The multiple functions of actin in apicomplexan parasites

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Abstract
The cytoskeletal protein actin is highly abundant and conserved in eukaryotic cells. It occurs in two different states- the globular (G-actin) form, which can polymerise into the filamentous (F-actin) form, fulfilling various critical functions including cytokinesis, cargo trafficking and cellular motility. In higher eukaryotes, there are several actin isoforms with nearly identical amino acid sequences. Despite the high level of amino acid identity, they display regulated expression patterns and unique non-redundant roles. The number of actin isoforms together with conserved sequences may reflect the selective pressure exerted by scores of actin binding proteins (ABPs) in higher eukaryotes. In contrast, in many protozoans such as apicomplexan parasites which possess only a few ABPs, the regulatory control of actin and its multiple functions are still obscure. Here, we provide a summary of the regulation and biological functions of actin in higher eukaryotes and compare it with the current knowledge in apicomplexans. We discuss future experiments that will help us understand the multiple, critical roles of this fascinating system in apicomplexans.

1 | ACTIN IN MOST EUKARYOTES

Actin is one of the most abundant proteins in eukaryotic cells and owing to its ability to polymerise into filaments (F-Actin) forming static or highly dynamic networks, plays an important function in many crucial cellular processes. The regulated dynamics of F-actin and crosslinking of individual filaments requires the integration of actin binding proteins (ABPs) with signalling cascades that ultimately regulate actin filament length, stability and anchorage (Hansen & Kwiatkowski, 2013). This control can be achieved at multiple levels, from direct binding of ABPs to post-translational modifications of actin and are based on influencing the basic polymerisation mechanism of this molecule (Dominguez & Holmes, 2011).

The atomic structure of monomeric G-actin was first described in 1990. With a molecular weight of 42 kDa (Pollard, 2016), the monomer consists of four subdomains (Kabsch, Mannherz, Suck, Pai, & Holmes, 1990); G-actin binds ATP or ADP in the cleft between subdomains 2 and 4 (Graceffa & Dominguez, 2003; Kabsch et al., 1990; Otterbein, Graceffa, & Dominguez, 2001). G-actin-ATP and G-ADP show conformational differences in subdomain 2 (Otterbein et al., 2001; Graceffa & Dominguez, 2003), imparting G-actin-ATP the ability to bind other ATP-bound actin monomers, facilitating polymerisation into F-actin (Pollard, 1984). Studies using electron microscopy in 1963 proposed that F-actin consists of two actin strands that intertwine to form a right-handed helical filament (Hanson, 1963). More recently, the F-actin structure was described by cryo-electron microscopy (Fujii, Iwane, Yanagida, & Namba, 2010; von der Ecken, Heissler, Pathan-Chhatbar, Manstein, & Raunser, 2016) and it has been suggested that it represents a single left-handed helix (Dominguez & Holmes, 2011). Dominguez and Holmes argued that, since the twist per actin molecule is $\sim 166^\circ$ (close to $\sim 180^\circ$), the F-actin filament structure only appears two-stranded that slowly turns in a right-handed fashion. Hence, the F-actin structure can either be described as a two-start right-handed helix or a single-start left-handed helix with a diameter close to 7–10 nm (Dominguez & Holmes, 2011). These arrangements of F-actin can result into different configurations and includes filaments orientated in the same direction, parallel and antiparallel bundles, and filaments forming actin lattices that enable it...
to fulfil different mechanical and functional roles (Chhabra & Higgs, 2007).

Actin polymerisation is a dynamic process that involves rapid changes in the assembly and disassembly of F-actin. One critical factor for the transition between G-actin and F-actin (actin treadmilling) is the hydrolysis of ATP (Korn, Carlier, & Pantaloni, 1987). The actin treadmilling cycle starts with the addition of G-actin-ATP to “barbed” (plus) end of the F-actin filament. During this polymerisation step, ATP is hydrolysed to ADP and inorganic phosphate (Pi). Hydrolysis of ATP results in stable F-actin filaments with bound ADP + Pi (F-actin-ADP-Pi). Slow release of Pi results in F-actin-ADP and destabilises the filament, resulting in the release of G-actin-ADP from the filament. In a growing F-actin strand, F-actin-ADP resides at the “pointed” (minus) end where the depolymerisation of G-actin-ADP occurs. Subsequently, ADP can be replaced with ATP in the monomer, generating a new G-actin-ATP molecule. The addition of a new G-actin-ATP molecule to the F-actin barbed end causes a conformational change in the adjacent actin molecule. Murakami and co-workers proposed this structural change to be responsible for the initiation of ATP hydrolysis (Murakami et al., 2010). Within subdomain 2 of the actin molecule lies the DNase I binding (D-) loop, which inserts itself into the hydrophobic cleft between subdomains 1 and 3 of the apposing monomer and is crucial for polymerisation (Fujii et al., 2010). Important differences in the amino acid residues of the D-loop between canonical and apicomplexan actins give rise to different filament polymerisation dynamics, which will be discussed in Section 2.2.

Of relevance is that the amount of G-actin has to be above a certain concentration threshold for polymerisation to occur. This threshold is referred to as the critical concentration, where the removal of G-actin occurs at the same rate as the addition of new monomers, but the net mass of the F-actin polymer does not change (Pollard & Borisy, 2003). Importantly, the critical concentration for polymerisation to happen at the barbed end is lower than the concentration needed for polymerisation at the pointed end or for the formation of an actin dimer (Pollard & Borisy, 2003; Pollard, 2016). This results in the typical behaviour of F-actin, where monomers at the barbed end are added 5–10 times faster than to the slow-growing pointed end. Together, this cooperative polymerisation process results in the so-called treadmilling of actin filaments (Figure 1a). It should also be mentioned that the critical concentration required for G-actin-ATP to polymerise was reported to be lower than the concentration required for G-actin-ADP (Cooke, 1975; Pollard, 1984). Consequently, actin polymerisation is more likely to occur at the barbed end of an F-actin strand by attaching G-actin-ATP monomers.

The first step of de novo F-actin filament formation is called actin nucleation, where a new F-actin filament is assembled from G-actin monomers (Pollard, Blanchon, & Mullins, 2000). Inevitably, this process requires the formation of actin dimers and trimers, a process that is kinetically unfavourable (Pollard, 2016; Sept & McCammon, 2001). From this point onwards, G-actin monomer assembly to the actin trimmer occurs with the same rate as G-actin polymerisation on existing F-actin filaments (Sept & McCammon, 2001). The assembly of this polymerisation nucleus therefore represents the critical step that has to be overcome for de novo F-actin formation (Sept & McCammon, 2001). To efficiently control kinetically unfavourable steps of actin dynamics, a vast number of actin binding proteins (ABPs) has been described, some highly conserved in most eukaryotes and others unique to certain species (see [Pollard, 2016] and Table 1).

In contrast to the huge repertoire of ABPs found in higher eukaryotes, only a handful of ABPs have been identified in most protozoa, including apicomplexan parasites (Baum, Papenfuss, Baum, Speed, & Cowman, 2006; Sattler et al., 2011; Schuler & Matuschewski, 2006) (Table 1).

The formation of actin polymers is a tightly regulated process that involves several steps: Nucleation, polymerisation and regulation of the polymer size by actin treadmilling and filament stabilisation. Nucleation of canonical acts depends on a set of ABPs to overcome the activation energy barrier of de novo filament formation. Three different types of actin nucleators have been described: the Arp2/3 complex, spire and the formin protein family (Goode & Eck, 2007). The Arp2/3 complex consists of seven subunits and promotes branching and formation of novel daughter F-actin filaments at an angle of 70° from an already existing filament (Mullins, Heuser, & Pollard, 1998). The Arp2/3 complex is an important nucleator of F-actin in lamellipodia and is involved in cell migration (Suraneni et al., 2012). Spire, first discovered in Drosophila, possesses four WH2 domains used for attracting four G-actin monomers to create a nucleation complex, and can collaborate with formin to build essential

### Take Away

- Disease-causing apicomplexan parasites such as Toxoplasma and Plasmodium possess significantly divergent actin genes compared to other eukaryotes. Differences in key amino acid residues contribute to important structural differences and actin filament instability in these parasites.
- Despite having only a basic set of actin binding proteins (ABPs), an extensive and dynamic formin-dependent F-actin network has been visualised in live parasites using actin-binding nanobodies fused to fluorescent tags (chromobodies).
- Actin dynamics control central biological processes such as invasion, gliding motility, vesicular transport, and apicoplast inheritance in apicomplexan parasites. Genus-specific functions such as haemoglobin uptake and completion of cytokinesis has been additionally observed in Plasmodium falciparum.

Use of actin-binding chromobodies combined with state-of-the-art reverse genetics and microscopy will be immensely useful in mechanistic dissection of the actin network and uncovering functions of known and novel ABPs.
FIGURE 1  Legend on next page.
Formins contain two formin homology (FH) domains, FH1 and FH2. The FH2 domain nucleates and elongates unbranched actin filaments by “processive capping” at the barbed end, while the FH1 domain can interact with the ABP profilin, release actin monomers sequenced by profilin and incorporate them into the growing filament (Courtemanche, 2018). In apicomplexans, only formin like proteins have been identified and are thought to represent the only actin nucleators in these parasites (Tosetti, Dos Santos Pacheco, Soldati-Favre, & Jacot, 2019).

Actin treadmilling is the continuous removal of monomers from the pointed ends of filaments and their simultaneous incorporation at the barbed end, a process in which the ABPs formin, actin depolymerizing factor (ADF)/cofilin and profilin are involved. ADF/cofilin family bind and destabilise F-actin filaments, thus increasing the amount of available G-actin monomers (Moon & Drubin, 1995; Nishida, Maekawa, & Sakai, 1984; Yonezawa, Nishida, & Sakai, 1985; Lappalainen & Drubin, 1997). Cofilin and ADF have higher affinity to G-Actin-ADP than to G-Actin-ATP, thereby increasing the depolymerisation rate of F-actin. While depolymerisation occurs at the pointed end during the treadmilling process, F-actin filament elongation takes place at the barbed end. One of the proteins involved in mediating filament assembly is the polymerisation factor profilin, which binds monomeric G-actin (Baum et al., 2006; Pollard, 2016; Carlsson, Nystrom, Sundkvist, Markey, & Lindberg, 1977; Pantalone & Carlier, 1993). Profilin binds to G-actin-ATP and G-actin-ADP with similar affinity while drastically increasing the exchange rate of actin-bound ADP for ATP (Selden, Kinosian, Estes, & Gershom, 1999). Actin treadmilling occurs by formins assembling a pool of regenerated G-actin-ATP above the critical concentration at the barbed end of an actin polymer (Romero et al., 2004; Kovar, 2006). Cyclase associated protein (CAP) is essential for most eukaryotes and can work in synergy with ADF/cofilin to increase F-actin depolymerization by almost 100-fold, and furthermore, can exchange ADP on depolymerized monomers with ATP to enable another round of F-actin assembly (Kotila et al., 2019). Coronins have been described as a “double-edged sword,” promoting F-actin disassembly in coordination with ADF/cofilin at ADP-rich pointed ends of networks, while promoting rapid F-actin growth at the ATP-rich barbed ends by recruiting the Arp2/3 complex for expansion of branches, thereby functioning during rapid actin-mediated processes such as endocytosis and cell migration (Gandhi & Goode, 2008). Capping proteins (CPs) are a heterodimer composed of the α and β subunits, bind to barbed ends of F-actin and prevent the addition or removal of monomers, thereby stabilising the filament (Edwards et al., 2014; Pollard, 2016). Interestingly, they also stabilise short filaments produced by the actin related protein 1 (ARP1) (Cooper & Sept, 2008).

2 | ACTIN IN APICOMPLEXAN PARASITES

2.1 | Introduction to Apicomplexa

The phylum Apicomplexa includes obligate intracellular protozoan parasites such as Plasmodium spp, Toxoplasma gondii and Cryptosporidium spp which cause devastating human and livestock diseases. Actin isoforms have been identified in all apicomplexans; in all of them, the amino acid sequence is highly divergent from canonical actin found in higher eukaryotes with only about 80% amino acid sequence similarity (Baum et al., 2006; Dobrowolski, Niesman, & Sibley, 1997). There are notable differences in apicomplexan actin, in both the number of isoforms and conservation in amino acid sequence. While Toxoplasma (and most other apicomplexans) possesses only a single gene for actin (Dobrowolski et al., 1997), Plasmodium species possess two isoforms, act-1 and act-2 (Wesseling, Smits, & Schoenmakers, 1988). Plasmodium falciparum ACT1 (PfACT1)
## TABLE 1  Summary of main roles of ABP and ABP regulatory protein partners

| Function                  | Protein                          | ABP regulatory proteins | Apicomplexa |
|---------------------------|---------------------------------|-------------------------|-------------|
| **Higher eukaryotes**     |                                 |                         |             |
| **Polymer**               | Actin isoform                    |                         |             |
|                           | α-skeletal                       |                         |             |
|                           | α-smooth                         |                         |             |
|                           | α-cardiac                        |                         |             |
|                           | β-cyttoplasmic                   |                         |             |
|                           | γ-enteric smooth muscle          |                         |             |
|                           | γ-cyttoplasmic                   |                         |             |
|                           | *P. falciparum*                  | *T. gondii*              | *C. parvum* |
|                           | Pf actin 1                       | Tg actin                | Cp actin    |
|                           | Pf actin 2                       |                         |             |
| **Monomer binding**       | Profilin                         |                         |             |
|                           | Thymosin-β4                      |                         |             |
|                           | WH2 (WASP-homology 2)            |                         |             |
|                           | *Pf profilin*                    | *Tg profilin*           | *Cp profilin* |
|                           | *Pf CAP-like*                    | *Tg CAP-like*           | *Cp CAP-like* |
| **Polymerase**            | Formin family proteins           | Rho GTPases             |             |
|                           | ENA                              |                         |             |
|                           | VASP                             |                         |             |
| **Nucleation**            | Initiation                       |                         |             |
|                           | Branching                        |                         |             |
|                           | Arp2/3                           | WASH/WASP               |             |
|                           | WASp, N-WASP                     | Reg complex; DIP1-Arpin |             |
|                           | SCAR/WAVE                        | Polyphosphoinositoides; SRC homology 3 domains | Rho GTPases; |
|                           | Lmod                             | SCAR/WAVE reg complex and RAC GTPases |             |
| **Alternative filament formation** | Tandem WH-2 (spire Cordon bleu, JMY) | Pf actin-like PfMTRAP-CT PIRH1-CT | Tg actin-like | Cp actin-like |
|                           | Cofilin                          |                         |             |
|                           | CP                               |                         |             |
|                           | CARMIL                           |                         |             |
|                           | Phosphoinositoides               |                         |             |
| **Capping proteins**      | Barbed ends                      |                         |             |
|                           | CP                               | Pf CAPα                 | Tg CAPα     | Cp CAPα    |
|                           | CARMIL                           | Pf CAPβ                 | Tg CAPβ     | Cp CAPβ    |
| **Pointed ends**          | Tropomodulin                     |                         |             |
|                           | Tropomyosin                      |                         |             |
|                           | Arp2/3                           |                         |             |
| **Severing proteins**     | Cofilin 1                        |                         |             |
|                           | Muscle-specific MS cofilin2      |                         |             |
|                           | Gelsoin family (barbed end)      |                         |             |
|                           | Pf ADF1                          | Tg ADF1                 | Cp ADF1     |
|                           | Pf ADF2                          |                         |             |
|                           | Pf Chronophin                    |                         |             |
is expressed in all life cycle stages, while ACT2 was found to be solely expressed in the sexual stages (Wesseling et al., 1989). *Toxoplasma gondii* actin (TgACT1) shares 93% amino acid sequence identity with PfACT1.

### 2.2 | Dynamics of apicomplexan Actin in vitro

While in most eukaryotes F-actin can form long filaments in vitro, apicomplexan actin forms only short filaments of less than 100 nm in the absence of filament-stabilising drugs such as jasplakinolide (Pospich et al., 2017; Schmitz et al., 2005). Apicomplexan actin has also been notoriously hard to visualise and characterise, both in vitro and in vivo, leading to conflicting interpretations regarding polymerisation mechanisms and functions. Early studies by Dobrowolski and colleagues used ultra centrifugation methods to investigate the state of the actin polymer (Dobrowolski et al., 1997); the findings failed to detect filaments, leading the authors to propose that actin is mainly in the monomeric G-actin state in *Toxoplasma*. A comparative study with recombinant actin in vitro showed that *Toxoplasma* actin formed short unstable filaments (Sahoo, Beatty, Heuser, Sept, & Sibley, 2006). Intriguingly, the critical concentration required for actin polymerisation in *Toxoplasma* was suggested to be lower compared to conventional actins, while F-actin assembly and turnover was suggested to occur very rapidly. It was proposed that amino acid residues on the *Toxoplasma* actin monomer surface differ from conventional actins and these differences contribute to filament instability, which could be an adaptation that enables fast parasite motility (Sahoo et al., 2006; Skillman et al., 2011). These results were supported by findings in *Plasmodium falciparum* (Schmitz et al., 2005), where short filaments (~100 nm) were observed in vitro when compared to rabbit actin (~350 nm). Further experiments using actin sedimentation assays led Skillman and co-workers to speculate on an isodesmic model for polymerisation in apicomplexan parasites, which would be a unique and surprising mechanism, only found in apicomplexans (Skillman et al., 2013). This model predicts that all polymerisation steps occur with the same rate, meaning that no critical concentration exists. In consequence, assembly and disassembly occur at the same rate, including the nucleation step prior to filament elongation. While this might explain the formation of short and highly dynamic actin filaments, it would complicate the regulation of actin dynamics in the cell, making nucleators such as formins redundant.

A recent study demonstrated that sedimentation assays are not reliable for the determination of critical concentration of apicomplexan actins (Kumpula et al., 2017). The isodesmic model has thus been readdressed using alternative technologies. Pyrene fluorescence assays convincingly demonstrated that PfACT1 polymerisation depends on a critical concentration, similar to canonical actins (Kumpula et al., 2017). However, the depolymerisation rate of PfACT1 appears to be very rapid and structural differences compared to canonical actins were proposed to cause instability and fragmentation of F-actin. In agreement, cryo-electron microscopy experiments revealed that the differences in contact sites between PfACT1 molecules...
were responsible for filament instability (Pospich et al., 2017). Further crystallography studies of PFAct1 identified the Arg178/Asp180-containing A-loop to be one of the factors responsible, which acts as a switch governing the relative stability of F-actin (Kumpula, Lopez, Tajedin, Han, & Kursula, 2019). Finally, a recent study used actin chromobodies and TIRF microscopy to visualise dynamics of PFAct1 (Lu, Fagnant, & Trybus, 2019) and a significantly higher critical concentration was determined. Here, the instability of F-actin was attributed to rapid filament shrinkage at the pointed end (Lu et al., 2019). The PFAct1 D-loop has important differences in amino acid residues compared to canonical actins, which contributes to natural filament instability essential for the parasite (Lu et al., 2019). A chimeric P. berghei actin-1 with a “canonical” D-loop could produce long filaments in vitro and restore gametocytogenesis in parasites lacking actin-2, indicating that the differential functional needs of the two actins rely heavily on differential filament stability (Vahokoski et al., 2014). In another study, a single point mutation N41H within the PFAct1 D-loop allowed PFAct1 incorporation into mammalian F-actin in a skin cell line (Douglas et al., 2018). Taken together, data obtained from recent in vitro experiments demonstrate that apicomplexan F-actin is more unstable than canonical actins. Differences are found in key residues in multiple regions of the G-actin monomers that appear to critically contribute to this phenomenon. Nonetheless, while different critical concentrations have been determined, a cooperative nucleation-elongation mechanism seems to be in place for F-actin polymerisation in apicomplexan parasites, as seen in all other eukaryotes studied thus far.

Interestingly, Theileria annulata parasites possess an actin isoform that has retained the amino acid residues Ser200, Met270 normally seen in canonical actins, which are mutated to Gly200, Lys270 in Plasmodium and Toxoplasma. Perhaps as a consequence, Theileria parasites make more stable F-actin structures, which were detected by cryo-electron tomography (Kuhni-Boghenbor et al., 2012). Indeed, TgACT1 produced more stable filaments with the reverse mutations Gly200Ser and Lys270Met (Skillman et al., 2011).

### 2.3 Apicomplexan Actin function and distribution in vivo

Until recently it was believed that actin is required primarily for parasite motility. This assumption was based on early inhibitor studies which suggested that microtubules, but not actin, are required for parasite replication (Shaw, He, Roos, & Tilney, 2000) and conversely, actin is required for motility and host cell invasion (Dobrowolski & Sibley, 1996). In the case of Plasmodium, a potential role for PFAct1 in haemoglobin uptake was suggested using inhibitors of actin dynamics (Smythe, Joiner, & Hoppe, 2008). In this study it was concluded that actin has a role in the fusion of endocytic vesicles or in vesicular transport. In Theileria long filamentous nanotubular protrusions of F-actin from the plasma membrane into the host cell cytoplasm were observed, which the authors hypothesise to aid in correct positioning of the parasite within the host cell (Kuhni-Boghenbor et al., 2012).

Multiple, additional functions of F-actin during intracellular parasite development have recently begun to unfold. Using a conditional mutagenesis approach based on DCre-recombinase it was demonstrated that TgACT1 is essential for maintenance of the apicoplast, an essential apicomplexa-specific chloroplast-like endosymbiotic organelle (Andenmatten et al., 2013; Whitelaw et al., 2017) and maturation of the parasite (Whitelaw et al., 2017). Furthermore, depletion of the unconventional myosin, MyoF, resulted in a similar phenotype, with parasites losing the apicoplast (Heaslip, Nelson, & Warshaw, 2016; Jacot, Daher, & Soldati-Favre, 2013). Importantly, the role of parasite F-actin in apicoplast inheritance appears to be conserved within the phylum, since disruption of pfact1 also resulted in loss of the apicoplast in *P. falciparum* (Das, Lemgruber, Tay, Baum, & Meissner, 2017).

While these studies suggested that parasite actin is involved in critical intracellular functions, the mechanisms involved remained obscure due to the inability of visualising F-actin dynamics in the parasite. Conventional actin probes, such as phalloidin, GFP-tagged actin, LifeAct or SiR-Act did not allow reliable detection of apicomplexan F-actin, either due to toxicity of the probe or their inability to stain apicomplexan F-actin. Recently, a new approach for the imaging of F-actin based on the expression of actin-chromobodies (Cb) was adapted to apicomplexan parasites. These single chain anti-actin camel antibodies (Rocchetti, Hawes, & Kriechbaumer, 2014) show significantly fewer effects on F-actin dynamics compared to other actin probes in all eukaryotes tested to date (Melak, Plessner, & Grosse, 2017; Panza, Maier, Schmees, Rothbauer, & Sollner, 2015). Using this tool it was demonstrated that F-actin is polymerised by formins in *T.gondii* and *P. falciparum* (Stortz et al., 2019; Tosetti et al., 2019) and thereby an extensive intravacuolar network is established that appears to be required for material exchange and cell-to-cell communication between individual parasites. This network is organised by short, bundled F-actin and not by long stable filaments, in good agreement with in vitro studies of apicomplexan actin (Kumpula et al., 2017; Kumpula & Kursula, 2015; Skillman et al., 2011; Skillman et al., 2013). Furthermore, actin nanobodies were tagged with standard fluorescent proteins, photoconvertible proteins mEos3.2 or multifunctional reporters HALO and SNAP enabling their use in diffraction limited microscopy and quantitative super-resolution microscopy methods - dual colour SMLM and live SIM (Periz et al., 2019). In combination with conditional mutants this approach has allowed investigation into the multiple functions of apicomplexan F-actin during the asexual life cycle.

### 2.4 The role of F-Actin during gliding motility and invasion of the host cell

Apicomplexan gliding motility and host cell invasion was believed to be a purely parasite actin-driven process (Dobrowolski & Sibley, 1996). According to the linear motor model (Figure 1b), short
F-actin filaments are polymerised between the plasma membrane (PM) and the inner membrane complex (IMC, a specialised structure found in apicomplexan parasites that consists of membranous cisternae and structural components located 20–30 nm beneath the PM). These short filaments interact with transmembrane proteins derived from secretion of micronemes (invasion related apical organelles) via the glideosome associated connector (GAC) and the myosin A motor complex that is anchored within the IMC (Jacot et al., 2016). Furthermore, it is believed that during gliding motility and invasion, F-actin is formed at the apical tip of the parasite, where formin-1 is localized (Baum et al., 2008; Jacot et al., 2016). While this model has been supported by several lines of evidence, it cannot explain recent findings that used reverse genetic and biophysical approaches to determine force production and transmission during gliding motility and invasion:

- Motility and invasiveness of conditional mutants for core components of the gliding and invasion machinery are affected, but not completely abrogated (Andenmatten et al., 2013; Egarter et al., 2014; Whitelaw et al., 2017)
- Plasmodium sporozoites do not move in a smooth fashion (as predicted by a linear motor), but in a slip–stick-mechanism, where F-actin appears to be required for the formation and release of attachment sites (Münter et al., 2009)
- Retrograde membrane flow, a prerequisite for gliding motility, occurs independent of the acto-myosin-system of the parasite (Gras et al., 2019; Quadt, Streichfuss, Moreau, Spatz, & Frischknecht, 2016; Whitelaw et al., 2017).
- During invasion by Toxoplasma, host cell actin is critical and in case of parasite mutants devoid of the glideosome, a macropinocytosis-like process allows the parasite to invade (Bichet et al., 2016).

While one explanation for these observations could be attributed to functional redundancies of paralogs, in particular in the repertoire of myosins and micronemal proteins (Frenal, Marq, Jacot, Polonais, & Soldati-Favre, 2014), the disruption of core components of this system—including the single copy gene for actin—mainly causes defects in the formation and regulation of attachment sites. The effect of these disruptions on gliding and invasion speed are to a much lesser extent (Whitelaw et al., 2017), raising the question whether the acto-myosin system is required as a molecular clutch in order to initiate motility and to transmit the force generated by retrograde membrane flow or other mechanisms (Bretscher, 2014; Whitelaw et al., 2017). Indeed, a recent study used a combination of traction force microscopy, quantitative RICM (reflection interference contrast microscopy), micro-patterning and expansion microscopy to determine the forces and mechanisms involved in parasite gliding. Together, the data suggest a mechanism, where the MyoA motor directs the traction force, allowing transient energy storage by the subpellicular microtubule cytoskeleton and therefore sets the thrust force required for gliding (Pavlou et al., 2020). Interestingly, using expansion microscopy, it was also demonstrated that MyoA, the central motor of the glideosome is coaligned with subpellicular microtubules, arguing for a direct or indirect connection between the acto-myosin system and subpellicular microtubules. This leads to provocative questions regarding the exact location and orientation of the actomyosin system at the IMC (Tardieux & Baum, 2016).

Current models (Soldati, Foth, & Cowman, 2004; Tardieux & Baum, 2016) suggest that the acto-myosin system is localized between the IMC and the PM of the parasite. This space is very narrow (20–30 nm) and surprisingly electron lucid, indicating a low density of proteins. In contrast, just below the IMC is the so called subpellicular network (SPN), an electron dense network consisting of interwoven 8–10 nm filaments which gives the parasite strength and stability (Mann & Beckers, 2001). The filaments making up this network are named alveolins, a family of intermediate filament-like proteins conserved between all members of the infrakingdom Alveolata (Gould, Tham, Cowman, McFadden, & Waller, 2008). Interestingly, F-actin was demonstrated to be localized just beneath the IMC in early EM studies and more recently actin has been purified from the SPN (Patron et al., 2005; Yasuda, Yagita, Nakamura, & Endo, 1988). In good agreement, analysis of actin dynamics during motility and host cell invasion demonstrated that most of the F-actin formation occurs within the cytosol of the parasite and that filaments are subsequently transported in a retrograde fashion along the surface, probably the IMC (Del Rosario et al., 2019). Furthermore, mutants where the retrograde flow of F-actin is blocked, such as a null mutant for myoA, demonstrated a partial colocalisation of F-actin with the subpellicular microtubules (Del Rosario et al., 2019), leading to the question if the molecular organisation and localisation of the actomyosin system could face towards the cytosol of the parasite, being in direct contact with subpellicular microtubules. In support of this hypothesis, recent studies demonstrate that subpellicular microtubules are connected and stabilised by proteins that are also crucial components of the glideosome, such as GAPM-proteins (Harding et al., 2019) or GAP40 (Harding et al., 2016), indicating that they are linked to the glideosome of the parasite (Harding et al., 2019). Together, these novel findings indicate that the parasite’s F-actin and microtubule system engage in extensive crosstalk to ensure parasite motility and host cell invasion (Figure 1c,d).

Furthermore, analysis of F-actin dynamics during invasion of Toxoplasma and Plasmodium (Del Rosario et al., 2019) revealed a novel role of F-actin during invasion that appears to act in parallel with the glideosome. Apicomplexan parasites establish their own entry portal, a ring-like junction through which they actively invade the host cell (Besteiro, Dubremetz, & Lebrun, 2011; Riglar et al., 2011). During this process the parasite is deformed and a recent study suggests that the host cell exerts counter-pressure on the junction, which can result in abortive invasion events, especially when components of the actomyosin system are disrupted (Bichet et al., 2014; Bichet et al., 2016). This situation is akin to other eukaryotes, where the nucleus represents a major obstacle for the migration through a constricted environment (McGregor, Hsia, & Lammerding, 2016). When F-actin dynamics of invading parasites were analysed, a meshwork surrounding the nucleus could be detected, leading to the hypothesis that F-actin, potentially in concert with the subpellicular microtubules...
facilitates nuclear entry through the junction in a push-and-pull mechanism, as observed for other motile eukaryotic cells when moving through constricted environments (Del Rosario et al., 2019) (Figure 1d). Indeed, an integration of the nucleus with the cytoskeleton is observed in most eukaryotes and this is facilitated by the so-called LINC-complex. However, to date, the components of the LINC complex in apicomplexan parasites (similar to many nuclear envelope proteins) remain unknown (Rout, Obado, Schenkman, & Field, 2017).

On the other hand, the requirement of the actomyosin machinery for invasion into erythrocytes by P. falciparum seems to be absolute. Three different components of the glideosome were conditionally knocked out in three independent studies – namely PfACT1 (Das et al., 2017), PfMyoA (Blake, Haase, & Baum, 2020) and PfGAP45 (Perrin et al., 2018), and in each of these cases, a complete abrogation of invasion was observed. These observations may be explained by the relatively larger size of the nucleus in comparison to the merozoite, differences in shape of the merozoite and tachyzoite, differences in stiffness of the erythrocyte membrane compared to other mammalian cell membranes, or by different requirements of the Plasmodium invasion machinery compared to Toxoplasma.

2.5 | The role of F-Actin during intracellular replication

Visualisation of F-actin in T. gondii and P. falciparum using Cbs demonstrated that individual parasites within the PV are connected via an extensive intravacuolar network that appears to be critical for the organisation of parasites within the PV, for regulation of parasite replication and material exchange between parasites (Periz et al., 2017; Periz et al., 2019; Stortz et al., 2019). In good agreement, conditional mutagenesis of Tgact1 results in asynchronous replication, aberrant parasite organisation within the PV and a blockade in co-ordinated parasite egress (Periz et al., 2017). Furthermore, the intravacuolar network is highly dynamic and its formation and disassembly during egress appears to be tightly regulated, suggesting the presence of unknown regulatory mechanisms (Periz et al., 2017).

2.5.1 | The F-Actin network is connected with the apicoplast

The use of actin-chromobody revealed that individual parasites in Toxoplasma (and Plasmodium with notable variations) are connected by a highly dynamic filamentous actin network. In T. gondii, this network flows through the parasite cytosol and through the posterior end, thereby forming a dynamic network in the parasitophorous vacuole that connects daughter parasites via the residual body. This network is in close association with formin-2 and the apicoplast in both T. gondii and P. falciparum (Stortz et al., 2019), (Figure 1e,f). A deletion of formin-2 resulted in disappearance of the F-actin cytosolic network followed by accumulation of apicoplast(s) within the residual body (T. gondii) or near the food vacuole (P. falciparum), showing that formin-2 acts as a nucleator for the cytosolic actin network and F-actin is essential for apicoplast inheritance to the daughter cells in both apicomplexan genera (Stortz et al., 2019). While formin-2 appears to be the only formin required in formation of the network in the case of P. falciparum, in the case of T. gondii a third formin, formin-3 is also involved in formation and organisation of the network (Tosetti et al., 2019).

Complementary data also supports the role of F-actin in apicoplast inheritance. Perturbation of actin dynamics by depletion of profilin, ADF or overexpression of the formin-2 FH2 domain led to defects in apicoplast inheritance. Finally, a conditional mutant for myoF, the gene encoding the unconventional Myosin F that localizes in proximity to the apicoplast also resulted in loss of the apicoplast and parasite death (Heaslip et al., 2016; Jacot et al., 2013).

2.5.2 | F-Actin network and vesicular transport

A detailed investigation of the functions of the actin network suggests that it contributes to vesicular trafficking between individual parasites and to the recycling of components of the IMC (Frenal et al., 2017; Periz et al., 2017; Periz et al., 2019). The exchange of material is also blocked in cell lines in which expression of myosins associated to actin is abrogated (Frenal, Dubremetz, Lebrun, & Soldati-Favre, 2017). In addition, F-actin dynamics facilitate transport and recycling of the micronemal protein TgMIC2 and the myosin TgMyoA (Periz et al., 2019).

In intracellular Toxoplasma parasites, time-lapse microscopy paired with kymograph-based intensity profiling revealed actin accumulation at apical and basal ends, the cytosolic actin region anterior to the nucleus and the periphery (Stortz et al., 2019). The cytosolic actin centre (cAC) anterior to the nucleus appears to be highly dynamic (Periz et al., 2017; Stortz et al., 2019) and interacts with peripheral actin (Periz et al., 2019, Stortz et al., 2019). Based on kymograph analysis, it was proposed that bi-directional actin flow along the tachyzoite periphery connects the various actin accumulation sites (Stortz et al., 2019). Bi-directional actin flow was also observed in resting extracellular parasites (Del Rosario et al., 2019). Interestingly, this actin flow did not depend on MyoA, a core component of the acto-myosin motor complex (Del Rosario et al., 2019; Frenal, Jacot, et al., 2017). Actin distribution in resting tachyzoites appeared similar to intracellular parasites (Del Rosario et al., 2019).

Multifunctional reporters and single molecule localisation microscopy were used to distinguish, track and quantify the synthesis of vesicles from the mother and the daughter (Periz et al., 2019). The results showed the presence of two populations of vesicles: one of recycled vesicles from the mother which are associated with actin bundles and a second population that is independent of actin bundles and associated to the endomembrane system. In support of these findings, new data demonstrate that vesicular transport, organelle positioning and morphology of the endomembrane compartments are controlled by TgMyoF and connected by filamentous actin (Carmelle, Schiano-Lomoriello, Devarakonda, Kellermeyer, & Heaslip, 2021).
**FIGURE 2** Legend on next page.
Together these results led us to propose that vesicle trafficking occurs via at least two inter-connected mechanisms (Figure 2a,b): one that is actin independent and is possibly associated to the microtubule network and a second mechanism that relies on F-actin in close association with the parasite membrane (Periz et al., 2019), (Figure 2c,d). The F-actin network may facilitate vesicle transport in two distinct ways: material transport can occur on actin tracks in a myosin-dependent manner as described for myosin VI in other eukaryotes (Frank, Noguchi, & Miller, 2004). Second, actin bundles appear to be highly mobile themselves and able to associate transiently to each other and thus, can transport and exchange associated vesicles between two membrane sites. This observation resembles data in transport associated with F-actin polymerisation driven by actin comet like trails, as discussed previously (Khaitlina, 2014).

As circular actin flow was observed within the residual body (RB), the RB was suggested to be a major sorting station for recycling and distribution of material between parasites (Periz et al., 2019). Using SIM, a close proximity between cytosolic F-actin, parasite membrane compartments and microtubules was demonstrated, suggesting a physical linkage between cytoskeletal components regulating cytosolic vesicular transport (Periz et al., 2019). The connection between actin and microtubules has been investigated in higher eukaryotes and it is becoming increasingly clear that a large number of protein complexes including myosins, plus end tracking factor, formins or septins can crosslink actin and microtubules directly or indirectly. This extensive crosslinking of microtubules and actin enables the sharing and co-ordination of material transport depending on these structures (Dogterom & Koenderink, 2019).

2.6 | F-actin in egress from the host cell

The importance of F-actin in egress was shown by conditional gene deletion studies. The results of these experiments show a complete abrogation of egress in *Toxoplasma gondii* but not in *Plasmodium falciparum*. This could be in part due to the fact that osmotic pressure and outward curling of the erythrocyte membrane plays an important role in the explosive dissemination of *P. falciparum* merozoites (Das et al., 2017). Another observation suggests that the actin network is important for preserving the structure of the parasitophorous vacuole and maintaining communication between parasites, so that they can respond in a co-ordinated fashion to external stimuli during egress. This idea is supported by experiments in which the collapse of the actin nanotubular network after treatment with calcium ionophore appears to be a prerequisite for the release of the tachyzoites from the parasitophorous vacuole (Periz et al., 2017).

### 2.7 | F-actin regulation: The role of ABPs in maintaining the Actin network

With the establishment of Cbs in apicomplexans, it is now possible to readdress the role of previously described ABPs and their influence on F-actin polymerisation and dynamics in vivo. Apicomplexan parasites, including *Toxoplasma* and *Plasmodium* species, possess a limited set of ABPs (Baum et al., 2006). For example, *Toxoplasma* encodes a single gene each for ADF and profilin, while three genes encode formins. *Plasmodium falciparum* encodes only two formins and two ADFs (Schuler, Mueller, & Matuschewski, 2005; Baum et al., 2006). In comparison, humans possess 5 profilin genes, 14 ADF/cofilin genes and 16 formin genes (Baum et al., 2006). Most actin nucleation factors, such as *spire* (Baum et al., 2006) or Arp2/3 (Gordon & Sibley, 2005; Baum et al., 2006) are missing in apicomplexan parasites.

Apicomplexan parasites retain most of their cellular actin as monomeric G-actin. While structural features of the molecule contribute to physiologically relevant filament instability in vivo, ABPs such as ADF, profilin, CAP and CPs also play important roles (Figure 1a). TgADF was first described in 1997 as a single copy gene (Allen,
Dobrowolski, Muller, Sibley, & Mansour, 1997). Recombinant TgADF is capable of binding to G-actin and of depolymerising F-actin in vitro (Allen et al., 1997). In vivo, a cytosolic localisation was reported for TgADF by antibody staining and endogenous tagging (Allen et al., 1997; Haase et al., 2015; Mehta & Sibley, 2011). Depletion of TgADF resulted in accumulation of actin structures and compromised host cell invasion, egress and overall gliding motility, making TgADF essential for the lytic replication cycle (Mehta & Sibley, 2011; Periz et al., 2017). Plasmodium falciparum has two adf genes, of which only ADF1 is essential and expressed throughout all life-cycle stages. Differing structurally from mammalian ADF/Cofilins, PIADF1 was shown to be unable to bind to F-actin in vitro, but functioned in sequestering G-actin, and surprisingly similar to mammalian profilins, promoted nucleotide exchange on monomers (Schuler et al., 2005). In a later report, PIADF1 demonstrated F-actin severing activity without stable binding, but via a low-affinity binding interface (Wong et al., 2014).

In divergence from classical profilins, apicomplexan profilins contain an additional β-hairpin loop, which is critically required for actin monomer binding, and a single point mutation in this loop region eliminates a hydrogen-bond, thereby abrogating fast motility seen in Plasmodium berghei sporozoites (Moreau et al., 2017). Recently, the same group produced mutations in another acidic loop in profilin, which did not affect actin polymerisation in vitro and yet affected gliding motility of Plasmodium berghei sporozoites, indicating that additional factors beyond actin polymerisation are at play during motility and invasion of apicomplexan parasites (Moreau et al., 2020). TgProfilin is critical for the completion of the lytic life cycle as depletion of TgProfilin rendered parasites defective in gliding motility, invasion and host cell egress (Plattner et al., 2008). Intracellular replication was not affected by TgProfilin loss. In a comparative study of yeast, mouse and Plasmodium falciparum cyclase associated protein (CAP), a common conserved mechanism of nucleotide exchange on G-actin-ADP monomers via a β-sheet domain was found. In comparison to PfCAP, higher CAPs contained additional domains which might have later evolved for more complex dynamics (Makkonen, Bertling, Chebotareva, Baum, & Lappalainen, 2013). Toxoplasma tachyzoites lacking CAP exhibited impaired motility, invasion and egress (Hunt et al., 2019). TgCAP also appears to play a role in dense granule trafficking. Depletion of TgCAP affects communication between parasites as TgCAP-KO tachyzoites lose their ability of rosetting and rapidly transferring proteins between distant parasites. Nevertheless, TgCAP-KO tachyzoites remained capable of mostly replicating synchronously. TgCAP was also reported to be critical for virulence in Toxoplasma type II strains (Hunt et al., 2019). In a departure from mammalian CPs, which function as a heterodimer, Plasmodium CPα subunit can function alone, likely as a homodimer, and CPβ can be ablated during the asexual life cycle in Plasmodium berghei parasites (Ganter, Rizopoulos, Schuler, & Matuschewski, 2015).

Formins are the only known actin nucleators in Toxoplasma and Plasmodium (Baum et al., 2006), (Figure 1e,f). TgFormin1 and 2 were initially localized to the tachyzoite pellicle (Daher, Plattner, Carlier, & Soldati-Favre, 2012). Initially, TgFormin3 was localized to the apical and the basal pole as well as around the mitochondrion (Daher et al., 2010). Instead, TgFormin1 is involved in tachyzoite motility and host cell invasion. A recent publication confirmed the importance of TgFormin1 in gliding and invasion, but also indicated a critical role in parasite egress (Daher et al., 2010). TgFormin2 was initially reported to have a diffuse cytoplasmic distribution in trophozoites (Baum et al., 2008). A comparative study of Formin-2 function in Toxoplasma and P. falciparum, revealed a localisation at the apicoplast vicinity in both genera (Stortz et al., 2019). Plasmodium falciparum parasites lacking functional Formin-2 showed a complete abrogation of detectable F-actin within growing and replicating asexual Plasmodium stages, making it the primary actin nucleator during asexual growth stages (Stortz et al., 2019).

TgFormin3-KO tachyzoites do not display any replication defects, making TgFormin3 dispensable for the lytic cycle (Daher, Klages, Carlier, & Soldati-Favre, 2012). Initially, TgFormin3 was localized to the apical and the basal pole as well as around the mitochondrion (Daher et al., 2012). In a later study TgFormin3 was localized to the apical and the residual body based on endogenous tagging (Daher et al., 2012). Asynchronous replication was observed in parasites lacking TgFormin3 (Tosetti et al., 2019). In addition, recovery of fluorescence in photobleaching experiments was slower for TgFormin3-KO parasites when compared to wild-type parasites. Tosetti and colleagues therefore suggested a role for TgFormin3 in cell–cell communication and organisation of the intravacuolar network.

### 2.7.1 The state of actin research in apicomplexans and future challenges

With the obstacle of actin visualisation overcome in Toxoplasma and Plasmodium (Periz et al., 2017; Stortz et al., 2019) we now have the chance of investigating the in vivo functions of actin and ABPs. Use of Cbs will improve our knowledge of actin during the entire life cycle of the parasite. The use of recently introduced probes, such as photoconvertible proteins for tracking subpopulations and quantitative analysis of protein interactions based on single molecule localisation microscopy (Periz et al., 2019) will be immensely useful to understand the diverse functions of F-actin in the parasite.
Together this will allow us to further understand actin dynamics and its regulation in apicomplexan parasites. As a starting point, we are proposing an actin treadmilling model that combines in vitro and in vivo data obtained from Toxoplasma and Plasmodium (Figure 1a). In this model, ADF acts to sever and depolymerise F-actin which re-introduces G-actin-ADP into the cytoplasmic G-actin pool. While Profilin sequesters G-actin, CAP proteins mediate the exchange of ADP to ATP. Formin nucleation factors can then enhance actin polymerisation, resulting in the quick assembly of F-actin at various polymerisation centres, akin to other eukaryotes. More stable actin structures such as the filamentous structures connecting individual parasites within the PV might be less affected by impaired actin treadmilling. Further in vivo research is needed to elucidate the precise functions of Profilin, ADF, CAP and CPs. In the future, the chromobody technology will be immensely useful to uncover novel ABPs and to further unravel the nature and function of the apicomplexan actin network.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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REFERENCES

Allen, M. L., Dobrowolski, J. M., Muller, H., Sibley, L. D., & Mansour, T. E. (1997). Cloning and characterization of Actin depolymerizing factor from toxoplasma gondii. Molecular and Biochemical Parasitology, 88(1–2), 43–52.
Andenmatten, N., Egarter, S., Jackson, A. J., Jullien, N., Herman, J. P., & Meissner, M. (2013). Conditional genome engineering in toxoplasma gondii uncovers alternative invasion mechanisms. Nature Methods, 10(2), 125–127.
Baum, J., Papenfuss, A. T., Baum, B., Speed, T. P., & Cowman, A. F. (2006). Regulation of apicomplexan Actin-based motility. Nature Reviews. Microbiology, 4(8), 621–628.
Baum, J., Tonkin, C. J., Paul, A. S., Rug, M., Smith, B. J., Gould, S. B., ... Cowman, A. F. (2008). A malaria parasite formin regulates Actin polymerization and localizes to the parasite-erythrocyte moving junction during invasion. Cell Host & Microbe, 3(3), 188–198.
Besteiro, S., Dubremetz, J. F., & Lebrun, M. (2011). The moving junction of apicomplexan parasites: a key structure for invasion. Cellular Microbiology, 13(6), 797–805.
Bichet, M., Joly, C., Henni, A. H., Guibert, T., Xemard, M., Tafani, V., ... Tardieux, I. (2014). The toxoplasma-host cell junction is anchored to the cell cortex to sustain parasite invasive force. BMC Biology, 12, 773.
Bichet, M., Touquet, B., Gonzalez, V., Florent, I., Meissner, M., & Tardieux, I. (2016). Genetic impairment of parasite myosin motors uncovers the contribution of host cell membrane dynamics to toxoplasma invasion forces. BMC Biology, 14(1), 97.
Blake, T. C. A., Haase, S., & Baum, J. (2020). Actomyosin forces and the energetics of red blood cell invasion by the malaria parasite Plasmodium falciparum. PLoS Pathogens, 16(10), e1009007.
Bretscher, M. S. (2014). Asymmetry of single cells and where that leads. Annual Review of Biochemistry, 83, 275–289.
Carlsson, L., Nystrom, L. E., Sundkvist, I., Markey, F., & Lindberg, U. (1977). Actin polymerizability is influenced by profilin, a low molecular weight protein in non-muscle cells. Journal of Molecular Biology, 115(3), 465–483.
Carmelle, R., Sciano Lomoriello, P., Devarakonda, P. M., Kellermann, J. A., & Heaslip, A. T. (2021). Actin and an unconventional myosin motor, TgMyoF, control the organization and dynamics of the endomembrane network in toxoplasma gondii. PLoS Pathogens, 17(2), e1008787.
Chhabra, E. S., & Higgs, H. N. (2007). The many faces of actin: Matching assembly factors with cellular structures. Nature Cell Biology, 9(10), 1110–1121.
Cooke, R. (1975). The role of the bound nucleotide in the polymerization of actin. Biochemistry, 14(14), 3250–3256.
Cooper, J. A., & Sept, D. (2008). New insights into mechanism and regulation of actin capping protein. International Review of Cell and Molecular Biology, 267, 183–206.
Courtemanche, N. (2018). Mechanisms of formin-mediated actin assembly and dynamics. Biophysical Reviews, 10(6), 1553–1569.
Daher, W., Klages, N., Carlier, M. F., & Soldati-Favre, D. (2012). Molecular characterization of toxoplasma gondii formin 3, an actin nucleator dispensable for tachyzoite growth and motility. Eukaryotic Cell, 11(3), 243–252.
Daher, W., Plattner, F., Carlier, M. F., & Soldati-Favre, D. (2010). Concerted action of two formins in gliding motility and host cell invasion by toxoplasma gondii. PLoS Pathogens, 6(10), e1001132.
Das, S., Lemgruber, L., Tay, C. L., Baum, J., & Meissner, M. (2017). Multiple essential functions of Plasmodium falciparum actin-1 during malaria blood-stage development. BMC Biology, 15(1), 70.
Del Rosario, M., Periz, J., Pavlou, G., Lyth, O., Latorre-Barragan, F., Das, S., ... Meissner, M. (2019). Apicomplexan F-actin is required for efficient nuclear entry during host cell invasion. EMBO Reports, 20(12), e48896.
Dobrowolski, J. M., Niesman, I. R., & Sibley, L. D. (1997). Actin in the parasite toxoplasma gondii is encoded by a single copy gene, ACT1 and exists primarily in a globular form. Cell Motility and the Cytoskeleton, 37(3), 253–262.
Dobrowolski, J. M., & Sibley, L. D. (1996). Toxoplasma invasion of mammalian cells is powered by the actin cytoskeleton of the parasite. Cell, 84(6), 933–939.
Dogterom, M., & Koenderink, G. H. (2019). Actin-microtubule crosstalk in cell biology. Nature Reviews. Molecular Cell Biology, 20(1), 38–54.
Dominguez, R., & Holmes, K. C. (2011). Actin structure and function. Annual Review of Biophysics, 40, 169–186.
Douglas, R. G., Nandekar, P., Aktories, J. E., Kumar, H., Weber, R., Sattler, J. M., ... Frischknecht, F. (2018). Inter-subunit interactions drive divergent dynamics in mammalian and plasmodium actin filaments. PLoS Biology, 16(7), e2005345.
Edwards, M., Zwolak, A., Schafer, D. A., Sept, D., Dominguez, R., & Cooper, J. A. (2014). Capping protein regulators fine-tune actin assembly dynamics. Nature Reviews. Molecular Cell Biology, 15(10), 677–689.

Egarter, S., Andermatten, N., Jackson, A. J., Whitelaw, J. A., Pall, G., Black, J. A., & Meissner, M. (2014). The toxoplasma Acto-MyoA motor complex is important but not essential for gliding motility and host cell invasion. PLoS One, 9(3), e91819.

Frank, D. J., Noguchi, T., & Miller, K. G. (2004). Myosin VI: A structural role in actin organization important for protein and organelle localization and trafficking. Current Opinion in Cell Biology, 16(2), 189–194.

Frenal, K., Dubremetz, J. F., Lebrun, M., & Soldati-Favre, D. (2017). Gliding motility powers invasion and egress in Apicomplexa. Nature Reviews. Microbiology, 15(11), 643–660.

Frenal, K., Jacot, D., Hammoudi, P. M., Graindorge, A., Maco, B., & Soldati-Favre, D. (2017). Toxoplasma gondii myosin F, an essential motor for centrosomes positioning and apicoplast inheritance. The EMBO Journal, 32(12), 1702–1716.

Frenal, K., Tosetti, N., Pires, I., Stock, J., Grindorge, A., Hung, Y. F., & Soldati-Favre, D. (2016). An apicomplexan actin-binding protein serves as a connector and lipid sensor to coordinate motility and invasion. Cell Host & Microbe, 20(6), 731–743.

Kabsch, W., Mannherz, H. G., Suck, D., Pai, E. F., & Holmes, K. C. (1990). Atomic structure of the actin:DNase I complex. Nature, 347(6288), 37–44.

Khaitлина, S. Y. (2014). Intracellular transport based on actin polymerization. Biochemistry (Moscow), 79(9), 917–927.

Korn, E. D., Carlier, M. F., & Pantaloni, D. (1987). Allosteric regulation of actin binding proteins. Science, 238(4827), 638–644.

Kotla, T., Wioland, H., Enkavi, G., Kogan, K., Vattulainen, I., Jegou, A., & Lappalainen, P. (2019). Mechanism of synergistic actin filament pointed end depolymerization by cyclase-associated protein and coflin. Nature Communications, 10(1), 5320.

Kovar, D. R. (2006). Molecular details of formin-mediated actin assembly. Current Opinion in Cell Biology, 18(1), 11–17.

Kuhn-Boghenbor, K., Ma, M., Lembgruber, L., Cyrlkafl, M., Frischknecht, F., Gaschen, V., & Baumgartner, M. (2012). Actin-mediated plasma membrane plasticity of the intracellular parasite Theileria annulata. Cellular Microbiology, 14(12), 1867–1879.

Kumpula, E. P., & Kursla, I. (2015). Towards a molecular understanding of the apicomplexan actin motor: On a road to novel targets for malaria remedies? Acta Crystallographica Section F: Structural Biology Communications, 71(Pt 5), 500–513.

Kumpula, E. P., Lopez, A. J., Tajedin, L., Han, H., & Kursula, I. (2019). Atomic view into plasmodium actin polymerization, ATP hydrolysis, and fragmentation. PLoS Biology, 17(6), e3000315.

Kumpula, E. P., Pires, I., Lasiwa, D., Pitrainen, H., Bergmann, U., Vahokoski, J., & Kursula, I. (2017). Apicomplexan actin polymerization depends on nucleation. Scientific Reports, 7(1), 12137.

Lappalainen, P., & Drubin, D. G. (1997). Cofilin promotes rapid actin filament turnover in vivo. Nature, 388(6637), 78–82.

Lu, H., Fagnant, P. M., & Trybus, K. M. (2019). Unusual dynamics of the divergent malaria parasite PlAAct1 actin filament. Proceedings of the National Academy of Sciences of the United States of America, 116(41), 20418–20427.

Makkonen, M., Bertling, E., Chebotareva, N. A., Baum, J., & Lappalainen, P. (2013). Mammalian and malaria parasite cyclase-associated proteins catalyze nucleotide exchange on G-actin through a conserved mechanism. The Journal of Biological Chemistry, 288(2), 984–994.

Mann, T., & Beckers, C. (2001). Characterization of the subpellicular network, a filamentous membrane skeletal component in the parasite toxoplasma gondii. Molecular and Biochemical Parasitology, 115(2), 257–268.

McGregor, A. L., Hsia, C. R., & Lammerding, J. (2016). Squish and squeeze: the nucleus as a physical barrier during migration in confined environments. Current Opinion in Cell Biology, 40, 32–40.

Mehta, S., & Sibley, L. D. (2011). Actin depolymerizing factor controls actin turnover and gliding motility in toxoplasma gondii. Molecular Biology of the Cell, 22(8), 1290–1299.
Pollard, T. D., Blanchoin, L., & Mullins, R. D. (2000). Molecular mechanisms controlling actin filament dynamics in nonmuscle cells. *Annual Review of Biophysics and Biomolecular Structure*, 29, 545–576.

Pollard, T. D., & Borisy, G. G. (2003). Cellular motility driven by assembly and disassembly of actin filaments. *Cell*, 112(4), 453–465.

Pospelovich, S., Kumpula, E. P., von der Ecken, J., Vahokoski, J., Kursula, I., & Raunser, S. (2017). Near-atomic structure of jasplakinolide-stabilized malaria parasite F-actin reveals the structural basis of filament instability. *Proceedings of the National Academy of Sciences of the United States of America*, 114(40), 10636–10641.

Quadt, K. A., Streichfuss, M., Moreau, C. A., Spatz, J. P., & Frischknecht, F. (2016). Coupling of retrograde flow to force production during malaria parasite migration. *ACS Nano*, 10(2), 2091–2102.

Quinlan, M. E., Heuser, J. E., Kerkhoff, E., & Mullins, R. D. (2005). Drosophila spire is an actin nucleation factor. *Nature*, 433(7024), 382–388.

Riglar, D. T., Richard, D., Wilson, D. W., Boyle, M. J., Dekiwiada, C., Turnbull, L., ... Baum, J. (2011). Super-resolution dissection of coordinated events during malaria parasite invasion of the human erythrocyte. *Cell Host & Microbe*, 9(1), 9–20.

Rocchetti, A., Hawes, C., & Kriechbaum, V. (2014). Fluorescent labelling of the actin cytoskeleton in plants using a camelid antibody. *Plant Methods*, 10, 12.

Romero, S., Le Clainche, C., Didry, D., Egile, C., Pantaloni, D., ... Carlier, M. F. (2004). Formin is a processive motor that requires profilin to accelerate actin assembly and associated ATP hydrolysis. *Cell*, 119(3), 419–429.

Rout, M. P., Obado, S. O., Schenkmans, S., & Field, M. C. (2017). Specialising the parasite nucleus: Pores, lamins, chromatin, and diversity. *PloS Pathogens*, 13(3), e1006170.

Sahoo, N., Beatty, W., Heuser, J., Sept, D., & Sibley, L. D. (2006). Unusual kinetic and structural properties control rapid assembly and turnover of actin in the parasite Toxoplasma gondii. *Molecular Biology of the Cell*, 17(2), 895–906.

Sattler, J. M., Granter, M., Hliass, M., Matuschewski, K., & Schuler, H. (2011). Actin regulation in the malaria parasite. *European Journal of Cell Biology*, 90(11), 966–971.

Schmitz, S., Grainger, M., Howell, S., Calder, L. J., Gaeb, M., Pinder, J. C., ... Veigel, C. (2005). Malaria parasite actin filaments are very short. *Journal of Molecular Biology*, 349(1), 113–125.

Schuler, H., & Matuschewski, K. (2006). Plasmodium motility: Actin not actin’ like actin. *Trends in Parasitology*, 22(4), 146–147.

Schuler, H., Mueller, A. K., & Matuschewski, K. (2005). A plasmodium actin-depolymerizing factor that binds exclusively to actin monomers. *Molecular Biology of the Cell*, 16(9), 4013–4023.

Selden, L. A., Kinosian, H. J., Estes, J. E., & Gershman, L. C. (1999). Impact of profilin on actin-bound nucleotide exchange and actin polymerization dynamics. *Biochemistry*, 38(9), 2769–2778.

Sept, D., & McCammon, J. A. (2001). Thermodynamics and kinetics of actin filament nucleation. *Biophysical Journal*, 81(2), 667–674.

Shaw, M. K., He, C. Y., Roos, D. S., & Tilney, L. G. (2000). Proteasome inhibitors block intracellular growth and replication of toxoplasma gondii. *Parasitology*, 121(Pt 1), 35–47.

Skillman, K. M., Diraviyam, K., Khan, A., Tang, K., Sept, D., & Sibley, L. D. (2011). Evolutionarily divergent, unstable filamentous actin is essential for gliding motility in apicomplexan parasites. *PloS Pathogens*, 7(10), e1002280.

Skillman, K. M., Ma, C. L., Fremont, D. H., Diraviyam, K., Cooper, J. A., Sept, D., & Sibley, L. D. (2013). The unusual dynamics of parasite actin result from isosomeric polymerization. *Nature Communications*, 4, 2285.

Smythe, W. A., Joiner, K. A., & Hoppe, H. C. (2008). Actin is required for endocytic trafficking in the malaria parasite *Plasmodium falciparum*. *Cellular Microbiology*, 10(2), 452–464.

Soldati, D., Foth, B. J., & Cowman, A. F. (2004). Molecular and functional aspects of parasite invasion. *Trends in Parasitology*, 20(12), 567–574.
Stortz, J. F., Del Rosario, M., Singer, M., Wilkes, J. M., Meissner, M., & Das, S. (2019). Formin-2 drives polymerisation of actin filaments enabling segregation of apicoplasts and cytokinesis in Plasmodium falciparum. *eLife, 8*, e49030.

Suraneni, P., Rubinstein, B., Unruh, J. R., Durnin, M., Hanein, D., & Li, R. (2012). The Arp2/3 complex is required for lamellipodia extension and directional fibroblast cell migration. *The Journal of Cell Biology, 197*(2), 239–251.

Tardieux, I., & Baum, J. (2016). Reassessing the mechanics of parasite motility and host-cell invasion. *The Journal of Cell Biology, 214*(5), 507–515.

Tosetti, N., Dos Santos Pacheco, N., Soldati-Favre, D., & Jacot, D. (2019). Three F-actin assembly centers regulate organelle inheritance, cell-cell communication and motility in toxoplasma gondii. *eLife, 8*, e42669.

Vahokoski, J., Bhargav, S. P., Desfosses, A., Andreadaki, M., Kumpula, E. P., Martinez, S. M., ... Kursula, I. (2014). Structural differences explain diverse functions of plasmodium actins. *PLoS Pathogens, 10*(4), e1004091.

von der Ecken, J., Heissler, S. M., Pathan-Chhatbar, S., Manstein, D. J., & Raunser, S. (2016). Cryo-EM structure of a human cytoplasmic actomyosin complex at near-atomic resolution. *Nature, 534*(7609), 724–728.

Wesseling, J. G., Smits, M. A., & Schoenmakers, J. G. (1988). Extremely diverged actin proteins in *Plasmodium falciparum*. *Molecular and Biochemical Parasitology, 30*(2), 143–153.

Wesseling, J. G., Snijders, P. J., van Someren, P., Jansen, J., Smits, M. A., & Schoenmakers, J. G. (1989). Stage-specific expression and genomic organization of the actin genes of the malaria parasite *Plasmodium falciparum*. *Molecular and Biochemical Parasitology, 35*(2), 167–176.

Whitelaw, J. A., Latorre-Barragan, F., Gras, S., Pall, G. S., Leung, J. M., Heaslip, A., ... Meissner, M. (2017). Surface attachment, promoted by the actomyosin system of toxoplasma gondii is important for efficient gliding motility and invasion. *BMC Biology, 15*(1), 1.

Wong, W., Webb, A. I., Olshina, M. A., Infusini, G., Tan, Y. H., Hanssen, E., ... Baum, J. (2014). A mechanism for actin filament severing by malaria parasite actin depolymerizing factor 1 via a low affinity binding interface. *The Journal of Biological Chemistry, 289*(7), 4043–4054.

Yasuda, T., Yagita, K., Nakamura, T., & Endo, T. (1988). Immunocytochemical localization of actin in toxoplasma gondii. *Parasitology Research, 75*(2), 107–113.

Yonezawa, N., Nishida, E., & Sakai, H. (1985). pH control of actin polymerization by cofilin. *The Journal of Biological Chemistry, 260*(27), 14410–14412.

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