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Actin assembly mechanisms at a glance

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ABSTRACT

The actin cytoskeleton and associated motor proteins provide the driving forces for establishing the astonishing morphological diversity and dynamics of mammalian cells. Aside from functions in protruding and contracting cell membranes for motility, differentiation or cell division, the actin cytoskeleton provides forces to shape and move intracellular membranes of organelles and vesicles. To establish the many different actin assembly functions required in time and space, actin nucleators are targeted to specific subcellular compartments, thereby restricting the generation of specific actin filament structures to those sites. Recent research has revealed that targeting and activation of actin filament nucleators, elongators and myosin motors are tightly coordinated by conserved protein complexes to orchestrate force generation. In this Cell Science at a Glance article and the accompanying poster, we summarize and discuss the current knowledge on the corresponding protein complexes and their modes of action in actin nucleation, elongation and force generation.

KEY WORDS: Actin cytoskeleton, Actin nucleation, Actin filament elongation, Myosin force generation, Membrane protrusion, Membrane contractions, Membrane trafficking, Rho-GTPase signalling

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Introduction

The cellular actin cytoskeleton emerges from the assembly of globular actin (G-actin) into double helical filaments (F-actin) (Fujii et al., 2010; Holmes et al., 1990; Oda et al., 2009). Generation and disassembly of F-actin has to be a very dynamic process in order to fulfil the diversity of actin functions (Blanchoin et al., 2014). The spontaneous polymerization of G-actin into filaments is prevented

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by the relative instability of the actin dimer or trimer, and by G-actin-binding and sequestering proteins, such as profilin and β-thymosin, respectively (Pantaloni and Carlier, 1993; Sept and McCammon, 2001; Xue and Robinson, 2013). To overcome these kinetic barriers, *de novo* actin filament polymerization is initiated by actin nucleators or nucleation complexes (Dominguez, 2016; Pollard and Cooper, 2009; Quinlan and Kerkhoff, 2008). Three major classes of actin nucleators have been identified, the Arp2/3 complex, which becomes activated, for example, by nucleation-promoting factors (NPFs) of the Wiskott–Aldrich syndrome protein (WASP)/WASP-family verprolin-homologous protein (WAVE) family; the formins, which assemble into a donut-shaped dimer, where each of the subunits can bind two actin monomers; and finally, the tandem actin-binding domain nucleators, such as Spire, Cobl and leiomodin, which promote actin filament initiation by binding of G-actin to two or more of their WH2 and other actin-binding domains (Ahuja et al., 2007; Chereau et al., 2008; Dominguez, 2016; Fowler and Dominguez, 2017; Machesky and Insall, 1998; Mullins et al., 1998; Otomo et al., 2005b; Pruyne et al., 2002; Quinlan et al., 2005; Sagot et al., 2002). In addition to nucleation, formins also accelerate elongation of the fast-growing (barbed) ends of actin filaments (Otomo et al., 2005a; Romero et al., 2004; Vavylonis et al., 2006), by both attracting profilin-bound G-actin and antagonizing the abrogation of barbed end elongation by capping protein. A similar function has been ascribed to members of the Ena/VASP family of actin filament polymerases, although the latter operate in protein clusters that can utilize actin and profilin–actin complexes for elongation (Breitsprecher et al., 2008; Brühmann et al., 2017; Disanza et al., 2013; Hansen and Mullins, 2015; Winkelman et al., 2014).

Many structures described here are driven, at least in part, by the activity of specific members of the Rho family of small GTPases, which are now recognized to operate through physical interaction with the aforementioned NPFs or formins (Chen et al., 2010; Lebensohn and Kirschner, 2009; Otomo et al., 2005a; Rohatgi et al., 1999; Rose et al., 2005) (see poster).

In recent years, the concerted action of actin nucleators and nucleation complexes with elongation factors and myosin motor proteins has become apparent. Here, we provide a summary of the composition, function and regulation of such actin-nucleation complexes by focusing on a selection of distinct subcellular structures that have been shown to exert important functions in mammalian cells and to be relevant in human disease.

The regulation of cell edge protrusions: lamellipodia and filopodia

Lamellipodia are major protrusions consisting of networks of actin filaments that are formed by various migrating cells on flat and rigid substrata (Small et al., 2002) (see poster). If they lift upwards and backwards onto the cell, lamellipodia are also called membrane ruffles and considered to harbour actin assembly machineries similar to those formed during micropinocytosis and phagocytosis. Furthermore, certain bacterial pathogens induce comparable, ruffle-like structures leading to their engulfment into non-phagocytic cells, allowing them to infect and spread within tissues and to escape the immune system (see also Box 1).

Lamellipodia represent branched actin networks that require the activity of the Arp2/3 complex (Suraneni et al., 2012; Svitkina and Borisy, 1999; Wu et al., 2012), which is activated downstream of the Rac subfamily of small GTPases (Ridley et al., 1992; Steffen et al., 2013). Rac targets the WAVE regulatory complex (WRC) through direct interaction with its Sra-1 (also known as CYFIP1) subunit

Box 1. Pathogen-derived nucleators and NPFs

A variety of pathogens, both bacteria and viruses, have developed distinct strategies to subvert the actin cytoskeleton of host cells for their own benefit. *Listeria monocytogenes* is able to move in host cell cytoplasm by generating actin comet tails that allow it to spread from cell to cell, thus enabling it to escape the host immune response. This tail formation requires activation of Arp2/3 by the *Listeria* surface protein ActA, the first NPF discovered (Welch et al., 1997). In contrast, intracellular *Shigella flexneri* moves through the cytosol using its IcsA/VirG to recruit the host cell NPF N-WASP (Egile et al., 1999). *Vaccinia* virus instead induces intracellular actin tail formation below the plasma membrane from the outside, but also usurps N-WASP by indirectly stimulating host cell signalling pathways by mimicking receptor tyrosine kinase signalling (Leite and Way, 2015). Similarly, enterohaemorrhagic and enteropathogenic *Escherichia coli* 'surf' on cell surface structures, so-called pedestals, on which these bacteria reside; both also depend on N-WASP and Arp2/3 complex, although they employ distinct host cell signalling pathways (Campellone, 2010).

Strategies for actin nucleation can even switch during the life cycle of the same bacterial species. For instance, invading *Rickettsia* spp. first use the bacterial protein RickA to activate Arp2/3 to drive the formation of short, flexible comet tails (Gouin et al., 2004), whereas later during infection, they use their bacterial surface protein and formin-mimic Sca2 to generate more rigid actin tails that allow a faster and more persistent motility (Haglund et al., 2010). Recent work on *Burkholderia* spp. revealed that the mechanism of actin nucleation can even differ between orthologous factors. Distinct *Burkholderia* species induce actin tails with divergent architectures in the host cytoplasm, depending on whether their orthologous actin inducer BimA has evolved to operate as an Arp2/3 complex activator or an Ena/VASP mimic (Benanti et al., 2015).

Finally, VopL and VopF are bacterial tandem WH2-domain-containing actin assembly factors of the Spire type, which support the colonization of infected epithelia by *Vibrio parahaemolyticus* (Liverman et al., 2007) and *Vibrio cholerae* (Tam et al., 2007), respectively. Although these proteins bind to both barbed and pointed actin filament ends (Pernier et al., 2013), nucleation was recently found to mostly occur from the pointed end (Burke et al., 2017).

(Innocenti et al., 2004; Leithner et al., 2016; Steffen et al., 2004) or its isogene PIR121 (CYFIP2), thereby driving Arp2/3 complex activation and lamellipodia formation (Steffen et al., 2014; Stradal and Scita, 2006).

Other crucial factors in lamellipodia include the heterodimeric capping protein (Mejillano et al., 2004), which accumulates close to lamellipodial edges (Iwasa and Mullins, 2007; Lai et al., 2008; Svitkina et al., 2003), and members of the ADF/cofilin family of actin disassembly factors that contribute to protrusion by maintaining cellular actin filament turnover (Hotulainen et al., 2005; Kanellos and Frame, 2016). Note that the maintenance of turnover will be essential for any of the actin structures discussed below, irrespective of the nucleation process employed, albeit to variable extents. Additional regulators include the Arp2/3 complex inhibitor Arpin, which has been implicated in steering Arp2/3-dependent protrusion and migration (Dang et al., 2013), or lamellipodin, an interactor of the enabled/vasodilator-stimulated phosphoprotein (Ena/VASP) family of actin polymerases, which has also been proposed to bridge Rac with the WRC (Krause and Gautreau, 2014; Law et al., 2013). Furthermore, actin turnover is fine-tuned in lamellipodia by the well-known lamellipodial marker cortactin (Lai et al., 2009) or the coronin family proteins, which are thought to regulate the destabilization of Arp2/3-dependent branches (Cai et al., 2008).

Finally, lamellipodial actin generation is boosted downstream of Cdc42 by the formins FMNL2 and FMNL3 (Block et al., 2012),

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which produce subpopulations of actin filaments that are independent of the Arp2/3 complex and crucial for mechanical stability and lamellipodial force development (Kage et al., 2017). As also shown in the latter study, the rate of network actin polymerization in lamellipodia can be uncoupled from protrusion efficiency, and Arp2/3-dependent actin branching alone is not sufficient to generate mechanically rigid lamellipodial actin networks (Kage et al., 2017). Notably, lamellipodial actin assembly rates are controlled by the number of available actin monomers rather than the actin polymerases positioned at their tips (Dimchev et al., 2017).

Filopodia, also termed microspikes if they are mostly embedded into the lamellipodium, are dynamic, finger-like cell surface protrusions found in most cell types (Mattila and Lappalainen, 2008; Small et al., 2002) (see poster). Filopodia have been implicated in navigation of neuronal growth cones, the fusion of epithelial sheets during morphogenesis (Millard and Martin, 2008; Viveiros et al., 2011) and also in promoting 3D migration and cancer cell invasion (Jacquemet et al., 2015). Canonical filopodia are usually a few micrometres wide and no more than 10 µm in length (Mellor, 2010), and comprise a few dozen parallel actin filaments that are compacted into dense bundles by cross-linkers, such as fascin and Daam1 (Hoffmann et al., 2014; Jaiswal et al., 2013; Vignjevic et al., 2006), although genetic data are lacking that would allow functional dissection of the role of fascin family members relative to other cross-linkers (Hashimoto et al., 2011; Yamakita et al., 2009). Additionally, filopodia can accumulate VASP (Svitkina et al., 2003), myosin-X (Bohil et al., 2006) and inverse Bin–amphiphysin–Rvs (IBAR) proteins such as the insulin receptor tyrosine kinase substrate p53 (IRSp53; also known as BAIAP2) at their distal tips, which are all thought to contribute to filopodial actin filament assembly, although a clear molecular understanding of their respective functions at these sites remains elusive (Mattila and Lappalainen, 2008). In general, filopodia are thought to grow by actin incorporation at their tips, and to shrink either by rearward pulling of filaments into the cell body followed by their depolymerization (Mallavarapu and Mitchison, 1999), and/or by cofilin-mediated actin filament disassembly initiated in tip regions or along their shafts (Breitsprecher et al., 2011).

Two major mechanisms employing distinct actin nucleators have been proposed to drive the formation of these structures (Faix et al., 2009; Faix and Rottner, 2006; Mattila and Lappalainen, 2008; Yang and Svitkina, 2011). In the ‘convergent elongation’ model, filopodial actin filaments arise from the dendritic network of Arp2/3 complex-nucleated filaments by selective elongation, coalescence and bundling (Korobova and Svitkina, 2008; Svitkina et al., 2003), whereas the ‘*de novo* nucleation’ model predicts that filopodial actin filaments are nucleated from scratch by mammalian Diaphanous-related formin mDia2 (also known as DIAPH3; Block et al., 2008) or the formin-like proteins FMNL2 and FMNL3 (Gauvin et al., 2015; Kage et al., 2017; Kühn et al., 2015) downstream of Rho-family GTPase signalling. Indeed, filopodia can form in the absence of Arp2/3 complex and/or its activators in different cell types, as well as in cells that lack any lamellipodia (Gomez et al., 2007; Nicholson-Dykstra and Higgs, 2008; Sarmiento et al., 2008; Steffen et al., 2006, 2013; Wu et al., 2012). The *de novo* nucleation model is further supported by the reduction of filopodia observed in *Dictyostelium* cells after genetic knockout of dDia2 (Schirenbeck et al., 2005), or after silencing of FMNL3 in mammalian cells (Gauvin et al., 2015). Extensions and/or amendments of both competing models have recently been described (Young et al., 2015). Notwithstanding this, future efforts are needed to unambiguously identify those nucleators and pathways that are obligatory for filopodia formation in mammals; this is currently lacking.

Invadosomes

Invadopodia and podosomes, collectively termed invadosomes, are adhesion and invasion structures that establish contact with the extracellular matrix through integrins (Pfaff and Jurdic, 2001; Teti et al., 1989) and CD44 (Chabadel et al., 2007), and recruit metalloproteases to degrade the extracellular matrix (Linder, 2007) (see poster). Podosomes reflect the physiological aspect of these structures and are formed in untransformed cells, including macrophages (Linder et al., 1999), dendritic cells (Burns et al., 2001), osteoclasts (Destaing et al., 2003), endothelial cells (Moreau et al., 2003) or neural crest cells (Murphy et al., 2011), whereas invadopodia are found in cancer cells, such as carcinoma (Lorenz et al., 2004) or melanoma cells (Monsky et al., 1993).

Podosomes consist of a core of branched actin filaments, which depends on WASP-mediated activation of the Arp2/3 complex (Linder et al., 2000, 1999), and is surrounded by a ring of integrins and adhesion-plaque proteins, such as talin or vinculin (Linder et al., 2000; Zambonin-Zallone et al., 1989) (Box 2), whereas a cap structure on top of the core contains the formins INF2 (Panzer et al., 2016) and FMNL1 (Mersich et al., 2010), and organizes lateral

Box 2. Actin assembly factors and genetic disorders

The pivotal role of actin structures in cell biological processes is reflected by deregulated actin functions, which frequently cause human diseases. A number of inherited diseases are directly linked to mutations in genes encoding actin regulators. Mutations in WASP, an activator of the Arp2/3 complex (Machesky and Insall, 1998) expressed in the hematopoietic lineage, are linked to Wiskott–Aldrich syndrome, which manifests in immune and blood clotting insufficiencies, as well as leukaemia (Derry et al., 1994). Loss of podosome formation in megakaryocytes may contribute to the reduced count of platelets in patients with Wiskott–Aldrich syndrome (Poulter et al., 2015; Schachtner et al., 2013), whereas loss of podosomes in immune cells, such as macrophages (Linder et al., 1999) and dendritic cells (Burns et al., 2001), could contribute to the observed defects in directional immune cell migration (Bouma et al., 2009). Mutations in the WASH complex subunit strumpellin cause autosomal dominant hereditary spastic paraplegia (HSP), a neurodegenerative disorder characterized by a progressive stiffness and contraction in the lower limbs (Jia et al., 2010; Valdmanis et al., 2007).

Inherited diseases have also been described for several members of the formin family. Autosomal dominant nonsyndromic auditory neuropathy, for instance, has been linked to a mutation in the 5′ untranslated region of the human *DIAPH3* gene, and results in an increased expression of the formin (Schoen et al., 2010). Transgenic mouse models overexpressing *Diaph3*, revealed that inner hair cells of the ear develop elongated stereocilia and fuse with neighbouring ones, coinciding with increasing hearing loss (Schoen et al., 2013). Moreover, homozygous loss of the related formin *DIAPH1* has recently been identified to cause microcephaly in humans (Ercan-Sencicek et al., 2015).

Mutations in two other formin family members, INF2 and FMN2, were found to cause severe human disorders (Boyer et al., 2011; Law et al., 2014). INF2 mutations are associated with the focal segmental glomerulosclerosis and with the Charcot–Marie–Tooth neurodegenerative disorder, a subtype of muscular dystrophy (Boyer et al., 2011), whereas homozygous mutations of *FMN2* cause nonsyndromic intellectual disability (Law et al., 2014). Interestingly, the functions of INF2 and FMN2 converge on the tandem WH2-domain-nucleator Spire, which cooperates with INF2 at mitochondria-ER contact sites and with FMN2 at Rab11-enriched vesicle membranes (Manor et al., 2015; Pfender et al., 2011; Schuh, 2011).

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unbranched filaments that are anchored at the ring (Akisaka et al., 2008); this enables podosomes to function as mechanosensors (Linder and Wiesner, 2016; van den Dries et al., 2013). A second set of unbranched actin filaments, regulated by the formin FHOD1 (Panzer et al., 2016), connects podosomes into higher-ordered groups (Luxenburg et al., 2007). Both sets of unbranched filaments contain myosin-IIA and are contractile (Bhuwania et al., 2012; van den Dries et al., 2013). Considering their biochemical activities, INF2 could function in both actin polymerization and depolymerization (Chhabra and Higgs, 2006), whereas FHOD1 likely bundles podosome-connecting filaments (Schönichen et al., 2013). FMNL1 regulates the stability of podosomes (Mersich et al., 2010), which could involve multiple activities, such as actin nucleation, elongation, bundling or severing (Harris et al., 2004, 2006).

Invadopodia form and mature in several steps, starting with an actin network that depends on N-WASP and the Arp2/3 complex (Gligorijevic et al., 2012; Yamaguchi et al., 2005) (see poster). This network is subsequently anchored at the plasma membrane by a ring structure of integrins and adhesion plaque proteins, which is also important for further growth (Branch et al., 2012), but often not detected in mature structures. It is unknown whether this ring structure is connected to the invadopodium core through contractile actomyosin fibres, in analogy to podosomes. Further protrusive growth of invadopodia requires both the Arp2/3 complex and formins (Schoumacher et al., 2010), as demonstrated for the Diaphanous-related formins DIAPH1–DIAPH3 (DRF1-DRF3) in breast cancer cells (Lizarraga et al., 2009), mDia2 (DIAPH3) in colon cancer (Schoumacher et al., 2010) and FHOD1 in squamous carcinoma cells (Gardberg et al., 2013). Diaphanous-related formins probably regulate nucleation and growth (Li and Higgs, 2003) of unbranched actin filaments along the shaft and tips of invadopodia as visualized by ultrastructural analysis (Schoumacher et al., 2010), with capping and bundling likely to be supported by FHOD1 (Schönichen et al., 2013). The pronounced protrusion of invadopodia, as opposed to the non-protrusive nature of podosomes, probably also underlies the respective differences in their lifetime, which can be over 60 minutes for invadopodia (Yamaguchi et al., 2005) compared with only several minutes for podosomes (Destaing et al., 2003).

Cell cortex

The contractile actin cortex of eukaryotic cells is a thin layer of about 100 nm that contains actin filaments cross-linked by specialized actin- and lipid-binding proteins of the ezrin–radixin–moesin (ERM) family (see poster). The cortex also contains myosin-II, which generates forces within this network beneath the plasma membrane (Salbreux et al., 2012; Sens and Plastino, 2015). The dynamic turnover of cortical actin enables cells to adapt and resist to extracellular stress, to perform mechanical work and quickly respond to external stimuli, as well as to drive cell shape changes. The tension generated by assembly and contraction of this layer plays a central role in various processes, including cell migration (Charras and Paluch, 2008), division (Stewart et al., 2011) or tissue morphogenesis (Munjal and Lecuit, 2014). Recent work using mitotic HeLa cells revealed that, in addition to Arp2/3 complex, mDia1 (DIAPH1) has a major role in nucleating cortical actin (Bovellan et al., 2014) (see poster). In mammalian amoeboid cells such as dendritic cells, but also in *Dictyostelium*, mDia1 and the mDia1-like formin ForA, respectively, are crucial when these cells migrate through mechanically confined environments (Ramalingam et al., 2015; Vargas et al., 2016). Loss of ForA in *Dictyostelium* cells causes failure to withstand hydrostatic pressure in confined environments, leading to ectopic blebbing at the rear and inefficient migration (Ramalingam et al., 2015). The fact that active mDia1 can also accumulate in the rear of migrating B16-F1 melanoma cells suggests evolutionarily conserved roles of cortical formins in contractility-assisted migration. However, the proposed role of mDia1 as a positive regulator of lamellipodial protrusion, through nucleation of mother filaments for subsequent branching by Arp2/3 complex (Isogai et al., 2015), appears incompatible with its strong accumulation in the rear (Ramalingam et al., 2015; Vargas et al., 2016) and with the observed antagonism between blebbing and lamellipodium formation (Bergert et al., 2012).

Stress fibres and contractile ring

The rate of assembly and turnover of actin filaments in stress fibres is much lower compared with levels in protrusions (Campbell and Knight, 2007; Lai et al., 2008), pointing to differences in regulation. The canonical type of stress fibre upon which we focus here has previously been referred to as a ventral stress fibre, which commonly terminates in so-called focal adhesions: cell–substratum contacts enriched for integrin transmembrane receptors connecting the extracellular matrix with the actin cytoskeleton inside through dozens of proteins, including vinculin and talin (Hotulainen and Lappalainen, 2006; Livne and Geiger, 2016) (see poster). Interestingly, actin filaments within such stress fibres and the contractile ring show a non-uniform polarity and are regulated by Rho-induced myosin-II-based contractility mostly mediated by Rho-associated kinase (ROCK) (Pellegrin and Mellor, 2007; Rottner and Stradal, 2011; Skau and Waterman, 2015). Regarding the generation of actin filaments in the contractile ring or in stress fibres and focal adhesions, there is little evidence for a prominent role of Arp2/3 complex in formation and turnover of these structures, except for vinculin–Arp2/3 hybrid complexes (Chorev et al., 2014). However, canonical Arp2/3 complex is not present in stress fibres and adhesions, and respective phenotypes are thus lacking in RNAi-depleted or genetic knockout cells (Suraneni et al., 2012; Wu et al., 2012). In contrast, formins have been implicated in filament nucleation in stress fibres and adhesions, and mDia1 was initially identified as a RhoA target, with the caveat of a lack of clear accumulation in RhoA-dependent structures (Watanabe et al., 1999). Subsequently, additional formins were described as operating in stress fibre and/or focal adhesion formation, including mammalian FHOD proteins (Schulze et al., 2014) and their *Drosophila* homologue Knittrig (Lammel et al., 2014), as well as, most recently, INF2, which stands out from the others by its clear accumulation in focal adhesions (Skau et al., 2015) (see poster).

Nevertheless, much remains to be learned regarding the regulation of actin assembly in stress fibres, focal adhesions or the mammalian contractile ring (Burridge and Guilluy, 2016; Skau and Waterman, 2015), and fundamental outstanding questions include determination of the obligatory factors and pathways for the formation and turnover of these different contractile arrays, the regulation of length and number of individual actin filaments contributing to these structures, as well as the coordination of their assembly and disassembly during contraction or constriction (Livne and Geiger, 2016; Pollard, 2014).

Actin assembly in intracellular membrane organization and dynamics

The actin cytoskeleton is not only essential for cell shape change and cell edge protrusion, but is also important for the organization and dynamics of endosomal vesicles and organelles (see poster). Actin filament formation at intracellular membranes provides

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the mechanical forces to drive intracellular processes, such as endocytosis, endosome sorting and recycling, exocytosis and autophagy (Gautreau et al., 2014). A key actin nucleator with roles in vesicle dynamics is the Arp2/3 complex, which is activated by different members of the WASP family of nucleation-promoting factors, such as N-WASP or WASH (depending on the specific intracellular membrane compartment) (Brüser and Bogdan, 2017; Galletta and Cooper, 2009; Gautreau et al., 2014; Girao et al., 2008; Rottner et al., 2010). N-WASP forms a stable complex with WIP/WIRE proteins that protect it from degradation (de la Fuente et al., 2007; Stewart et al., 1999). Once N-WASP is recruited to sites of endocytosis and activated by Cdc42 and its effector, the F-BAR protein Cip4/Toca, it activates the Arp2/3 complex, facilitating membrane invagination of endocytic vesicles and/or promoting movement by actin comet tail formation (Benesch et al., 2002, 2005; Fricke et al., 2009; Ho et al., 2004; Takano et al., 2008) (see poster). Recruitment of myosin-Ie coincides with a burst of actin assembly at sites of clathrin-mediated endocytosis, suggesting that the mammalian class I myosins function in the regulation of actin-driven endocytosis (Cheng et al., 2012) (see poster).

In contrast to N-WASP, WASH associates into a heteroheptameric complex known as the WASH regulatory complex (SHRC) together with strumpellin, FAM21, SWIP, CCDC53 and heterodimeric capping protein (CapZ) (Derivery et al., 2009; Gomez and Billadeau, 2009; Jia et al., 2010) (see Box 2). The SHRC localizes to F-actin-enriched microdomains at the surface of different endosomal compartments, including early endosomes, late endosomes and lysosomes (see poster). Accordingly, loss of WASH function results in diverse trafficking defects in different endocytic routes, including endosome-to-Golgi transport via the retromer complex (Gomez and Billadeau, 2009; Harbour et al., 2012; Kvainickas et al., 2017; Piotrowski et al., 2013; Zech et al., 2011) and endosome-to-plasma membrane recycling of various cargos (Derivery et al., 2009; Nagel et al., 2017; Zech et al., 2011). Endosomal sorting defects in WASH-deficient cells are accompanied by a loss of branched actin networks at the surface of endosomes and an increased formation of endosomal tubular membranes (Gomez and Billadeau, 2009; Piotrowski et al., 2013). The exact role of WASH-dependent actin patches on endosomes, however, is not yet clear. WASH-mediated, branched actin filaments might physically stabilize the tubular neck of budding sorting endosomes and provide additional forces to push against any tension exerted by the membrane, thereby facilitating endosomal fission in a fashion analogous to the role of WASP in endocytic vesicle fission (Fricke et al., 2010, 2009). Branched actin networks might also stabilize distinct endosomal tubules, thereby allowing for cargos to diffuse into these vesicles before pinching off (Puthenveedu et al., 2010). Alternatively, actin-stabilized microdomains could function as platforms that not only cluster cargo, but also localize signalling factors, including lipids or proteins, to mediate efficient endosomal scission (Dong et al., 2016).

At lysosomes, WASH-dependent actin polymerization directly drives the removal of vacuolar H^+-ATPase (V-ATPase) and its recycling into small vesicles, which allows the lysosome to become neutralized (see poster). Accordingly, loss of WASH function in both *Dictyostelium* and *Drosophila* results in strong defects in lysosome and phagosome neutralization (Carnell et al., 2011; King et al., 2013; Nagel et al., 2017). Furthermore, increased autolysosomal acidification upon starvation appears to promote autophagy in *wash* mutant flies and significantly reduces their life span. A dramatic induction of autophagy might also be causative for embryonic lethality of WASH-knockout mice (Xia et al., 2013). In contrast to flies, mammalian WASH appears to directly inhibit autophagosome formation, a role appearing independent of its function as Arp2/3 complex activator (Xia et al., 2014).

Roles in autophagosome formation have also been identified for two related WASP protein family members, the vertebrate proteins WHAMM and JMY (Coutts and La Thangue, 2015; Kast et al., 2015), as discussed in detail in a recent review (Alekhina et al., 2017). In contrast to mammalian WASH, WHAMM promotes autophagosome formation and motility (Kast et al., 2015) (see poster). Consistently, overexpression of WHAMM increases size and number of autophagosomes formed at the ER, whereas its suppression reduces both autophagosome size and number, as well as that of actin comet tails on ER membranes (Kast et al., 2015). Thus, a model has been proposed, in which WHAMM-dependent branched actin network formation by the Arp2/3 complex promotes the biogenesis and motility of autophagosomes from the ER membrane (Kast et al., 2015).

An important role in early autophagosome biogenesis and motility has also been described for JMY, a WHAMM-related protein that also accumulates at LC3-positive ER sites where autophagosomes form and at the *trans*-Golgi network (Coutts and La Thangue, 2015; Schlüter et al., 2014). It is currently unclear whether JMY and WHAMM co-operate or serve differential, perhaps consecutive functions in the autophagosome biogenesis pathway.

Apart from Arp2/3 complex and its activators, members of the Spire protein family also have crucial roles in intracellular membrane organization and transport (Dietrich et al., 2013; Kerkhoff, 2011; Kerkhoff et al., 2001) (see poster). Spire proteins contain a FYVE-type membrane-binding domain, which targets them to negatively-charged endosomal membranes (Pylypenko et al., 2016; Tittel et al., 2015). In metaphase mouse oocytes, Spire proteins, in cooperation with formin-2 (FMN2), nucleate actin filaments at Rab11-marked vesicles, which serve as tracks for myosin-V-mediated long-range transport towards the oocyte cortex (Pfender et al., 2011; Schuh, 2011) (see Box 2) (Pylypenko et al., 2016). The direct interactions of myosin-V with Spire and Rab11 specify the membrane of Rab11-containing vesicles for Spire function (Pylypenko et al., 2016). As a result of alternative splicing, the Spire1 protein (Spire1C) can also be targeted to the outer membranes of mitochondria (Manor et al., 2015). In cooperation with the ER-anchored isoform of inverted formin-2 (INF2), Spire proteins assemble actin filaments at ER–mitochondria intersections. Based on this knowledge, a model has been proposed, in which polymerized actin filaments along these zones provide the forces required for inducing mitochondrial constriction and subsequent division (Manor et al., 2015) (see also Box 2).

Conclusions

Tuning of nucleation and turnover of actin filaments is no doubt instrumental in ensuring specificity and appropriate functioning of a given actin filament structure *in vivo*. The mechanisms of formation of the selected actin structures presented here clearly illustrate two points: (1) the unexpected diversity of factors that co-operate in assembly of individual structures, and (2) the multitude of actin regulators that display crucial functions in more than one structure or process. The Arp2/3 complex certainly represents an extreme case with regard to multi-functionality, but the specific functions of its activators and other actin assembly factors are also emerging as increasingly diverse. All this suggests that we continue to underestimate the connections between seemingly distinct

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Journal of Cell Science (2017) 130, 3427-3435 doi:10.1242/jcs.206433

processes, and instead prefer simplified, isolated views over delving into their complexity. Therefore, the challenge for the future will be to develop more universally applicable models of actin-based motile processes *in vivo* that also include more indirect connections between seemingly independent structures, and thus move beyond the sole characterization of isolated processes.

Acknowledgements
Research on actin assembly mechanisms has been generously funded by the Deutsche Forschungsgemeinschaft (DFG) from 2010 to 2017 within the priority programme SPP1464 "Principles and evolution of actin nucleator complexes", which also included support for international conferences and training courses. The close cooperation within the priority programme, as well as within the wider international community of actin researchers very much inspired and promoted our research. We would like to thank all our colleagues for precious contributions and fruitful scientific discussions. We further thank Tobias Welz for his help in image design.

Competing interests
The authors declare no competing or financial interests.

Funding
Work in the authors' labs is funded by Deutsche Forschungsgemeinschaft grants RO2414/3-2 and RO2414/5-1 to K.R.; FA330/6-2 and FA330/10-1 to J.F.; BO1890/3-2 and BO1890/4-1, cluster of excellence "Cells in Motion" (CIM) to S.B.; LI 925/2-2 and LI 925/8-1 to S.L.; KE 447/10 and KE 447/11 to E.K.

Cell science at a glance
A high-resolution version of the poster and individual poster panels are available for downloading at http://jcs.biologists.org/lookup/doi/10.1242/jcs.206433.supplemental.

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