Apicomplexans pulling the strings: manipulation of the host cell cytoskeleton dynamics

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SUMMARY

Invasive stages of apicomplexan parasites require a host cell to survive, proliferate and advance to the next life cycle stage. Once invasion is achieved, apicomplexans interact closely with the host cell cytoskeleton, but in many cases the different species have evolved distinct mechanisms and pathways to modulate the structural organization of cytoskeletal filaments. The host cell cytoskeleton is a complex network, largely, but not exclusively, composed of microtubules, actin microfilaments and intermediate filaments, all of which are modulated by associated proteins, and it is involved in diverse functions including maintenance of cell morphology and mechanical support, migration, signal transduction, nutrient uptake, membrane and organelle trafficking and cell division. The ability of apicomplexans to modulate the cytoskeleton to their own advantage is clearly beneficial. We here review different aspects of the interactions of apicomplexans with the three main cytoskeletal filament types, provide information on the currently known parasite effector proteins and respective host cell targets involved, and how these interactions modulate the host cell physiology. Some of these findings could provide novel targets that could be exploited for the development of preventive and/or therapeutic strategies.

Key words: Apicomplexa, host–parasite interaction, actin, microtubules, intermediate filaments.

INTRODUCTION

Once intracellular pathogens have invaded their host cell, they explore and/or modulate its intracellular organization at different levels. This includes gene expression patterns, defined structures and compartments, the host cell metabolism and also signalling transduction pathways. Bacteria, viruses and parasites have evolved common strategies to modulate the living conditions within their host cell according to their needs, in order to optimize survival, replication and finally dissemination. During these processes pathogens not only induce remodelling of the cytoskeleton, but they also modify diverse pathways and key components involved in regulation of the host cell cytoskeleton dynamics (Haglund and Welch, 2011).

The cytoskeleton is a complex network of different polymers involved in a plethora of cellular functions, including the maintenance of cell shape, the organization and positioning of intracellular organelles, the establishment of a physical structure for intracellular cargo transportation, cell division and motility. To perform these diverse activities, the dynamic elements of the cytoskeleton interact with several distinct proteins, ranging from motor proteins to proteins involved in regulation of polymer length, stability and organization. In turn this enables the cell to respond to environmental challenges, including those involved in cell–cell contact, cell–matrix interactions and cell polarization and migration. By taking over certain aspects of host cell cytoskeleton organization, pathogens can exploit host cell resources, while simultaneously evading intracellular defence mechanisms, such as avoiding the destruction by lysosomes following phagocytosis by manipulating the actin cytoskeleton (Lang *et al.* 2010).

Recent studies revealed how bacteria and viruses manipulate the host cell cytoskeleton, and many molecular mechanisms subjacent to cytoskeleton organization and function were discovered (Lai *et al.* 2014; Fernandez *et al.* 2015; Foo and Chee, 2015; Sana *et al.* 2015). However, the knowledge on how protozoan parasites, such as those of the phylum Apicomplexa, explore the host cell cytoskeleton is less well established.

Apicomplexan parasites grow and replicate inside a host cell, the majority of them forming a parasitophorous vacuole (PV). They are the aetiological agents of several human and animal diseases, such

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as malaria (*Plasmodium* spp.), toxoplasmosis (*Toxoplasma gondii*), besnoitiosis (*Besnoitia* spp.), neosporosis (*Neospora caninum*) cryptosporidiosis (*Cryptosporidium* spp.), theileriosis (*Theileria* spp.) or babesiosis (*Babesia* spp.), among others. Being intracellular parasites they have been observed to modulate several aspects of the host cell. This includes repositioning of host cell organelles [centrosome, mitochondria, endoplasmic reticulum, Golgi complex (Coppens *et al*. 2006; Wang *et al*. 2010; Romano *et al*. 2013; Cardoso *et al*. 2014; Pernas *et al*. 2014; Nolan *et al*. 2015)], scavenging of host cell nutrients (Blader and Saeij, 2009), modulate the host cell cycle (Dobbelaere and Kuenzi, 2004), they interfere with signalling pathways (Dobbelaere and Kuenzi, 2004; Yang *et al*. 2004), and influence host cell migration (Lambert *et al*. 2010; Collantes-Fernandez *et al*. 2012).

In this review we focus on how apicomplexan parasites act upon the three main filament types that constitute the cytoskeleton of their host cell, namely intermediate filaments, actin filaments (F-actin) and microtubules. While intermediate filaments provide mechanical strength to the cell and many organelles are physically linked to the network, actin filaments determine the shape of the cell surface, provide its stability and mediate cell motility. Microtubules are structures that are involved in many crucial cellular functions such as cell division, motility, intracellular transport and various cell signalling pathways (Gundersen and Cook, 1999; Pollard, 2003). We review the current knowledge on how these parasites interact with each of these cytoskeletal components during host cell invasion and parasite replication, and how this modulation could be advantageous for parasite survival, replication and/or development.

INTERACTIONS OF APICOMPLEXAN PARASITES WITH HOST CELL MICROTUBULES

Several studies (see Table 1 for an overview) have described the remodelling of the host cell microtubule cytoskeleton during invasion by apicomplexan parasites (Coppens *et al*. 2006; Hermosilla *et al*. 2008; Walker *et al*. 2008; Romano *et al*. 2013; Woods *et al*. 2013; Cardoso *et al*. 2014; Nolan *et al*. 2015). As such, modulation of the microtubule network by *T. gondii* and *B. besnoiti* occurs early during invasion and extends to the formation and establishment of the PV (Sehgal *et al*. 2005; Coppens *et al*. 2006; Walker *et al*. 2008; Romano *et al*. 2013; Cardoso *et al*. 2014). Reis *et al*. (2006) demonstrated that upon *B. besnoiti* host cell entry, microtubules start to surround the parasite upon the first minutes of invasion forming a cone shape microtubule network; and a microtubule ring on the host cell is observed around the parasite entry site, probably corresponding to the moving junction (MJ) – a structure mediating a tight connection between the invading parasite and the host plasma membrane (reviewed in Tyler *et al*. 2011). In *T. gondii*, and most likely also in other Apicomplexa, the MJ is assembled by a macromolecular complex comprising several rhoptry neck proteins (RON2, RON4, RON5, RON8) and AMA1 (micronemal apical membrane antigen 1) (Alexander *et al*. 2005; Lebrun *et al*. 2005; Besteiro *et al*. 2009; Straub *et al*. 2009). Other studies also showed that host cell microtubules surrounded the PV of *T. gondii* and *B. besnoiti* already after 15 min post invasion (Walker *et al*. 2008; Cardoso *et al*. 2014). Indeed, the presence of microtubule filaments at or near the MJ prior to invasion has been shown to increase the speed at which *T. gondii* starts to initiate the host cell invasion process after adhering to the host cell surface (Sweeney *et al*. 2010). On the other hand, host cell microtubule depolymerization effectively delayed parasite invasion, which could be explained by the fact that microtubules mediate the delivery of the RON complex to the plasma membrane at the site of the MJ (Sweeney *et al*. 2010). This hypothesis is supported by the observation that host cell β-tubulin binds to TgRON4, and that the C-terminal domain of β-tubulin interacts with TgRON4 during invasion (Takemae *et al*. 2013). However, not all apicomplexans require the presence of a MJ during invasion: *Theileria* sporozoites enter their host cells through other mechanisms, which is morphologically characterized as a 'self-zippering process' between parasite and host cell surface membrane, also resulting in intracellular parasites surrounded by a parasitophorous vacuole membrane (PVM). However, in the case of *Theileria*, this membrane is rapidly degraded, and parasites reside free in the cytoplasm. In addition, no remodelling of the microtubule filament network has been described on the surface of host cells during *Theileria* invasion (reviewed in Shaw, 2003).

Upon the initiation of intracellular replication, *T. gondii* and *B. besnoiti* exhibit distinct differences. In cells infected with replicating *B. besnoiti* tachyzoites, the microtubules present a complex arrangement around the PV, creating a clearly visible alveolus-like structure (Fig. 1A). In contrast, microtubules in *T. gondii* infected host cells exhibit a basket-like structure (Fig. 1B), which is distinct from the alveolar arrangement found in *B. besnoiti* infected cells (Coppens *et al*. 2006; Reis *et al*. 2006; Walker *et al*. 2008; Cardoso *et al*. 2014). In a recent study that compared microtubule interactions in *N. caninum* and *T. gondii* infected cells, microtubules were also closely apposed to *N. caninum* containing PVs, but at a statistically significant lower density compared with what was observed in *T. gondii* infected cells (Nolan *et al*. 2015). Overall, this suggests that throughout invasion, the PVs of these three

Table 1. Rearrangement of host microtubules and parasite and host molecules that interact with the host microtubule cytoskeleton during invasion and replication

Species	Rearrangement of host microtubules	Parasite molecules associated with tubulin/microtubules	Host molecules associated with tubulin/microtubules
Besnoitia besnoiti	Organization of a microtubule cone shape structure at the MJ (Reis *et al*. 2006; Cardoso *et al*. 2014). Host microtubules create an alveolus-like structure around the PV clearly seen at 24 h post invasion; no recruitment of the host cell centrosome (Cardoso *et al*. 2014)	?	?
Toxoplasma gondii	Microtubules recruited to the MJ (Sweeney *et al*. 2010). Host microtubules form a basket-like structure around the PV; recruitment of the host cell centrosome (Coppens *et al*. 2006; Walker *et al*. 2008; Cardoso *et al*. 2014). Invaginations of the PVM induced by host microtubules into the lumen of the PV: host organelle sequestering tubulo structures (H.O.S.T.) (Coppens *et al*. 2006)	TgRON4 interacts with β-tubulin (Takemae *et al*. 2013)	Infection of primary fibroblasts results in stable activation of the host Ser/Thr kinase Akt Ser/Thr kinase Akt (Wang *et al*. 2010)
Neospora caninum	Host microtubules closely apposed to the PVM (Nolan *et al*. 2015). Presence of an analogue of the H.O.S.T. system with proteins originating from the dense granule organelles (Hemphill *et al*. 2004). Recruitment of the host cell centrosome (Nolan *et al*. 2015)	?	?
Plasmodium	Presence of an analogue of the H.O.S.T. system in infected erythrocytes (Haldar *et al*. 2001)	?	?
Eimeria bovis	Microtubule remodelling of the host cell around the PV within 3 days post-infection (Hermosilla *et al*. 2008)	?	TUBB, TUBB4, TUBB6, TPPP, DOCK7, CKAP4, and DCT3 are enhanced specially (Taubert *et al*. 2010; Hermosilla *et al*. 2012)
Theileria	Microtubules facilitate the movement of the parasite to the perinuclear region (Frénal and Soldati-Favre, 2009). Tight association of the schizont and the host cell microtubules of the mitotic apparatus (Dobbelaere and Kuenzi, 2004)	TaSE (Schneider *et al*. 2007). TaSP (Seitzer *et al*. 2010). p104-functioning as an EB1-binding protein (Woods *et al*. 2013)	Plk1: association of the parasite with central spindles (von Schubert *et al*. 2010)

EB1, end-binding protein 1; MJ, moving junction; PV, parasitophorous vacuole; PVM, parasitophorous vacuole membrane; (?), not known.

apicomplexan parasites interact with the host microtubule cytoskeleton, but they promote distinct arrangements of these polymers.

Walker *et al*. (2008) observed that in macrophages actively invaded by *T. gondii* tachyzoites the host microtubule rearranged into ordered circular arrays, while this was not the case during phagocytosis of heat-killed tachyzoites. This suggested that these rearrangements require an active parasite intervention, and this raises the possibility that the delivery of parasite-secreted proteins into the host cell could be crucial for the microtubule rearrangement. Secretory proteins have been shown to mediate other intracellular interactions: for instance mitochondrial association factor 1 is a *T. gondii* protein that mediates the association of the PVM with host cell mitochondria (Pernas *et al*. 2014), and ER–PV interactions are mediated by *T. gondii* dense granule protein 3 (GRA3), which binds to calcium modulating ligand, an ER-anchoring host cell protein (Kim *et al*. 2008). Thus, the importance of host cell microtubules during PV establishment could serve to position replicating parasites near to important host cell organelles such as the nucleus and centrosome (Coppens *et al*. 2006; Wang *et al*. 2010; Cardoso *et al*. 2014), the host endoplasmic reticulum (Coppens *et al*. 2006), mitochondria (Sinai *et al*. 1997; Pernas *et al*. 2014) and the Golgi complex (Coppens *et al*. 2006; Wang *et al*. 2010; Cardoso *et al*. 2014). This could probably facilitate the acquisition of several important molecules and nutrients, but could also facilitate the delivery of

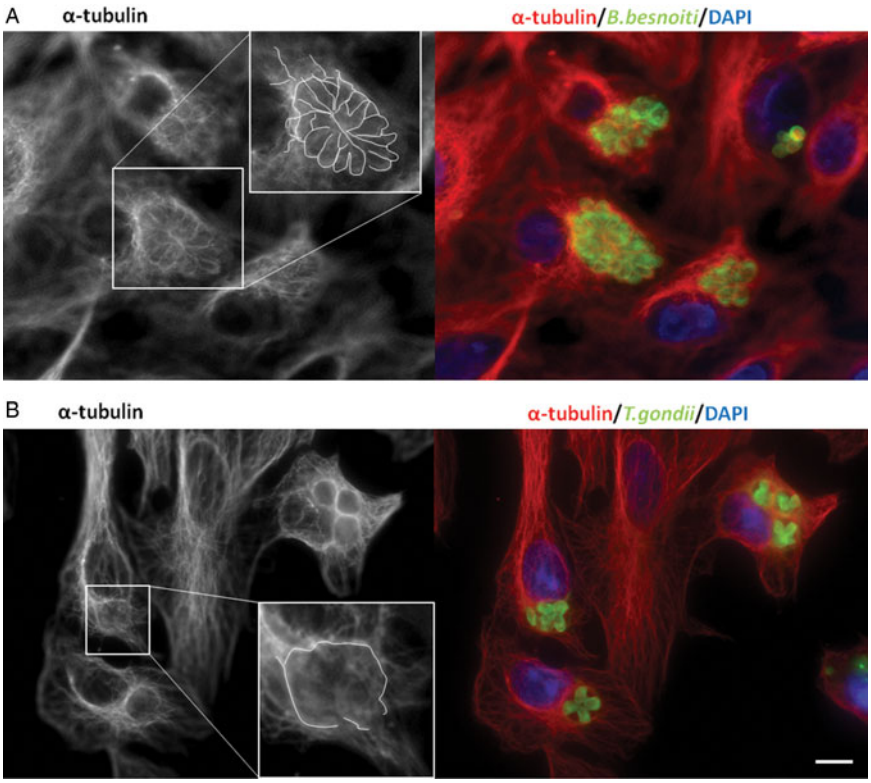

Fig. 1. Host microtubule cytoskeleton rearrangement around the parasitophorous vacuole of *Besnoitia besnoiti* and *Toxoplasma gondii*. Indirect immunolocalization of host cell α-tubulin during infection by *B. besnoiti* and *T. gondii*. On the left side, in grey, α-tubulin shows the structure of host cell microtubules, and their organization surrounding the PVs. Zoomed areas correspond to detailed views of the close interaction between host cell microtubules and PVs, with microtubules delineated in white. On the right side α-tubulin is shown in red, parasites in green (polyclonal antibodies against *B. besnoiti* and *T. gondii* were used), and blue for DNA staining with DAPI. (A) *Besnoitia besnoiti* infecting Vero cells. (B) *Toxoplasma gondii* infecting Vero cells. Scale bar = 5 μm. Abbreviation: DAPI, 4′, 6-diamidino-2-phenylindole; PVs, parasitophorous vacuoles.

parasite components into these organelles. This is supported by the observation that microtubules surrounding the PVM are short and stable microtubules, which maintain the PV structure and position near the host organelles (Coppens *et al.* 2006). Another hypothesis is that the recruitment of the host microtubules provides the mechanical support needed for the tachyzoites to divide and acquire a correct positioning inside the PV.

Toxoplasma gondii infected cells were shown to exhibit microtubule-induced double-membrane invaginations of the PVM into the lumen of the PV, which have been named Host Organelle Sequestering Tubulo structures (H.O.S.T.) (Coppens *et al.* 2006). H.O.S.T. are required for the scavenging of host's cholesterol and possibly other nutrients, serving as conduits for the delivery of host lysosomes and endocytic vesicles into the PV (reviewed in Laliberté and Carruthers, 2008). The tubular conduits are coated by a dense collar of parasite proteins, including GRA7, which facilitates the sequestering of host organelles in the PV lumen, and consequently the scavenging of nutrients by *T. gondii* (Coppens *et al.* 2006).

Once inside the host cell, secreted GRA2, GRA4, GRA6 and GRA9 (Mercier *et al.* 2002; Adjogble *et al.* 2004) by *T. gondii* into the intravacuolar space, interact with a complex network of membrane-like material within the PV, being required for its reorganization. This network of thin tubules fills the lumen of the PV and is known as the intravacuolar network and probably it is responsible for the rosette arrangement of parasites inside the PV (Magno *et al.* 2005). A PV tubular network that forms the PV matrix has also been described in *N. caninum* PVs, and dense granule proteins are integral components of the PV matrix (Hemphill *et al.* 2004; Guionaud *et al.* 2010).

In *P. falciparum* infected erythrocytes, the PV extends into the cytosol as a network of tubovesicular membranes to the periphery of the host cell [tubule vesicular network (TVN)]. The TVN might facilitate the parasite access to nutrients, and could be involved in lipid import from the erythrocyte surface (Lauer *et al.* 1997; Haldar *et al.* 2001; reviewed in Mbengue *et al.* 2012).

Wang *et al.* (2010) were the first to provide evidence for the involvement of a host signalling pathway in the reorganization of the cytoskeleton of cells invaded by *T. gondii*. Infection of primary fibroblasts resulted in stable activation of the host Ser/Thr kinase Akt, a signalling regulatory kinase implicated

in cell polarization during migratory responses in fibroblasts (Onishi *et al*. 2007; Wang *et al*. 2009), which is the upstream activator of Akt, phosphatidylinositol 3-kinase (PI3 K) and mTOR complex 2 (mTORC2) (reviewed in Franke, 2008). mTORC2 is also able to directly regulate spatial cell growth through actin cytoskeleton remodelling by acting upstream Rho-GTPases (reviewed in Soares *et al*. 2014). Wang *et al*. (2010) showed that host mTORC2-Akt signalling is important for several aspects relating to cell reorganization, including the localization of the host centrosome, the organization of microtubules and the distribution of mitochondria and lysosomes surrounding the PV of *T. gondii*.

Microtubule remodelling of the host cell around the PV has also been observed in bovine endothelial cells infected with *Eimeria bovis*. This event begins within 3 days post infection (dpi), and becomes evident in the end stages of meront maturation. One part of these microtubules is post-translationally modified by acetylation, which is linked to the regulation of microtubule dynamics, mainly by increasing the binding of motor proteins, and thus promoting microtubule stability (reviewed in de Forges *et al*. 2012). This could serve the acquisition of organelles such as mitochondria and the endoplasm reticulum to the closer vicinity of the *E. bovis* PV during replication (Hermosilla *et al*. 2008). In *E. bovis* infected cells, the transcription levels of several tubulins (TUBB, TUBB4 and TUBB6) were increased. In the more advanced stage of infection, transcription was enhanced for proteins involved in microtubule cytoskeleton organization and regulation such as: (i) the polymerization-promoting protein (TPPP) that induces microtubule bundling; (ii) the cytoskeleton-associated protein 4 (CKAP4) involved in the anchoring of microtubules to membranes (e.g. endoplasmic reticulum); (iii) the dedicator of cytokinesis 7 (DOCK7) a guanine nucleotide exchange factor (Taubert *et al*. 2010; reviewed in Hermosilla *et al*. 2012). Thus *E. bovis* infection impacts on molecular mechanisms that regulate the modulation of the host cell microtubule cytoskeleton.

Even in apicomplexan parasites that do not form a PV, the interaction with host microtubules during intracellular development seems to be a critical aspect. For example, in the case of *Theileria* schizonts, which are located freely in the host cell cytoplasm, the cross-talk between parasite and host microtubules facilitates the movement of the parasite to the perinuclear region of the host cell (reviewed in Shaw, 2003; Frénal and Soldati-Favre, 2009). Moreover, *Theileria* proliferation is synchronized with host cell division, and the schizont is tightly associated with the host cell mitotic apparatus during host cell cytokinesis (reviewed in Dobbelaere and Kuenzi, 2004). The host cell mitotic kinase polo-like kinase 1 (Plk1) plays a fundamental role in the association of the parasite with central spindles (von Schubert *et al*. 2010). Nevertheless, the identity of parasite proteins involved in the interaction and association with host microtubules remains, for the most part, unknown. Surface-associated or secretory schizont proteins are most likely of major relevance. As an example, the *Theileria annulata* secreted protein TaSE was detected in infected cells in association with the parasite, and in the host cell cytoplasm co-localizing with α-tubulin (Schneider *et al*. 2007). Taking in consideration this co-localization with α-tubulin, TaSE seems to play a role in the segregation of the schizont into the two daughter cells, which is supported by the fact that during mitosis and cytokinesis of the host cell, TaSE co-localizes with components of the mitotic spindle (reviewed in Frénal and Soldati-Favre, 2009). In addition TaSE not only binds to α-tubulin, but indirectly also interacts with microtubules via scaffold proteins or with motor proteins (Schneider *et al*. 2007).

Another *T. annulata* protein that stably localizes to the schizont surface, TaSP, could also be involved in mediating the interaction between the parasite surface and host cell microtubules. TaSP has an extracellular domain that could interact with the host cell microtubule network, contributing to the schizont separation during host cell division and to the regulation of microtubule assembly in the host cell. TaSP was found to co-localize with α-tubulin and γ-tubulin at the centrosome, and immunoprecipitated with γ-tubulin (Seitzer *et al*. 2010). Another schizont surface protein, p104, functions as an EB1-binding protein, which is an important regulator of microtubule dynamics and belongs to a vast family of +TIPs proteins that interact with the microtubule plus ends of the host cell. Thus, *Theileria* hijacks EB1 that docks to a consensus SxIP motif present in p104, thus gains access to the regulatory mechanisms that control the microtubule cytoskeleton dynamics. Other molecules could be involved in microtubule stabilization at the schizont surface, such as CLASP1 (CLIP-170 associating protein 1), another +TIP protein that stabilizes microtubules. This protein was found in great amounts at the schizont surface, and it also contains two SxIP motifs that could provide additional EB1 docking sites (Woods *et al*. 2013). Therefore, the surface antigens p104 and TaSP [which are highly phosphorylated in a cell cycle dependent manner, during host cell S-phase (Wiens *et al*. 2014)] are involved in the interaction between *Theileria* and the host cell microtubule cytoskeleton, and could be of major importance for ensuring equal partitioning of the schizont to the two host cells during host cell division.

SOME, BUT NOT ALL, APICOMPLEXA RECRUIT THE HOST CELL CENTROSOME

The centrosome has a major role in organizing the microtubule cytoskeleton in animal cells. Usually

the centrosome is closely associated with the nucleus. However, during *N. caninum* (Nolan *et al.* 2015) and *T. gondii* invasion (Coppens *et al.* 2006; Walker *et al.* 2008; Wang *et al.* 2010; Romano *et al.* 2013; Cardoso *et al.* 2014; Nolan *et al.* 2015), the host cell centrosome is recruited away from the nuclear membrane and positioned near to the PVM (Fig. 2A). This process could be crucially involved in the mechanism by which these parasites effectively remodel host microtubules. In contrast, in *B. besnoiti* infected cells, the recruitment of the host cell centrosome towards the PV does not occur (Fig. 2B), and no additional foci of γ-tubulin that could act as non-centrosomal MTOCs were observed at the PV membrane. This must have implications regarding host cell microtubule dynamics in *Besnoitia* infected cells compared with cells infected with *T. gondii* or *N. caninum*. Thus, it seems that *B. besnoiti* remodelling of the host cell cytoskeleton depends neither on a centrosomal MTOC, nor on non-centrosomal MTOCs (Cardoso *et al.* 2014). Non-centrosomal MTOCs, similar to centrosomes, catalyze γ-tubulin-dependent microtubule nucleation and anchor microtubules (reviewed in Lüders and Stearns, 2007). Interestingly, in *T. gondii*-infected cells, no additional clusters of γ-tubulin located in the vicinity of PVs could be detected, indicating that the rearrangement of host microtubules in *T. gondii* invasion/replication is probably dependent mostly on the interaction with the host centrosome that is recruited by the parasite (Cardoso *et al.* 2014).

Considering that the centrosome organizes microtubules that participate in intracellular trafficking, cell motility, cell adhesion, cell polarity and formation of the mitotic spindle (reviewed in Azimzadeh and Bornens, 2007), the repositioning of the centrosome in the vicinity of *T. gondii* PV could be important for the parasite to control the distribution of host organelles, modulation of the host cell cycle and cell migration. Meanwhile, the recruitment of the centrosome towards *T. gondii* PV is more evident in advanced stages of infection (24 h post invasion), without any significant difference in centrosome positioning in early infection (6 h post invasion) (Romano *et al.* 2013; Cardoso *et al.* 2014). This again suggests that the remodelling of the host microtubules is not solely dependent on centrosome recruitment, especially in the first stages of PV establishment, since microtubules are observed at the PV after a few minutes following host cell entry, and the centrosome is detached from the nucleus only several hours later.

Since *T. gondii* infection induces the arrest of infected fibroblasts in the G2 phase of the cell cycle (Brunet *et al.* 2008), it is conceivable that the recruitment of the host cell centrosome facilitates the control over the host cell cycle. It is important to mention that in infected cells the recruited centrosome apparently maintains its integrity, as it consists of centrioles surrounded by a pericentriolar matrix [at least for NEDD1 and pericentrin (Romano *et al.* 2013)].

Furthermore, overexpression of TBCCD1 [a centrosomal protein important for centrosome–nucleus connection in *T. gondii* infected cells (Gonçalves *et al.* 2010)], inhibits the recruitment of the centrosome at 18 h of invasion. Moreover, in these cells, *T. gondii* replication is significantly slower, suggesting that tachyzoite proliferation requires an efficient recruitment of the host cell centrosome, with the manipulation of the molecular mechanisms involved in the nucleus-centrosome connection. The observation that invasion of *T. gondii* into cells overexpressing TBCCD1 is not inhibited supports the hypothesis that the recruitment of the centrosome is important only for tachyzoites undergoing endodyogeny (Cardoso *et al.* 2014).

Thus, different apicomplexan parasites have evolved mechanisms to modulate the structure and functional activity of the microtubule cytoskeleton. However, some species such as those in the genus *Toxoplasma, Neospora* and *Besnoitia* remodel host cell microtubules already during host cell entry or at an early stage post invasion, while for others such as *Eimeria* and *Theileria* the current evidence suggests that they act on microtubule dynamics mainly at a later stage during intracellular proliferation.

Although several hypotheses have been proposed to explain these interactions, it is still not clear why parasites manipulate the host cell microtubule cytoskeleton to establish a successful infection, and the molecular mechanisms are only now starting to be uncovered.

ACTIN FILAMENT DYNAMICS IN CELLS INFECTED BY APICOMPLEXA

Several studies indicate that invasion by apicomplexan parasites triggers an important reorganization of the host cells actin microfilament system. The current data on host cell actin remodelling induced by apicomplexan parasites is summarized in Table 2. Even though the molecular mechanisms used by the parasites to trigger actin-filament rearrangements in infected cells are better understood compared with those responsible for the remodelling of the host cell microtubule cytoskeleton, they remain for the most part elusive.

De novo polymerization of host actin is an important prerequisite for the entry of *T. gondii* tachyzoites into host cells. A host cell derived ring-shaped F-actin structure is formed at the MJ that disappears 10 min after tachyzoite entrance, which provides a solid anchor mechanism for pulling the parasite inside the host cell (reviewed in Frénal and Soldati-Favre, 2009). Adding to this, the presence

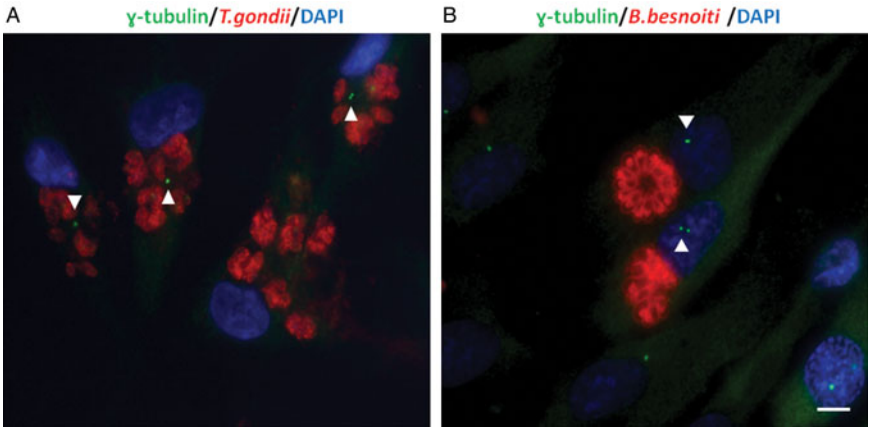

Fig. 2. Host centrosome–nucleus position in RPE-1 host cells infected with *Toxoplasma gondii* or *Besnoitia besnoiti*. Indirect immunolocalization of *T. gondii* and *B. besnoiti* infecting RPE-1 cells. Antibodies against γ-tubulin, *B. besnoiti* (polyclonal antibody) and *T. gondii* (polyclonal antibody) were used. DNA was stained with DAPI. The position of the centrosomes can be seen in green (γ-tubulin staining), in relation to the position of the parasites in each PV (red) and host cell nucleus (blue). Arrowheads indicate the host centrosome positioning. (A) *Toxoplasma gondii* 24 h post invasion – displacement of the centrosomes away from the nuclei and closer to the PVs. (B) *Besnoitia besnoiti*, 24 h post invasion – the position of the centrosome is maintained close to the host cell nucleus. Scale bar = 5 μm. Abbreviations: DAPI, 4′, 6-diamidino-2-phenylindole; PV, parasitophorous vacuole; RPE, retinal pigment epithelial.

at the MJ of the host Arp2/3 complex involved in actin polymerization control, together with the nucleation-promoting factor, cortactin, supports *de novo* polymerization of the host actin (Gonzalez *et al*. 2009). It is likely that the changes in the actin microfilaments during invasion include not only the induction of actin polymerization, but also a local disorganization of the cortical actin network to facilitate tachyzoite entrance. Considering this, and probably to overcome the resistance conferred by the host cortical actin, *T. gondii* expresses a protein named toxofilin (Bradley *et al*. 2005). This protein remodels the host cytoskeleton by upregulating actin filament turnover and retrograde flow, which loosens the local host cell actin meshwork and facilitates parasite internalization and invasion (Frénal and Soldati-Favre, 2009). In addition, *T. gondii* tachyzoites lacking toxofilin had an increased difficulty in inducing cortical actin disassembly and exhibited delayed invasion kinetics (Delorme-Walker *et al*. 2012). A recent study showed that about 20% of *T. gondii* toxofilin mutants fail to enter into the host cell and end up disengaging from the host cell membrane. This is in agreement with the view that both cortical disassembly and free actin availability is required for *T. gondii* complete invasion through the toxofilin activity (Bichet *et al*. 2014).

Moreover, a functional relationship has also been established between host cell GTPases involved in cytoskeleton regulation and *T. gondii* invasion. For instance, host cell ADP-ribosylation factor-6 (ARF6) was shown to be recruited to the PVM (da Silva *et al*. 2009). ARF6 is a small GTPase that is known to regulate membrane trafficking and actin cytoskeleton rearrangements at the plasma membrane. The RhoA and Rac1 GTPases, which are involved in the regulation of the organization and dynamics of the actin cytoskeleton (Hall, 1998), were also observed to accumulate and be activated at the PVM upon *T. gondii* host entrance (Na *et al*. 2013). The recruitment and activation of these two small host GTPases could be implicated in the actin microfilament reorganization observed during PV formation.

Furthermore, by performing a high-throughput siRNA screen of a human siRNA library, a subset of host factors that appear to influence *T. gondii* invasion by regulating the host actin cytoskeleton were identified (Gaji *et al*. 2013). Six of these identified factors (PTK9L – Twinfilin, actin binding protein homolog 2; PHPT1-Phosphohistidine phosphatase 1; MAPK7-Mitogen activated protein kinase 7; PTPRR – Protein tyrosine phosphatase, receptor type, R; MYLIP-Myosin regulatory light chain interacting protein; and PPIL2 – Peptidylprolyl isomerase (cyclophilin)-like 2) are antagonists of the actin cytoskeleton, as the downregulation of their expression causes increased development of cortical actin microfilaments with deposition of more F-actin near the PV periphery. Despite the fact that the mechanisms underlying how these proteins influence actin polymerization are unclear, the identification of these novel proteins can be of significance not only to understand *T. gondii* host cell invasion, but also to the understanding of other actin-dependent processes such as host cell migration (Gaji *et al*. 2013).

Parasite–host cell F-actin interactions have also been described for other apicomplexans. In *N. caninum* infected cells, the actin microfilaments associate with the periphery of the PV, which might

Table 2. Rearrangement of host actin filaments and parasite and host molecules that interact with the host actin cytoskeleton during invasion and replication

Species	Rearrangement of host actin filaments	Parasite molecules that associate with actin filaments	Host molecules that associate with actin filaments
Toxoplasma gondii	*De novo* polymerization of host actin: formation of a host ring-shaped F-actin structure at the MJ (Gonzalez *et al*. 2009). In infected DCs: actin redistribution with the disappearance of podosome structures and the appearance of a rounded cellular morphology with veils, ruffles, filopodia and lamellipodia (Weidner *et al*. 2013)	Toxofilin promotes cortical disassembly and free actin availability (Delorme-Walker *et al*. 2012; Bichet *et al*. 2014)	Presence at the MJ of the host Arp2/3 complex and cortactin, (Gonzalez *et al*. 2009). Recruitment of ARF6 to the PVM (da Silva *et al*. 2009). RhoA and Rac1 GTPases accumulation and activation at the PVM (Na *et al*. 2013). PTK9L; PHPT1; MAPK7; PTPRR; MYLIP; PPIL2: antagonists of the actin cytoskeleton (Gaji *et al*. 2013). Redistribution of integrins (CD18 and CD11c) in DCs (Weidner *et al*. 2013)
Neospora caninum	Association with the periphery of the pseudocyst (Hemphill *et al*. 2004)	?	?
Plasmodium falciparum	Formation of membrane-limited vacuolar structures in the RBC cytoplasm upon infection: MC; formation of knobs through the remodeling of spectrin, ankyrin and actin (Mbengue *et al*. 2012)	MESA interacts with Band 4·1 in the erythrocyte cytoskeleton; KAHRP links PfEMP1 to the cytoskeleton through Spectrin-actin-4·1 complex binding (Oh *et al*. 2000). RESA stabilizes the spectrin tetramer (Silva *et al*. 2005). LyMP interacts directly with the membrane skeleton (Proellocks *et al*. 2014). PfPTP1 links MC to the host cell actin cytoskeleton (Rug *et al*. 2014). SBP1 binds specifically to 4·1R and spectrin, components of the RBC membrane skeleton (Kats *et al*. 2015)	?
Eimeria bovis	Accumulation of host cell actin at the PV in 3 days after infection (dpi); presence of a thick layer 15–20 dpi (Hermosilla *et al*. 2008). Accumulation of host cell spectrin around the PV 8 dpi (Hermosilla *et al*. 2008)	?	Upregulation of vinculin and ezrin and other actin-related genes (CAPG, CNN2, TAGLN, and PALLD) during macromeront development; downregulation of several proteins interacting with actin: α-actin-1, gelsolin, actin-like protein-2, gelsolin-like capping protein, tropomodulin-3, and transgelin at the end of macromeront development (Lutz *et al*. 2011; Hermosilla *et al*. 2012)
Theileria annulata	PTAs formation and triggering the organization of a single lamellipodium (Baumgartner, 2011). *De novo* actin polymerization of F-actin at host cell protrusions (Ma and Baumgartner, 2013)	?	Induction of TNFα, phosphorylation of the ERM family of cytoskeleton regulatory proteins (ezrin, radixin and moesin) in a MAP4K4 dependent manner to control actin cytoskeleton dynamics (Ma and Baumgartner, 2014)
Cryptosporidium	Accumulation of host cell actin filaments at the site of entrance (Elliott *et al*. 2001; Feng *et al*. 2006)	?	PI3 K and cdc42 seem to induce actin remodelling during host cell invasion; Integrins (ITGA2/ITGB1) could be an alternative pathway for parasite induced actin remodeling (Lendner and Daugschies, 2014)

DC, dendritic cell; ERM, ezrin/radixin/moesin; MESA, mature-parasite-infected erythrocyte surface antigen; KAHRP, knob-associated histidine-rich protein; MJ, moving junction; PV, parasitophorous vacuole; PVM, parasitophorous vacuole membrane; PTA, podosome-type adhesions; RBC, red blood cells; (?) not known.

suggest a physical link between actin filaments and the pseudocyst membrane (Vonlaufen *et al*. 2002). The molecular mechanisms and pathways underlying these actin rearrangements are far from being understood. *Cryptosporidium parvum* initiates structural changes in the host cell cortical actin upon attachment to the host cell surface, resulting in an accumulation of host cell actin filaments at the site of infection (Elliott *et al*. 2001; Feng *et al*. 2006). It is possible that this parasite employs signal cascades that are also involved in the formation of lamellipodia/filopodia. In this view the host phosphoinositide 3-kinase (PI3K) and Cdc42 (a small GTPase of the Rho-subfamily) are good candidates to induce actin remodelling during *Cryptosporidium* host cell invasion. In fact, the PI3K/cdc42/N-WASP pathway activates the Arp2/3 complex leading to the elongation and branching of actin filaments and formation of protrusions. Interestingly, inhibition of host Cdc42 reduced *Cryptosporidium* invasion up to 80% but never blocked host cell entry completely, which suggests that one or several alternative pathways exist. A possible, but not yet proven, alternative pathway for *Cryptosporidium* induced actin remodelling, involves integrins (like ITGA2/ITGB1, fundamental for the control of actin-based processes in lamellipodia and filopodia in cells), that could pass a signal via a tyrosin Src kinase, with the subsequent activation of cortactin and recruitment of the Arp2/3 complex (reviewed in Lendner and Daugschies, 2014).

Interestingly a similar strategy seems to be used by *T. annulata* that specifically promotes the formation of podosome-type adhesions (PTAs) and induces host cell polarization by triggering the organization of a single lamellipodium. *Theileria annulata* can manipulate the actin cytoskeleton by increasing the assembly of functional adhesion and signalling complexes, controlling proteins involved in cell–matrix interactions and cell signalling in lamellipodia and PTAs of infected macrophages (Baumgartner, 2011). Moreover, there seems to be *de novo* actin polymerization of F-actin in parasitized macrophages at host cell protrusions in the leading edge, allowing the forward extension of these cells necessary for their invasive characteristics (Ma and Baumgartner, 2013).

Thus, host cell actin cytoskeleton remodelling is a universal event during the apicomplexan infection process. Nevertheless parasites seem to have adapted their invading strategies to the specificities of the cytoskeleton of their target cells. For example, in the case of *E. bovis* entry into bovine endothelial cells, there is an accumulation of host cell actin at the PV that stabilizes the growing intracellular macromeront. Actin surrounds the PV within 3 dpi, and actin filament bundles form a thick layer in the final stages of meront maturation (15–20 dpi) (Hermosilla *et al*. 2008). Several actin-related genes are upregulated in cells containing developing macromeronts. This includes vinculin and ezrin (involved in actin cytoskeleton signalling) and others such as CAPG, CNN2, TAGLN and PALLD (Taubert *et al*. 2010). Meanwhile, in the final stages of macromeront development, when macromeronts rupture and merozoites are released, the expression of proteins that interact with actin, including α-actin-1, gelsolin, actin-like protein-2, gelsolin-like capping protein, tropomodulin-3 and transgelin are downregulated, and the same accounts for transcripts coding for β-tubulin 5 and 6. This suggests that the mechanisms regulating the microtubule and actin cytoskeleton are different, depending on the developmental stage of meront maturation. Thus, an upregulation is observed during meront formation (probably explained by the increasing need of structural support), and downregulation at the final phase of merogony, which could facilitate the release of merozoites (Lutz *et al*. 2011; reviewed in Hermosilla *et al*. 2012). In addition to the alterations in the actin filament system, *E. bovis* infected cells also exhibit alterations in the localization and overall organization of spectrin. Spectrin is a cytoskeletal protein that is closely linked to the cortical actin network lining the intracellular side of the plasma membrane. Spectrin forms tetramers that associate with short actin filaments, and provides a scaffold that ensures maintenance of plasma membrane integrity. Infection of endothelial cells with *E. bovis* results in accumulation of host cell spectrin around the PV 8 dpi, at the onset of parasite replication (Hermosilla *et al*. 2008).

Plasmodium falciparum infection of red blood cells (RBCs) induces dramatic host cell remodelling, which includes overall changes in shape and surface structures, and formation of several compartments in the host cell cytoplasm. Many of these alterations are also related to changes in the spectrin-actin cytoskeleton. During infection, the host spectrin elongates, the spectrin network becomes sparser and the spectrin tetramers appear more extended (Shi *et al*. 2013). Maurer's clefts (MC) are the best-studied compartments formed in the RBC cytoplasm upon infection, but how these are formed is unclear and questions on their functional role have not been completely resolved so far. MC function as membrane-limited vacuolar structures important for trafficking of parasite-encoded proteins destined for the RBC membrane. Most of these proteins confer cytoadherence of infected RBCs to the endothelium of the microvasculature but it remains elusive how proteins are sorted and transported to and from these structures (reviewed in Mundwiler-Pachlatko and Beck, 2013). The parasite adhesin *P. falciparum* erythrocyte membrane protein-1 (PfEMP1) is one of these proteins, and it mediates cytoadhesion by binding

to host receptors at the surface of vascular endothelial cells. PfEMP1 localizes in specific regions at electron-dense protrusions of the erythrocyte membrane, referred to as knobs. Knob formation is a dynamic process. As the parasite matures from trophozoite to schizont, the density of knobs increases and in the end, knobs cover the entire RBC surface. The formation of these structures implicates several RBC cytoskeletal components, such as spectrin, ankyrin and actin, by probably altering the architecture of the sub-membrane skeleton and its interactions with membrane proteins. This results in increased rigidity and adhesiveness of the erythrocyte membrane (reviewed in Mbengue *et al*. 2012). Recent electron microscopy studies revealed that each knob contains a spiral filament that coils into a shallow cone, and a layer of diffuse material that coats the upper surface of the conical frame. The outer coat of the knob skeleton connects with the erythrocyte spectrin cytoskeleton, mostly around the knob base. This interaction with the surrounding cytoskeleton could suggest a mechanical explanation for the enhanced cytoadherence of knobs (Watermeyer *et al*. 2016).

There are several proteins at MCs required for PfEMP1 trafficking (Maier *et al*. 2007; Spycher *et al*. 2008), including PfEMP1 trafficking protein 1 (PfPTP1) that was recently shown to be required for transport of PfEMP1 to the surface of *P. falciparum*–infected erythrocytes. In the absence of PfPTP1, MC are reduced to globular structures, thus the protein is important for maintenance of the MC architecture. PfPTP1 has a role in linking MC to the host actin cytoskeleton, and is integrated in a large complex that includes skeleton-binding protein 1 (SBP1). It influences actin filament length or organization, suggesting that PfPTP1 has a possible function in host cell actin filament stabilization (Rug *et al*. 2014). SBP1 is another MC protein that is essential for the placement of PfEMP1 at the erythrocyte surface, and it has a defined region that binds specifically to defined sub-domains of two major components of the RBC membrane skeleton, protein 4·1R and spectrin (Kats *et al*. 2015).

Several cytoskeleton binding proteins have been identified in *P. falciparum*, such as the Mature-parasite-infected erythrocyte surface antigen (MESA) that interacts with Band 4·1 in the erythrocyte cytoskeleton; or *P. falciparum* knob-associated histidine-rich protein (KAHRP), that binds the acidic terminal sequence (ATS) of PfEMP1 (termed VARC) and Spectrin-actin-4·1 complex, thus linking PfEMP1 to the cytoskeleton (Oh *et al*. 2000). It was recently demonstrated that the full-length proteins KAHRP and PfEMP1 co-localized to membrane knobs in erythrocytes infected with the trophozoite stage *P. falciparum*. Four positively charged linear sequence motifs of high intrinsic mobility on KAHRP that interact electrostatically with VARC have been identified (Ganguly *et al*. 2015). Another important protein is a PHISTb protein, ring-infected erythrocyte surface antigen (RESA) that contains a C-terminal spectrin-binding domain, which stabilizes the spectrin tetramer (Foley *et al*. 1991; Silva *et al*. 2005). Also, Tarr *et al*. (2014) identified a cytoskeleton binding domain in RESA that again indicates that PHISTb proteins may act as cross-linkers of the erythrocyte cytoskeleton. Finally, another example of PHISTb proteins interaction with the erythrocyte cytoskeleton was recently demonstrated to be a member of the *P. falciparum* lysine-rich membrane-associated PHISTb (LyMP) protein family. It was shown that LyMP is exported into the RBC, where it interacts directly with the RBC membrane skeleton (Proellocks *et al*. 2014).

As it was already mentioned, after *P. falciparum* infection, the erythrocytes become more rigid, less deformable and more fragile (reviewed in Mbengue *et al*. 2012). Parasite-exported proteins [like KAHRP and *P. falciparum* erythrocyte membrane protein 3 (PfEMP3)] and the extended spectrin tetramers may be responsible for the increased rigidity of the host cell cytoskeleton (Glenister *et al*. 2002; Shi *et al*. 2013).

APICOMPLEXAN PARASITES AND HOST CELL INTERMEDIATE FILAMENTS

Information on potential modulation of intermediate filaments by apicomplexan parasites is sparse. In fibroblasts, vimentin intermediate filament distribution markedly changed upon infection with *T. gondii* tachyzoites. While vimentin was largely localized around the nuclear periphery in non-invaded cells, filaments rapidly associated with the PVM periphery within 30 min post invasion. This association progressively became more pronounced with time, and at 18 h post invasion, an important network of intermediate filaments bundles encaged the PV (Halonen and Weidner, 1994). In contrast, in endothelial cells infected with *E. bovis*, no marked changes in host cell vimentin intermediate filament distribution were evident during first merogony (Hermosilla *et al*. 2008).

During the development of *T. gondii* tissue cysts in murine astrocytes, immunostaining revealed the presence of glial fibrillary acidic protein (GFAP) intermediate filaments. The GFAP filaments were present on the cytoplasmic side of the cysts in close apposition to the cyst wall and arranged in a concentric layer measuring 5–10 μm in thickness. The GFAP filament wrapping seems to be important mainly in early cyst development since more mature cysts were surrounded by a GFAP layer that was diminished when compared with younger cysts (Halonen *et al*. 1998). The same intermediate

filaments of GFAP were found in *N. caninum* infection, in close juxtaposition to the cytoplasmic side of the PVM in organotypic rat brain slice cultures (Vonlaufen *et al*. 2002).

APICOMPLEXAN PARASITES AFFECT HOST CELL MIGRATION

Considering the pronounced effects of apicomplexan parasites on the host cell cytoskeleton, and the fact that some of these parasites affect the architecture of the sub-membrane cytoskeleton and the cell-matrix junctions, it seems likely that these changes could reflect on an altered migration of the host cell.

Most studies dealing with this aspect were carried out in *T. gondii* infected cells. For instance, *T. gondii* infection of dendritic cells (DCs) alters their motility and confers a hypermigratory phenotype (Lambert *et al*. 2006, 2010). Thus, DCs could play an important role in the dissemination of *T. gondii* in an infected host. This is supported by the observation that parasitized DCs adoptively transferred to uninfected mice disseminate more quickly than non-infected DCs (reviewed in Blader and Saeij, 2009). Interestingly, the induction of increased cell migration through *T. gondii* infection is also exhibited by infected macrophages, but not by infected monocytes, T-cells, B-cells or NK-cells (Lambert *et al*. 2010).

The observed hypermigratory phenotype in DCs consists in rapid morphological transformation, altered adhesive properties, hypermotility, transmigration across endothelial monolayers *in vitro*, and responsiveness to chemokines, and was shown to account for *T. gondii* lineages types I, II and III (Weidner and Barragan, 2014). Already 5–10 min following invasion of DCs, cytoskeletal actin redistribution occurred, leading to the disappearance of podosomes, which are known to limit rapid migration by strongly binding to the extracellular matrix. Other features included the appearance of a rounded cellular morphology with veils, ruffles, filopodia and lamellipodia linked to the protrusive activity of DCs. In addition, *T. gondii* invasion caused the redistribution of integrins (CD18 and CD11c), with a more prominent integrin staining of CD18 at the periphery of the cell membrane, and of CD11 in veil structures (Weidner *et al*. 2013). *Toxoplasma gondii* infected DCs exhibited increase secretion of γ-aminobutyric acid (GABA), which is a neurotransmitter involved in cell migration, immunomodulation and metastasis. Interestingly, inhibition of GABA transporters or the GABA synthesizing enzyme, as well as blocking of the GABAA receptor, resulted in a reduction of hypermotility and transmigration, but did not alter the morphological changes in infected DCs described above (reviewed in Weidner and Barragan, 2014). Finally, the hypermigratory phenotype in *T. gondii* infected DCs does not require *de novo* protein synthesis, since inhibition of parasite or host cell protein synthesis did not influence the morphological changes or hypermotility (Weidner *et al*. 2013). Nearly identical features including a hypermigratory phenotype occur in DCs infected with *N. caninum* tachyzoites (Collantes-Fernandez *et al*. 2012).

The impact of *T. gondii* infection on host cell migration has also been addressed in other cell types, such as fibroblasts (Wang *et al*. 2010) and epithelial cells (Cardoso *et al*. 2014). In these cases, infection leads to a loss of host cell motility. In an *in vitro* wound-healing assay, the migration of parasitized human foreskin fibroblasts (HFF) and retinal pigment epithelial cells (RPE-1) was delayed (Wang *et al*. 2010; Cardoso *et al*. 2014). In contrast, RPE-1 cells infected with *B. besnoiti* tachyzoites did not show any motility impairment in wound-healing assays (Cardoso *et al*. 2014). It is possible that this difference in motile behaviour could be related to the above mentioned differences in recruitment of the centrosome (e.g. recruitment to the PV in *T. gondii* infected cells, but not in *B. besnoiti* infected cells) (Coppens *et al*. 2006; Walker *et al*. 2008; Wang *et al*. 2010; Romano *et al*. 2013; Cardoso *et al*. 2014). The participation of the centrosome in migration was previously documented by results showing that stimulation of cell motility was accompanied by centrosome reorientation towards the leading edge (Schmoranzer *et al*. 2009; Vinogradova *et al*. 2009). This cell polarization could be compromised in *T. gondii* infected cells, but not in *B. besnoiti* infected cells. However, the mean angle of the centrosome towards the wound was quite similar in non-invaded RPE-1 cells, and in *B. besnoiti* and *T. gondii* infected cells (Cardoso *et al*. 2014). Overall, infection of RPE-1 cells by *B. besnoiti* and *T. gondii* did not have an obvious effect in directional cell polarization, but cell migration in *T. gondii* infected cells was impaired.

Transforming species of *Theileria* such as *T. annulata* trigger the motility and invasive behaviour of infected macrophages by controlling host cell membrane protrusions through F-actin dynamics, which assists parasite dissemination in the host (Baumgartner, 2011; Ma and Baumgartner, 2013, 2014). More recent studies determined that the parasite drives host cell motility and invasiveness by activating the host proto-oncogenic ser/thr kinase MAP4K4, which is induced by the inflammatory cytokine TNFα. TNFα induces the phosphorylation of the ERM (ezrin/radixin/moesin) family of cytoskeleton regulatory proteins in a MAP4K4-dependent manner, with this ERM phosphorylation likely occurring in lamellipodia, thus controlling parasite-dependent actin dynamics regulation. Supporting this, blocking TNFα secretion or MAP4K4 expression compromised the formation of polar F-actin-rich

invasion structures, impairing host cell motility. Thus, *Theileria* has the capacity of inducing TNFα, activating the conserved kinase MAP4K4, in order to control actin cytoskeleton dynamics and thus cell motility (Ma and Baumgartner, 2014).

CONCLUDING REMARKS

Apicomplexan parasites exhibit a number of common morphological characteristics, which have been used to place them into the same phylum. However, they also share a number of features related to their interactions with the host cell, which is essential for intracellular survival, replication and further development. One aspect is their ability to modulate the host cell cytoskeletal filamentous networks composed of microtubules, actin microfilaments and intermediate filaments. In many instances, it still remains elusive to what extent this interaction and cross talk with the host cell cytoskeleton is beneficial for these parasites, but one can speculate that it enables them to (i) reposition crucial host cell organelles, (ii) hijack host cell nutrients, (iii) gain control of signalling pathways, (iv) interfere or promote host cell motility, and (v) control the host cell shape and adherence properties. Several recent studies have revealed effector parasite proteins and respective host cell targets that enable apicomplexans to modulate the host cell cytoskeleton.

Although being so similar in many ways, each apicomplexan species has its own peculiarities and requirements, and these are reflected in the differential influence that distinct apicomplexan species exert on the host cell cytoskeleton. As a word of caution, one should mention the possibility that some of the rearrangements induced upon the host cell cytoskeleton by invading parasites could just simply reflect host cell specific responses upon invasion, which are not actively induced by the parasites *per se*, and future approaches should address this point. In addition, it would be of great benefit to focus on three-dimensional cell culture models or organoid culture rather than on the two-dimensional *in vitro* cell culture that is currently mostly employed, simply to more accurately mimic what is going on *in vivo*. In any case, the elucidation of the molecular effector pathways that allow apicomplexan parasites to manipulate the host cell cytoskeleton in order to establish infection and propagate in their host is of major importance. These efforts could reveal new aspects of host–parasite interactions and parasite vulnerabilities that may be exploited for an efficient therapeutic and prophylactic research.

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