

## Trends Box

- Several Arp2/3 hybrid complexes are detected in focal adhesions, where the classical adhesome component vinculin together with or without the actin cross-linker  $\alpha$ -actinin, associates with the core subunits Arp2, Arp3 and ARPC2 to promote actin polymerization and to regulate focal adhesion formation.
- Arp2/3 subunits ARPC1A, ARPC4, and ARPC5 are not required for cytoskeleton rearrangements upon host cell invasion by *Listeria monocytogenes*, while subunits ARPC5 and ARPC1B are dispensable for bacterial actin-based motility.
- Arp2/3 complexes containing the subunits ARPC1B and ARPC5B are significantly more efficient at promoting actin assembly by Vaccinia virus.
- ARPC1B and ARPC5 are not essential for *in-vitro* actin nucleation.
- Phosphorylation of Arp2 and ARPC1B enhances actin nucleation, while phosphorylation of Arp3 attenuates this process.

## Keywords

Arp2/3, actin polymerization, focal adhesions, *Listeria monocytogenes*, vaccinia virus

## Text Boxes

**Box 1.** Activation of the Arp2/3 complex by NPFs.

The Arp2/3 complex is by itself an inefficient actin nucleator, and requires the binding of nucleation promoting factors (NPFs) to activate its nucleation activity. While preformed actin filaments are also Arp2/3 complex activators [20], the most efficient NPFs include the bacterial surface proteins ActA and RickA from *L. monocytogenes* and *R. conorii* respectively [15,33,61] as well as the eukaryotic WASP (Wiskott-Alrich syndrome protein) [62], SCAR/WAVE (suppressor of cyclic AMP repressor/WASp-family verprolin-homologous protein) [20], WASH (WASP and Scar homologue) [63], WHAMM (WASP

homolog associated with actin, membranes and microtubules) [64], and JMY (junction mediating and regulatory protein, p53 cofactor) [65]. These NPFs characteristically display a WCA domain (W: WASP-homology 2 domain or WH2, C: Connector, A: Acidic; this domain is also known as VCA domain: Verprolin-Cofilin-homology and Acidic-rich) that increases the affinity of Arp2/3 complex for the mother filament, activating the complex [4,66]. NPFs like ActA and WASP dimerize to deliver actin monomers to the Arp2/3 complex [67]. NPFs dissociate from the Arp2/3 complex and may participate in multiple rounds of activation [68].

### **Box 2.** Actin branching by the Arp2/3 complex.

The Arp2/3 complex generates new actin filaments that branch-off from the side of pre-existing filaments at a 70° angle to form a Y-branched network [23]. The use of electron tomography to reconstruct the branch junction suggested that the Arp2 and Arp3 subunits reorganize into a dimer, providing a template for elongation of the daughter filament [52]. The remaining subunits, in particular ARPC2 and ARPC4 were reported to make substantial contacts with the mother filament and allow the anchoring of Arp3 as the first subunit of the daughter filament [52]. Subsequent mutagenesis studies have confirmed a role for ARPC2 and ARPC4 in providing an actin-filament-binding interface which is critical for nucleation and branch stability [69] and suggest for the yeast Arc40 (ARPC1 in mammalian cells, see the nomenclature in **Table 1**) multiple essential roles including suppression of spontaneous nucleation by the Arp2/3 complex and propagation of NPFs activation signals [70].

### **Glossary Box**

- **Focal adhesions:** large multi-protein complexes that act as transmembrane links between the extracellular matrix and the actin cytoskeleton. The protein network associated to integrin receptor that bridges between the extracellular matrix and the termini of actin stress fibres is called 'the adhesome' [71].

- ***Listeria monocytogenes:*** a Gram-positive bacterium responsible for a food-borne disease named listeriosis, which can lead to meningitis in the new born and abortions in pregnant women. *L. monocytogenes* is the prototype intracellular pathogen, inducing its

internalization within non phagocytic cells, lysing its phagosome and using an actin-based motility system to promote cell-to-cell spread [26].

- **NPFs**: nucleation promoting factors activate the Arp2/3 complex to polymerize actin above the basal nucleation rate threshold displayed by the native complex. The activities of the NPFs are regulated by signal-transduction pathways that coordinate actin cytoskeleton polymerization in time and space [4,5].

- **Vaccinia virus**: a prototypic poxvirus closely related to variola virus, the causative agent of smallpox. Poxviruses are enveloped DNA viruses that have the particularity to exist in two infectious forms: mature virions are viral particles contained within a single membrane, while extracellular virions are contained within two concentric membranes. Vaccinia virus particles are propelled on the tip of actin tails at the surface of infected host cells [36].