather than enhance, actin nucleation via this pathway. It seems that profilin will remain something of an enigma for some time to come.

The Arp2/3 complex in vivo

The conservation of the Arp2/3 complex throughout evolution indicates that its control of the actin cytoskeleton is critical for all organisms. Different species use actin filaments for a wide range of tasks and, even within a single

cell, F-actin can be organised into a multitude of different arrangements; parallel 'cables', cross-linked networks or contractile rings, for example. Over evolutionary time the Arp2/3 complex has been drafted in to create many of these structures.

Insights from yeast

Early genetic screens suggested that *ARP3* was an essential gene in both *S. cerevisiae* (in which it was originally named *ACT4*) [104] and in *Sch. pombe* (in which it was originally named *ACT2*) [105]. Similarly, the *ARP2* gene (also known as *ACT2*) was proposed to be essential for viability in *S. cerevisiae* [106] as was *ARPC2* [107]. Winter et al. have challenged these findings by producing *ARP2*, *ARP3* and *ARPC2* mutants in *S. cerevisiae* that are viable, the discrepancy with earlier studies being attributed to differences in yeast strain and growth media [16]. Indeed, this work found only *ARPC1* to be essential for viability, with the loss of other subunits producing viability ranging from 2.5 to 88% [16]. *S. cerevisiae* is therefore the only organism so far shown to be capable of life in the absence of a fully functional Arp2/3 complex.

Although yeast do not produce the dramatic actin-rich structures found in higher organisms (lamellipodia, filopodia, phagocytic cups, and so on) they nonetheless possess two clearly-defined structures composed of F-actin: cytoplasmic actin cables and cortical actin patches (fig. 5c) [108, 109]. Actin patches are highly motile structures that contain a variety of cytoskeletal components, including the Arp2/3 complex [9, 16, 100, 101, 110, 111]. Mutations in subunits of the Arp2/3 complex disrupt actin patch formation and motility [9, 16, 111, 112] and inhibit endocytosis [113]. Intriguingly, ARPC2 interacts with calmodulin in yeast [114], and overexpressing calmodulin can compensate for the loss of this subunit [107]. The biological significance of this is currently unclear. The Arp2/3 complex has also been implicated in the movement of yeast mitochondria, which use the complex to nucleate actin filaments at their surface [115]. Clearly the Arp2/3 complex plays a critical role in actin-mediated events in yeast as well as in higher organisms.

The Arp2/3 complex in plants – a field ripe for exploration

Although plants have a well-defined cytoskeleton that is essential for a variety of cellular processes [116], the investigation of actin dynamics in this kingdom has lagged far behind that in animals or fungi. It is not yet known whether plants have a complete Arp2/3 complex, although an *ARP2* gene has recently been cloned from *Arabidopsis thaliana* [117]. Expression of this gene is very low in most plant tissues, in contrast to the high expression observed in most animal cells, but high expression is observed around the xylem and in pollen grains [117]. By sequence similarity it appears that *A. thaliana* also has *ARP3* and *ARPC1* genes, although close homologues of the other four subunits are not apparent in the database.

Life without a cell wall: the Arp2/3 complex as an actin-remodelling machine

Changes in the actin cytoskeleton regulate cell morphology and movement, so it is perhaps unsurprising that research into actin dynamics has focussed primarily on cells that are not constrained by a cell wall. In particular, motile cells such as mammalian neutrophils and macrophages, keratocytes from fish and unicellular amoeboid cells such as *Dictyostelium discoideum* and *Acanthamoeba castellanii* have provided powerful models in which to study actin remodelling.

Early experiments showed that the Arp2/3 complex localises to the cell cortex of *Acanthamoeba* [2]. In mammalian fibroblasts the complex localises to lamellipodia, sheetlike protrusions of the plasma membrane at the edge of the cell [5, 6], where it assembles a dense network of branched actin filaments (fig. 5d) [18]. This localisation to the leading edge is seen in a variety of other cells and appears to be essential for chemotactic locomotion [118]. The Arp2/3 complex also localises to nascent phagosomes (fig. 5b) [119], to the immunological synapse formed by T cells [120], to neurite protrusions [121] and to podosomes, actin-rich adhesion structures found in specialised cells such as macrophages [122]. The Arp2/3 complex appears to play a critical role in all of these structures, since delocalising the complex inhibits their formation. Interestingly, the Arp2/3 complex does not localise to sites where actin filaments are arranged in parallel arrays, such as stress fibres (fig. 5e) or filopodia [5, 6], although it appears that the complex may be required to initiate and/or maintain these structures by nucleating new actin filaments [14, 123].

A surprising discovery that has recently been made is the involvement of actin polymerisation in the movement of vesicles within the cell. Membrane-bound vesicles in *Xenopus* egg extracts assemble F-actin at their surface [66], and in cells endocytic vesicles can be propelled through the cytoplasm by F-actin 'tails' [124, 125] in a similar way to some intracellular bacteria (see below). In both mammalian cells and *Xenopus* extracts, actin polymerisation by vesicles appears to require the Arp2/3 complex [125, 126].

To date, all experimental evidence on the role of the Arp2/3 complex in mammalian cells has been either observational or has relied on the expression of mutated or truncated regulators of the complex. For example, a small C-terminal fragment of WASP (or WASL/WASF) is sufficient to bind to the Arp2/3 complex. Expressing this fragment in cells delocalises the complex by preventing its interaction with endogeneous WASP-family regulators [14, 55, 119, 120,

122]. Similarly, removing the acidic (Arp2/3-binding) domain from WASP family proteins prevents them from interacting with the Arp2/3 complex. Thus 'acidic domain-removed' WASP-family proteins act as dominant negative constructs by interacting with upstream signalling components but failing to transmit this signal to the Arp2/3 complex. Such constructs have been used to block Arp2/3-dependent actin polymerisation induced by enteropathogenic *Escherichia coli* [127]. Research has been impeded, however, by the lack of a genuine, preferably reversible, inhibitor of the Arp2/3 complex. A significant step forward in this direction was recently reported by Bailly et al. [22], who have developed a blocking antibody that selectively prevents the complex from binding to actin filaments but does not affect its nucleating activity. The development of similar tools will undoubtedly stimulate rapid advances in the field.

Hijacking the Arp2/3 complex

Many pathogenic organisms have evolved mechanisms that allow them to enter and multiply within host cells. For these intracellular pathogens the ability to move through the host cytoplasm from cell to cell confers a strong selective advantage, allowing the rapid spread of infection without exposure to the host immune system. Several pathogens have independently evolved mechanisms that use the polymerisation of host cell actin to provide the driving force for entry and/or movement within the host cell.

Many intracellular pathogens remodel the host cytoskeleton in order to effect entry, although the morphology of internalisation varies dramatically. The best-studied entry processes are those carried out by *Salmonella*, *Shigella* and *Listeria*. *Salmonella* and *Shigella* 'inject' bacterial proteins into the host cell using a type III secretion system. Several of these bacterial effectors manipulate host-signalling molecules such as the small GTPases and induce dramatic ruffling of the cell membrane [128]. The attached bacteria are then internalised into 'spacious phagosomes' [129]. Since surface ruffles are thought to be adapted lamellipodia, and the formation of lamellipodia is Arp2/3 dependent [14], it is perhaps unsurprising that the complex has been implicated in *Salmonella* entry [130]. Several cytoskeletal proteins are involved in the uptake of *Shigella,* but a possible role for the Arp2/3 complex has yet to be explored. The Arp2/3 complex has not been implicated in the uptake of *Listeria,* and indeed uptake is only mildly inhibited by delocalising the host cell's Arp2/3 complex [55]. This may reflect a greater dependence on the reorganisation of pre-existing actin filaments than on de novo filament nucleation.

The Arp2/3 complex is also involved in the bizarre coiling phagocytosis that allows the spirochete pathogen *Borrelia* to enter macrophages [131] – it remains to be seen whether the complex will also have a role in other 'coiling' uptake processes such as that of *Legionella pneumophila* [132]. Enteropathogenic *E. coli* (EPEC) do not enter the host cell, but still remodel the actin cytoskeleton. By recruiting WASP and Arp2/3 complex to the site at which they are attached, EPEC produce actin-rich pedestals that anchor the bacteria to the cell surface [127, 133].

Once internalised, several pathogens continue to exploit the actin cytoskeleton in order to propel themselves through the cytoplasm. This 'rocketing' or 'comet tail' motility has been a powerful tool with which to dissect actin dynamics and is remarkably similar to that which propels endocytic vesicles (discussed above). The bacteria *L. monocytogenes* and *Shigella flexneri*, and the virus *Vaccinia*, all 'hijack' the Arp2/3 complex in the host cell. By recruiting and activating the complex at their surface, the pathogens produce an actin filament comet tail (fig. 5 a) that propels them through the cytoplasm by continual polymerisation (at the proximal end) and depolymerisation (at the distal end) [134, 135].

All four organisms have evolved molecules that mimic host cell signals upstream of the Arp2/3 complex [136]. For *Listeria* this is ActA [52], a transmembrane protein that imitates WASP-family proteins and directly recruits the Arp2/3 complex, as discussed earlier. *Shigella* has developed a mimic one step 'upstream' of this, the protein VirG/IcsA, which is unrelated to ActA [137]. IcsA contains glycine-rich repeats that are able to bind and activate WASL [138], which in turn activates the Arp2/3 complex to produce an actin tail [47]. More complicated still is the *Vaccinia* virus, which produces a membrane-anchored protein, termed A36R that is essential for movement [139], A36R is phosphorylated by host cell kinases during infection [79] and is then able to recruit the adaptor protein Nck [79] and the WASP-interacting protein (WIP or WASPIP) [80]. Nck and WIP are both able to bind WASL, which is recruited to the viral particle where it activates the Arp2/3 complex, producing an actin tail.

Several bacterial species within the genus *Rickettsia* exhibit actin-tail propulsion. Interestingly, the tail formed by *Rickettsia* is very different in structure to that produced by *Listeria* and *Shigella* and does not appear to contain the Arp2/3 complex [140–142]. Unlike the dynamic, densely branched plume of actin filaments produced by *Listeria* and *Shigella*, *Rickettsia* tails are composed of long, roughly parallel actin filaments that sometimes coil around each other [141, 142]. This structure is more like that found in filopodia or stress fibres than the lamellipodial-type structure of *Listeria* and *Shigella* tails and may suggest a role for the Arp2/3 complex in the initiation, but not the maintenance, of *Rickettsia* tails [135]. Similarly, actin tail formation has recently been described for the bacterium *Burkholderia pseudomallei* [143]. It is not yet known whether this motion is also Arp2/3 dependent.

Enduring mysteries of the Arp2/3 complex

It is only 7 years since the first isolation of the Arp2/3 complex [2], and yet in that short time tremendous progress has been made in understanding its function. However, we are still a considerable distance from a coherent picture of the 'life and times' of the Arp2/3 complex – many questions remain:

At the mechanistic level

An elegant model exists to envision how the Arp2/3 complex could stimulate actin nucleation, but how do activators such as WASP or ActA convert the complex from an essentially inactive state into an actin-nucleating machine? What are the other cellular activators of the Arp2/3 complex, and how are signals integrated to trigger actin nucleation at specific sites? Similarly, how does side binding to actin filaments act as a secondary stimulus for nucleation?

At the structural level

Some of the subunits within the Arp2/3 complex have clearly defined roles (Arp2 and Arp3, for example), but what do the others do? The Arp2 and Arp3 subunits are closely related to actin and thus bind ATP – can they also hydrolyse this nucleotide and, if so, what purpose does this serve? It now appears that isoforms exist for many of the Arp2/3 complex subunits – can these all assemble as part of an Arp2/3 complex (producing a massive number of potential 'alternative complexes') and, if so, what does this variability achieve? How is the complex synthesised – are the subunits cooperatively transcribed, and do they assemble in a particular order?

Mystery binding partners

The Arp2/3 complex was originally isolated by virtue of its ability to bind profilin [2]. What is the role of profilin in Arp2/3-mediated nucleation? ARPC3 binds to WASP family proteins in the yeast two-hybrid assay [14] but not via chemical cross-linking [26] – why?

Debranching

Debranching must precede remodelling or disassembly of actin networks. How is debranching achieved in vivo? What role does the ADF/cofilin family play in this process? Is debranching a precursor to the formation of parallel F-actin assemblages, or are these produced by a totally different mechanism?

Concluding remarks

The astounding dynamism of the actin cytoskeleton has been a subject of fascination for a long time. Just as with the scaffolding of an architectural monument, the cell's actin 'scaffold' is produced by the coordinate activity of numerous cellular machines, from assemblers and dismantlers to cross-linkers and bundlers. In the Arp2/3 complex we can glimpse one of these machines in action, a precision device that takes the nuts and bolts of a cellular scaffold and assembles it into a beautiful framework. Like a supernal observer studying the rise of an office block, we have seen both the scaffolders and their creation. The task now is to understand the way in which they work.

Note added in proof: Two new Arp2/3 complex-binding partners have recently been identified. Pan1p in S. cerevisiae is proposed to couple actin polymerisation to the endocytic process [Duncan et al., 2001], whilst the Dictyostelium protein CARMIL couples the Arp2/3 complex to Type I myosins and capping protein [Jung et al., 2001].

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