Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Microbiology

Intracellular protozoan parasites: living probes of the host cell surface molecular repertoire

Georgios Pavlou[1], Geneviève Milon[2] and Isabelle Tardieux[1]

Intracellular protozoans co-evolved with their mammalian host cells a range of strategies to cope with the composite and dynamic cell surface features they encounter during migration and infection. Therefore, these single-celled eukaryotic parasites represent a fascinating source of living probes for precisely capturing the dynamic coupling between the membrane and contractile cortex components of the cell surface. Such biomechanical changes drive a constant re-sculpting of the host cell surface, enabling rapid adjustments that contribute to cellular homeostasis. As emphasized in this review, through the design of specific molecular devices and stratagems to interfere with the biomechanics of the mammalian cell surface these parasitic microbes escape from dangerous or unfavourable microenvironments by breaching host cell membranes, directing the membrane repair machinery to wounded membrane areas, or minimizing membrane assault using discretion and speed when invading host cells for sustained residence.

Addresses
[1] Institute for Advanced Biosciences (IAB), Team Membrane Dynamics of Parasite-Host Cell Interactions, CNRS UMR5309, INSERM U1209, Université Grenoble Alpes, Grenoble 38700, France
[2] Institut Pasteur, Paris 75015, France

Corresponding author: Tardieux, Isabelle (isabelle.tardieux@inserm.fr)

Current Opinion in Microbiology 2019, **52**:116–123

This review comes from a themed issue on **Host–microbe interactions: parasites**

Edited by **Norma Windsor Andrews** and **Dario SimoÄ es Zamboni**

For a complete overview see the Issue and the Editorial

Available online 23rd July 2019

https://doi.org/10.1016/j.mib.2019.06.007

Introduction

Over the last few decades, researchers have shed light on how micron-size single-celled eukaryotes – that is, protozoan microbes – utilize diverse tactics to overcome the challenge of invading cells from a range of mammalian hosts. In close partnership with either ectotherm or warm-blooded mammalian hosts, those protozoan microbes sculpt the dynamic protective and feeding niches to sustain transient to prolonged-time intracellular residence. Regardless of the 'manoeuvres' co-evolved by each pair of invasive protozoan–host cell to build heterotypic associations of distinct life-time and fate, the parasites must inevitably cope with the structural and functional complexity of the host cell surface. This complexity is dictated by the host cell identity alongside a micro-environment that usually consists of scaffolding bioactive matrices and, in the context of barrier tissues, of connecting neighbouring cells. In this short review only a few examples have been selected to illustrate how Trypanosomatid and Apicomplexan intracellular protozoan parasites make use of their target cell properties to generate selective paths towards the intracellular world, where they unfold developmental programs responsible for further producing transmissible populations.

What invasive protozoan microbes face/perceive when meeting the surface of targeted mammalian cells

When confronted with the surface of the mammalian permissive host cell, pre-invading protozoan parasites encounter a continuous, non-uniform interface that behaves as a dual-active composite [1]. While a fluid bilayer predominantly composed of phospholipids and loaded with hundreds of proteins and protein modules is positioned at the extracellular interface – namely the Plasma Membrane (PM) –, underneath is tethered a thin ActoMyosin Contractile Meshwork (AMCM) known as cortex [2]. The dynamic coupling that operates between the two composite entities over a range of spatiotemporal scales is key for ensuring vital contributions and adjustments of the PM, which concurrently maintain cellular homeostasis. Through the control of nano-domain to micro-domain assembly, PM-AMCM active coupling allows 'out–in' signals to be sensed, amplified and conveyed before promoting a choreography of events that reshape the cell surface architecture. Recently, *in vitro* minimal composite models revealed how cortex contractile myosins [1] (including mammalian myosin 2 isoforms, myosin 1 and myosin 17) [3] can drive mini-filament assembly alongside the actin architecture to precisely regulate cortical tension gradients, thus sculpting the cell surface into distinct shapes [2].

In this context, when protozoans and host cells come into contact with each other, signals emitted by the single-celled eukaryotic parasite – either through surface-exposed or secreted ligands – are expected to be captured by host cell surface through lectin-carbohydrate, protein–protein or protein–lipid molecular interactions and processed to regulate PM-AMCM dynamics in a way compatible with

the safe intracellular installation of the parasitic microbes. However, to reach the selected residence cell/tissue alive, some protozoans need first to take tough measures to deal with barriers/hurdles impeding their intruding progression.

The extreme case: the protozoan parasite disrupts the mammalian cell surface, *en route* to safer residence in *bona fide* hosting cells

A somehow 'drastic' strategy was reported for parasites encountering a continuous host cell layer before reaching a growth-permissive cell/tissue. Such layers called endothelia or epithelia are composed of type-specific cells that collectively develop robust apico-basal polarity through engagement with specialized cell–cell and cell–substratum molecular anchors, forming a physical barrier to the progression of non-parasitic intruders [4]. As a way to circumvent this obstacle, some parasitic intruders such as protozoan parasites can literally cross cells by causing the PM-AMCM to irreversibly breach, imposing a rapid exit of the invader from the sacrificed host cell. This cell traversal (CT) scheme seems to be a quasi-obligate path for the malaria-causing *Plasmodium* spp. Sporozoites, which are delivered into the dermis of warm-blooded mammals by blood-feeding anopheline mosquitoes. A fraction of these sporozoites eventually initiate the intra-hepatocyte phase of the *Plasmodium* spp. intracellular development, provided that the parasites first achieve traversal of endothelial cells of the dermal blood microvascular bed, of the liver sinusoid endothelial cells, as well as resident Küpffer macrophages [5–7]. Relying on *Plasmodium* gene disruption, the latter teams provided insights on a required discharge of stage-specific pore-forming or putative pore-forming proteins by sporozoites (e.g. sporozoite-restricted SPECT1 and SPECT2/PLP1, sporozoite/ookinete-restricted CelTOS) that enable cell traversal. Upon exocytosis from a calcium-responsive secretory organelle called microneme, the SPECT1 and SPECT2 plasmodial proteins were proposed to promote destabilization of the PM by forming transmembrane channels in Küpffer macrophages and fenestrated sinusoidal endothelial cells, ultimately promoting PM rupture and parasite translocation across the cell layer adjacent to hepatocytes. Tandem cooperation between the PM-inserted channels and yet undefined sporozoite effectors has also been speculated as a way to impact the cell cortex integrity, contributing to the extent of PM wounds and facilitating CT. However, *in vivo* live imaging of pore formation in endothelial cells and macrophages targeted by sporozoites in experimentally relevant <hosts – mice or humanized mice – remains a challenge. These limitations preclude definitive conclusions on which membrane domains, apical versus basal, are targeted by the plasmodial molecules, and how the sporozoite intracellular gliding behavior contributes to achieve CT. To add complexity to the CT issue, it was recently reported that CT can occur without detectable lesions at the site of entry, whereas the SPECT2/PLP1-triggered membrane disruption actually accounts only for exit out of entry vesicles and host cells to complete rapid CT [8]. Of note, this study interrogated CT of rodent-associated *Plasmodium* spp. in human – possibly not fully polarized – hepatocytes, mammalian cells that typically support both rapid parasite CT and progeny production. Thus, this scenario might not be valid for CT of macrophages and endothelial cells. Interestingly, comparative analysis of CT and productive invasion processes using hepatoma cell lines [8] established a link with a large body of work performed in several Apicomplexa underscoring the crucial post-entry role of a large family of canonical pore-forming proteins {excellently reviewed in Ref. [9]}. Finally, an intriguing structure depicting a 4-helix bundle connected to a helical hook was identified between the rodent-associated *Plasmodium berghei* SPECT1 and the mammalian sperm protein Izumo, the latter being proposed as a critical member of an egg-sperm fusogenic complex in mice [10]. This finding raised even more perplexity about the mechanisms by which SPECT 1 could perhaps cooperate with the pore-forming SPECT2 [5] and other partners to orchestrate CT [11,9].

Do invasive protozoan microbes design selective paths within permissive mammalian host cells by benefiting from PM-AMCM dynamic features?

In contrast to *Plasmodium* sporozoites that cause crude, deadly cell surface attack when confronted with a non-growth permissive cellular barrier, a large number of protozoan parasites from different lineages have evolved developmental stages that tackle the cell surface in a more subtle way to ensconse the parasite intracellularly. They have elaborated an array of unrelated procedures that all (i) serve the same goal – that is, preserve cell viability for longer pairing – and (ii) obey a common principle – that is, benefit from PM-AMCM dynamic features for designing selective paths of entry. Depending on the mode of entry, the intracellular microbe subsequently either escapes from or reconfigures the PM-derived entry compartment to satisfy its metabolic needs and to perceive and counteract any endogenous cell defences. Because these crucial determinants for successful parasitism operate post-cell invasion, they are beyond the scope of this review and have been discussed elsewhere [12,13].

Taking advantage of PM-AMCM dynamics driving multiple types of endocytosis to gain intracellular residence

Among the predominant strategies elaborated to preserve PM integrity are those relying on (i) parasite surface-exposed ligands – such as glycoproteins, proteins and lipids – that recognize surface-exposed receptor molecule(s) of the target cell or (ii) specific molecular sets of parasite delivery systems and effectors, the first directing the second

to differentially target host cell elements. Both designs modulate signalling pathways, most commonly PIP3 phosphoinositide-centred pathways, that in turn promote the formation of PM outward protrusions through processes derived from the ancestral feeding mode of free phagotrophic predators and broadly dedicated to nutrient uptake in mammalian cells (for recent reviews see Refs. [14,15]). While these processes typify a large set of closely related endocytic events, they invariably proceed at the expense of PM regions that shape ruffles, filopodia and blebs or pseudopods during macropinocytosis and phagocytosis, respectively. Driven by a variety of stimuli that promote the activation of cortical F-actin assembly, these PM folds emerge and expand to engulf micrometer sized droplets of medium or/and solid materials within a PM-derived bud that is subsequently removed from the cell surface through the action of a multi-component and multistep fission force machinery [16]. The PM-AMCM dynamic coupling driving macropinocytosis is so robust that it allows tissue-patrolling immune cells to both overcome hydraulic resistance and clear undesired materials [17]. As an efficient way to safely access the cytoplasm of mammalian cells, a range of medically relevant parasitic protozoans – among them the Trypanosomatids and Apicomplexans discussed in Ref. [18] – but mostly non-protozoan invasive microbes – including viruses, bacteria and fungi – have exploited facets of the endocytic potential of their hosts [19,20]. Likewise, functional reprogramming of macrophages from receptor-mediated phagocytosis to macropinocytosis has been observed upon activation by non-protozoan microbes, leading to increased bacterial killing in the case of *Mycobacterium* [21]. In the case of the protozoan *Leishmania amazonensis* (Trypanosomatids), amastigotes in the progeny released from reprogrammed macrophages display phosphatidylserine lipid on their outer leaflet, which serves as a signal to promote uptake via macropinocytosis and subsequent rapid amastigote cycling [22]. These examples underline that alternative uptake modes may be used by a parasite developmental stage population that is not as homogeneous as often perceived, considering the inflammatory/counter-inflammatory milieu operating in the hosting tissue(s). Future investigation should continue documenting the contrasting immune-metabolic features of two clinically silent tissues – the dermis and skin-draining lymph nodes – populated with (i) a large number of reprogrammed macrophages hosting rapidly cycling *Leishmania* amastigotes and (ii) a small number of the following reprogrammed populations: monocyte-derived macrophages, monocyte-derived dendritic cells and fibroblasts hosting persistent *Leishmania* amastigotes.

Promoting cooperation between PM-AMCM dynamics and endo-exocytic organelle functions of mammalian cells to shape a hybrid entry compartment

Invasive stratagems that mitigate the cell surface composite contribution have been evolutionary retained by some protozoan developmental stages. For instance, *Leishmania* spp. and *Trypanosoma* spp. have evolved slender, flagellated invasive parasite stages of up to a few tenths of a micron in length that do not exclusively rely on macropinocytic or phagocytotic routes to access their host cells. Indeed, when targeting non-professional vertebrate phagocytic cells, these 'actively swimming stages' are endowed with the capacity to subvert intracellular membrane pools from the host cell endo-exocytic pathway as a way to assist the PM in generating a nascent entry compartment while alleviating the risk of irreversible damage to the PM. First uncovered for fibroblast or epithelial cell invasion by the trypomastigote stage of the Chagas disease-causing *Trypanosoma cruzi* and recently demonstrated for fibroblast invasion by the metacyclic promastigote stage of *L. amazonensis* [23], this path of entry is proposed to rely at least in part on parasite mechanical forces to actually inflict local PM injury to the target cell. In response to such microbe-induced cell surface lesions and initiated by the resulting calcium influx, a local disruption of the cortex promotes exocytosis of a specific subset of PM-associated (*T. cruzi*) or PM-redistributed (*T. cruzi* and *L. amazonensis*) lysosomes at the mammalian cell surface. Explored in the *T. cruzi* model and still to be investigated for *L. amazonensis*, the concomitant release of acid sphingomyelinase to the PM outer leaflet was identified as the trigger for massive caveolae endocytosis [24]. This PM remodelling event drives removal of the wounded cell surface area, hence heading off any threats of prolonged PM leakage. To achieve efficient PM lesion mending, a contribution of the Exocyst complex to direct cytoplasmic <vesicles – lysosomes and/or endosomes – to the cell surface was also observed during *T. cruzi* trypomastigote entry [25] and it awaits confirmation in the case of invading *Leishmania* promastigotes. In addition to protecting the PM integrity, this ordered sequence of events provides the required extra membrane to enable host cell entry by both parasites in a PM-endosome-lysosome hybrid/mosaic compartment, and independently of any target cell AMCM-generated force. In this peculiar invasive scenario that unifies two groups of Trypanosomatidae, the early event(s) responsible for PM rupture of the target cells are not yet fully characterized. How the parasites mechanically assault the cell surface and how the interplay between parasite-mammalian cell surface molecules [26] could act as additive mechanisms for ensuring calcium-mediated effector functions to ensure parasite entry are issues to be addressed.

Designing a protozoan molecular device to target the PM-AMCM and to allow traction into a non-fusogenic entry compartment with minimal disturbance

In contrast to the aforementioned parasites, some invading Apicomplexa parasites can perform discreet space and time minimally imposing, although not silent,

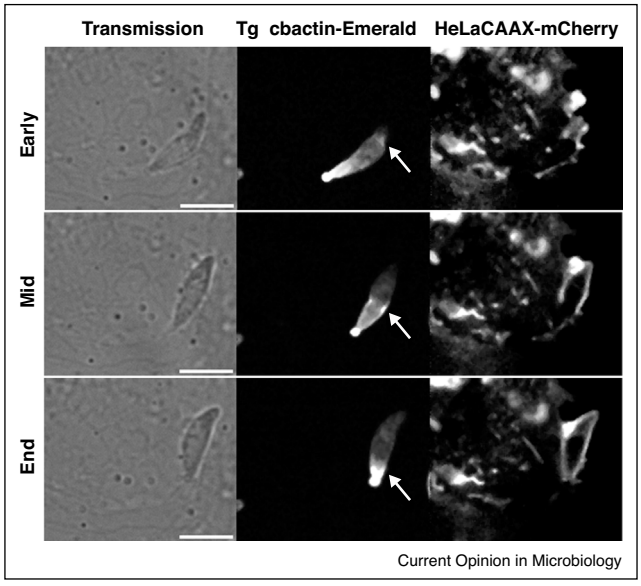
remodelling of the PM-AMCM. Achieved in only a few tens of seconds, this entry process is not associated with (i) large membrane ruffling; hence it does not co-opt with any of the 'uptaking'cortical forces driven by the mammalian cell PM-AMCM, nor with (ii) significant surface damage, not relying on endocytic/exocytic sources of membrane to support formation of an entry compartment. Falling in this category are the well-studied *Plasmodium* merozoite and *Toxoplasma gondii* tachyzoite developmental stages. Lacking motile appendages these parasites show a robust polarized architecture, primarily featured by an apical complex whose functions govern the parasite invasive skills. Indeed, within the club-shaped rhoptry organelles that populate the zoite apex, is stored a very unique invasive nanodevice designed to timely direct both the development of actomyosin-based invasive forces and the formation of a PM bud containing the invading zoites. The discovery of the RhOptry Neck (RON) multi-subunit complex and its regulated discharge once the zoite is apically positioned on the cell surface [27–29] were key clues for solving these parasites' invasive stratagem.

Despite notorious differences between the molecular composition and biomechanical properties of the PM-AMCM in the a-nucleated and organelle-free erythrocyte – the only cell targeted by the *Plasmodium* ovoid merozoite – and the wide repertoire of non-erythrocyte nucleated cells targeted by the *Toxoplasma* ellipsoidal tachyzoite, the two zoites still initiate invasion *per se* by releasing the rhoptry-derived RON complex into the mammalian cell surface. Likewise, while several specific duos of merozoite ligand-erythrocyte receptor molecules have been characterized and their timely interaction shown to initiate specific changes in architecture of the very peculiar erythrocyte PM-AMCM before RONs' discharge [30], the upstream molecular events directing the latter event remain less defined for the invasive tachyzoite. However, a first clue on the identity of a molecular trigger for RONs' delivery was provided by the recent discovery of Ferlin2, a calcium sensor strictly required for this step. This was an important finding that also provided insights into the mode of RON delivery into the target PM [31].

Visualizing the *Toxoplasma* nanodevice functions after assembly in the mammalian cell surface

Genetically transformed *T. gondii* tachyzoites engineered to express a fluorescent version of RON2, a member of the complex that spans the mammalian cell PM, combined to genetically transformed mammalian cells expressing chimeric fluorescent proteins of interest and high speed live cell imaging allowed assessment in real time the 'one shot process' of RON material release, and its assembly as a toroidal platform in the recipient PM-AMCM composite [32,33]. Such approaches have proven particularly fruitful to dissect salient features of the tachyzoite invasive stratagem, taking advantage of the 5–7 μm size of the tachyzoite, its wide host cell repertoire and its genetic tractability. The toroidal nanodevice can – within a few seconds – stretch and shrink in coordination with forces applied by the tachyzoite, which rapidly moves its body through the device into a budding portion of the PM [32]. Such a force, which is sensed by the nanodevice, is generally thought to arise from a divergent parasite actomyosin motor housed in the space between the parasite PM and the Internal Membrane Complex (IMC) underneath the PM [34]. Transmission of this force across the torus occurs through an evolutionary conserved structural interaction between the ectodomain of RON2 and the surface-exposed AMA1 protein [35,36], a surface-exposed protein that is secreted from the cigar-shaped, apically positioned microneme organelles. Although the intrinsic dynamic properties of *Plasmodium* and *Toxoplasma* actin have been extensively debated – and are not discussed here –, let us mention that the original model for motion implies that the myosin motor is fixed in place on the IMC and pulls actin filaments toward the basal end of the zoite [37]. Alternative, related [38] or more divergent [39] models assign force generation or force anchorage to the parasite actomyosinA system during both gliding and invasive motions. Overall it is clear that the actomyosinA motor drives tachyzoite gliding in 2D and 3D conditions [39] as well as during cell invasion [40]: the visualization of F-actin dynamics using a *T. gondii* tachyzoite line expressing a fluorescent chromobody actin chimera [41] confirmed that a F-actin ring is co-aligned with the nanodevice site in most invading parasites (Figure 1). Recent biophysical approaches have allowed detecting and measuring of gliding force at the PM for *Plasmodium* sporozoites [42] while laser trap-devices revealed a typical reorganization of the motor machinery along the main axis only under conditions promoting tachyzoite directional motility [43]. However, directly observing the invasive force, which might not be simply overimposed to the motile force of the free gliding tachyzoite, remains a challenge considering the second-scale time frame of interest. The most convincing datasets in support of a tachyzoite traction force applied on the torus during invasion was brought on by tracking the xyt trajectory of the apex of the parasite and the nanodevice, while the former experienced resistance to forward progression by hitting an obstacle ahead from it [32]. In this setting, the torus together with the PM into which it is inserted were seen to translocate backward. Even stronger evidence is now available thanks to a peculiar situation where invading conjoined parasites are simultaneously pulled back by each other, resulting in both being impeded in their forward motion (Figure 2). As recently attributed to force application on the nanodevice, this peculiar parasite arrangement allowed visualization of the mechanism, by which the budding entry vesicle actually separates from the PM it is derived from, to give birth to a

Figure 1



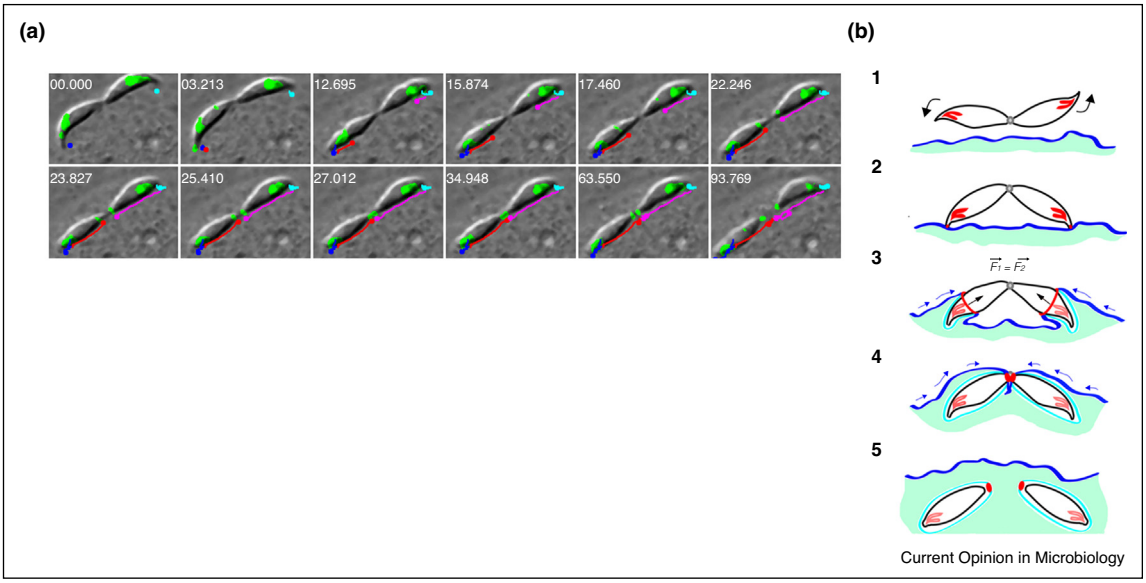
T. gondii tachyzoite F-actin re-localizes nearby the RON nanodevice insertion site in the metazoan target cell surface during the entry process.
Evidence has accumulated that the tachyzoite relies on its actomyosin motor to propel itself into a unique nascent parasitophorous vacuole, but the parasite F-actin has remained difficult to visualize in the parasite. The recent availability of a tachyzoite line expressing a chimeric F-actin chromobody-Emerald GPF has allowed visualization of F-actin dynamics during invasion in real time. The movie stills show the tachyzoite at early (top panel), mid (middle panel) and end (bottom panel) stages of the invasion event. The columns show the two cells by transmission light (left), the actin-chromobody-Emerald GPF in the tachyzoite (middle) and the CAAX-mCherry construct expressed in HeLa cells and targeted to the plasma membrane (right). White arrows point to the enrichment of F-actin aligning with the site where the nanodevice is inserted, and from where emerges the plasma membrane-derived bud that allows parasite entry. Scale bar: 5 μm.

Parasitophorous Vacuole (PV) enclosing the tachyzoite in the mammalian cell cytoplasm. Unlike previously thought, this fission step does not require PM fission specialized mechanoenzymes, namely dynamins, but instead relies on a parasite twisting motion that imposes a spiral motion of the tachyzoite basal end. The compressive forces applied on the nanodevice co-drive mechanical closure of the torus and tight sealing of the bud [33]. The authors of this study proposed that torsion and constriction during torque achieve PM fission upstream of the nanodevice, similar to what is commonly performed in concert by dynamins, actin and associated proteins to complete fission of endocytic vesicles [16]. As a result, the nanodevice incorporates in the cytoplasmic PV, thereby being removed from the cell surface. Whether the host cell PM is left totally unscathed during the torus functions has not been formerly investigated (see below), but it already became clear that unusual PM-AMCM reorganizations including PM defects are associated with the minute-range persistence of the nanodevice in the cell surface in the case of impaired invasion events [40,33].

Optimal function of the nanodevice requires PM specificities and AMCM anchoring points: can these requirements be associated with cell surface damage, hence activating membrane repair mechanisms?

To sustain insertion of the nanodevice and the resulting tensile stress force application while avoiding excessive alterations in local PM tension, proper anchoring of the RON nanodevice on the host cell is expected. RON2 partners (RON4, 8) that are retained in the cortex through interactions with the RON2 N terminal region were reported to selectively interact with the F-actin [44] and microtubule cytoskeletons [45], suggesting how the torus might acquire the ability to handle the mechanical load imposed by AMA1–RON2 during invasion. Although details on molecular links between specific RON proteins and the cytoskeleton await confirmation, anchoring reinforcement through local assembly of cortical actin is supported by the transient recruitment of the ARP2/3 actin nucleator complex and its regulatory partner cortactin to the site of parasite entry [46]. Two novel mammalian proteins that bind directly to different regions of RON4 were recently identified: ALIX, an accessory member of the endosomal sorting complex required for transport (ESCRT), and the adaptor protein CIN85 (Cbl-interacting protein of 85 kDa) which acts as a scaffold for a variety of endocytic accessory proteins, including ALIX [47]. However, ALIX-associated ESCRT components required for membrane fission and repair were not detected at the nanodevice site [47] and the invasion process remains unaltered in ALIX null fibroblasts [33]. Interestingly, Poupel *et al.* proposed that ALIX and CIN85 might assist the actin cytoskeleton for cortical anchoring of the nanodevice through their ability to bind cortactin, an activator of the ARP2/3 actin nucleator complex. In addition, the rhoptry effector toxofilin protein was shown to directly impact the AMCM actin dynamics [48]. Delivered by the tachyzoite upon apical contact with the host cell surface [49], toxofilin promotes severing of actin filaments, causing local release of actin monomers [50]. Therefore, the actin monomer pool could potentially fuel the ARP2/3 complex-driven actin assembly seen at the nanodevice site. It is still unclear if toxofilin-mediated loosening of the cortex, known to facilitate formation of the tachyzoite entry compartment, can also facilitate attachment of the RON nanodevice to the F-actin cortex. Major challenges to be overcome include deciphering the spatial organization of the RON members within an active torus, and better understanding the torus capacity to withstand the parasite invasive force when bridged to specific components of both parasite and mammalian cells. Similarly, there is a need to clarify the unique sieving properties of the invasive nanodevice, which allows selection of PM components that

Figure 2



The tachyzoite applies an invasive force on the RONs nanodevice to propel itself into the metazoan host cell.
Evidence has accumulated that the nanodevice acts as a traction point for tachyzoite invasion of the host cell. A unique demonstration of this is shown with two *Toxoplasma* tachyzoites attached at their posterior ends through the maternal residual body. Both parasites simultaneously assemble a nanodevice and start to invade the same cell, thereby pulling on each other and imposing reciprocal resistance. Because the two invasion events are precisely synchronized, both parasites attempt to apply traction force that translates into a symmetrical rearward displacement and closure of their junctions, eventually isolating each in a distinct vacuole (*unpublished data*). Movie stills are shown in **(a)** schematic is shown in **(b)**.
1_ Constrained extracellular motility: posteriorly connected tachyzoites extrude their conoids and attempt to move forward in opposite directions on top of a Ptk cell monolayer; by applying similar force this results in almost no net displacement of the parasites, which remain attached to each other.
2_ Torus assembly: both tachyzoites simultaneously release RONs (visualized by RON2-mCherry) that assemble a torus in the host cell surface.
3_ Force engagement on the torus: both tachyzoites pull simultaneously on the torus inserted in the PM and impose resistance to forward movement on each other. This situation leads to a symmetrical rearward displacement of the torus, suggesting that the forces applied by both zoites are of similar intensity.
4_ Membrane sealing: complete translocation of the torus to the posterior pole coincides in both tachyzoites, leading to concomitant closure of the PM at the torus site.
5_ Intracellular vacuole: each tachyzoite ends up in their respective vacuoles.

incorporate into the parasite-driven PM bud. By retaining most PM-associated proteins outside from this bud, the nanodevice controls the emergence of a lipid-enriched and fluid membrane around the invading tachyzoite, probably easing the invasion process. In addition, the PVM is rapidly remodeled post PV fission [33] and remains excluded from the endocytic pathway thereby provide a safe growth-permissive residence to the parasite [51].

Conclusions

The diversity of invasive stratagems evolved by intracellular protozoans to access mammalian cells are obviously not restricted to the situations depicted in this review. For instance, some medically important Apicomplexa including *Cryptosporidum* spp. in humans or *Theileria* spp. in non-human animals do not rely on the RON nanodevice to remodel the PM-AMCM when they infect target cells. Here we have highlighted recent advances in molecular and imaging technologies that have brought insights on the fascinating 'molecular language' repertoire unfolding at the cell surface composite of a range of mammalian cells, as they interact with Apicomplexan and Trypanosomatidae protozoans. Yet, the distinct mammalian cell lineages operating as either host cells or parasite-shuttling cells are durably or transiently anchored to basement membranes or the extracellular matrix (ECM), so how the dynamic ECM features contribute to the molecular language repertoire remains understudied. We foresee that these gaps may be approached by (i) implementing biomechanics and mechanotransduction analysis to determine how bilateral force generation and cellular responses are integrated during microbe-induced PM-AMCM coupling, and (ii) engineering organoids of metazoan host cells where single-celled eukaryotic parasites can deploy the complex stepwise developmental programs on which their fitness relies.

Conflict of interest

Nothing declared.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.mib.2019.06.007.

Acknowledgements

We thank the Foundation for Innovation in Infectiology (FINOVI) for funding our research program (France), the University Grenoble-Alpes for the AGIR PhD fellowship support to Georgios Pavlou, and Prof. P. Hainaut (IAB, Grenoble, France) for internship support to the IT team. We thank Dr MA. Hakimi for sharing discussions along the writting of this review.

References

1. Köster DV, Husain K, Iljazi E, Bhat A, Bieling P, Mullins RD, Rao M, Mayor S: **Actomyosin dynamics drive local membrane component organization in an in vitro active composite layer**. *Proc Natl Acad Sci U S A* 2016, **113**:E1645-E1654.

2. Chugh P, Clark AG, Smith MB, Cassani DAD, Dierkes K, Ragab A, Roux PP, Charras G, Salbreux G, Paluch EK: **Actin cortex architecture regulates cell surface tension**. *Nat Cell Biol* 2017, **19**:689-697.

3. Chugh P, Paluch EK: **The actin cortex at a glance**. *J Cell Sci* 2018, **131** jcs186254.

4. Luscinskas FW, Leick M, Newton G, Nusrat A: **Introduction for the special issue on "Tissue Barriers in Inflammation."**. *Tissue Barriers* 2015, **3**:e1015825.

5. Ishino T, Chinzei Y, Yuda M: **A plasmodium sporozoite protein with a membrane attack complex domain is required for breaching the liver sinusoidal cell layer prior to hepatocyte infection: role of a MACPF-related protein in the malarial life cycle**. *Cell Microbiol* 2004, **7**:199-208.

6. Tavares J, Formaglio P, Thiberge S, Mordelet E, Van Rooijen N, Medvinsky A, Ménard R, Amino R: **Role of host cell traversal by the malaria sporozoite during liver infection**. *J Exp Med* 2013, **210**:905-915.

7. Yang ASP, O'Neill MT, Jennison C, Lopaticki S, Allison CC, Armistead JS, Erickson SM, Rogers KL, Ellisdon AM, Whisstock JC *et al.*: **Cell traversal activity is important for plasmodium falciparum liver infection in humanized mice**. *Cell Rep* 2017, **18**:3105-3116.

8. Risco-Castillo V, Topçu S, Marinach C, Manzoni G, Bigorgne AE, Briquet S, Baudin X, Lebrun M, Dubremetz J-F, Silvie O: **Malaria sporozoites traverse host cells within transient vacuoles**. *Cell Host Microbe* 2015, **18**:593-603.

9. Guerra Alfredo, Carruthers Vern: **Structural features of apicomplexan pore-forming proteins and their roles in parasite cell traversal and egress**. *Toxins* 2017, **9**:265.

10. Inoue N, Hagihara Y, Wright D, Suzuki T, Wada I: **Oocyte-triggered dimerization of sperm IZUMO1 promotes sperm–egg fusion in mice**. *Nat Commun* 2015, **6**.

11. Nishimura K, Han L, Bianchi E, Wright GJ, de Sanctis D, Jovine L: **The structure of sperm izumo1 reveals unexpected similarities with Plasmodium invasion proteins**. *Curr Biol* 2016, **26**:R661-R662.

12. David Sibley L: **Invasion and intracellular survival by protozoan parasites: parasite invasion strategies**. *Immunol Rev* 2011, **240**:72-91.

13. Walker DM, Oghumu S, Gupta G, McGwire BS, Drew ME, Satoskar AR: **Mechanisms of cellular invasion by intracellular parasites**. *Cell Mol Life Sci* 2014, **71**:1245-1263.

14. King JS, Kay RR: **The origins and evolution of macropinocytosis**. *Philos Trans R Soc B Biol Sci* 2019, **374** 20180158.

15. Bloomfield G, Kay RR: **Uses and abuses of macropinocytosis**. *J Cell Sci* 2016, **129**:2697-2705.

16. Antonny B, Burd C, De Camilli P, Chen E, Daumke O, Faelber K, Ford M, Frolov VA, Frost A, Hinshaw JE *et al.*: **Membrane fission by dynamin: what we know and what we need to know**. *EMBO J* 2016, **35**:2270-2284.

17. Moreau HD, Blanch-Mercader C, Attia R, Maurin M, Alraies Z, Sanséau D, Malbec O, Delgado M-G, Bousso P, Joanny J-F *et al.*: **Macropinocytosis overcomes directional bias in dendritic cells due to hydraulic resistance and facilitates space exploration**. *Dev Cell* 2019, **49**:171-188.e5.

18. de Carvalho TMU, Barrias ES, de Souza W: **Macropinocytosis: a pathway to protozoan infection**. *Front Physiol* 2015, **6**.

19. Fernandes MC, Flannery AR, Andrews N, Mortara RA: **Extracellular amastigotes of *Trypanosoma cruzi* are potent inducers of phagocytosis in mammalian cells: *T. cruzi* amastigotes induce phagocytosis**. *Cell Microbiol* 2013, **15**:977-991.

20. Mercer J, Helenius A: **Virus entry by macropinocytosis**. *Nat Cell Biol* 2009, **11**:510-520.

21. BoseDasgupta S, Pieters J: **Inflammatory stimuli reprogram macrophage phagocytosis to macropinocytosis for the rapid elimination of pathogens**. *PLoS Pathog* 2014, **10**:e1003879.

22. Wanderley JLM, Deolindo P, Carlsen E, Portugal AB, DaMatta RA, Barcinski MA, Soong L: **CD4+ T cell-dependent macrophage activation modulates sustained PS exposure on intracellular amastigotes of *Leishmania amazonensis***. *Front Cell Infect Microbiol* 2019, **9**.

23. Cavalcante-Costa VS, Costa-Reginaldo M, Queiroz-Oliveira T, Oliveira ACS, Couto NF, dos Anjos DO, Lima-Santos J, Andrade LO, Horta MF, Castro-Gomes T: ***Leishmania amazonensis* hijacks host cell lysosomes involved in plasma membrane repair to induce invasion in fibroblasts**. *J Cell Sci* 2019, **132** jcs226183.

24. Fernandes MC, Cortez M, Flannery AR, Tam C, Mortara RA, Andrews NW: ***Trypanosoma cruzi* subverts the sphingomyelinase-mediated plasma membrane repair pathway for cell invasion**. *J Exp Med* 2011, **208**:909-921.

25. Fernandes MC, Corrotte M, Miguel DC, Tam C, Andrews NW: **The exocyst is required for trypanosome invasion and the repair of mechanical plasma membrane wounds**. *J Cell Sci* 2015, **128**:27-32.

26. Maeda FY, Cortez C, Yoshida N: **Cell signaling during *Trypanosoma cruzi* invasion**. *Front Immunol* 2012, **3**.

27. Lebrun M, Michelin A, El Hajj H, Poncet J, Bradley PJ, Vial H, Dubremetz JF: **The rhoptry neck protein RON4 re-localizes at the moving junction during *Toxoplasma gondii* invasion**. *Cell Microbiol* 2005, **7**:1823-1833.

28. Alexander DL, Mital J, Ward GE, Bradley P, Boothroyd JC: **Identification of the moving junction complex of *Toxoplasma gondii*: a collaboration between distinct secretory organelles**. *PLoS Pathog* 2005, **1**:e17.

29. Besteiro S, Michelin A, Poncet J, Dubremetz J-F, Lebrun M: **Export of a *Toxoplasma gondii* rhoptry neck protein complex at the host cell membrane to form the moving junction during invasion**. *PLoS Pathog* 2009, **5**:e1000309.

30. Weiss GE, Gilson PR, Taechalertpaisarn T, Tham W-H, de Jong NWM, Harvey KL, Fowkes FJI, Barlow PN, Rayner JC, Wright GJ *et al.*: **Revealing the sequence and resulting cellular morphology of receptor-ligand interactions during *Plasmodium falciparum* invasion of erythrocytes**. *PLOS Pathog* 2015, **11**:e1004670.

31. Coleman BI, Saha S, Sato S, Engelberg K, Ferguson DJP, Coppens I, Lodoen MB, Gubbels M-J: **A member of the ferlin calcium sensor family is essential for *Toxoplasma gondii* rhoptry secretion**. *mBio* 2018, **9**:14.

32. Bichet M, Joly C, Henni AH, Guilbert T, Xémard M, Tafani V, Lagal V, Charras G, Tardieux I: **The toxoplasma-host cell junction is anchored to the cell cortex to sustain parasite invasive force**. *BMC Biol* 2014, **12**:773.

33. Pavlou G, Biesaga M, Touquet B, Lagal V, Balland M, Dufour A, Hakimi M-A, Tardieux I: **Toxoplasma parasite twisting motion**

mechanically induces host cell membrane fission to complete invasion within a protective vacuole. *Cell Host Microbe* 2018, **24**:81-96.e5.

34. Meissner M, Schlüter D, Soldati D: **Role of *Toxoplasma gondii* myosin A in powering parasite gliding and host cell invasion**. *Science* 2002, **298**:837-840.

35. Tonkin ML, Roques M, Lamarque MH, Pugniere M, Douguet D, Crawford J, Lebrun M, Boulanger MJ: **Host cell invasion by apicomplexan parasites: insights from the co-structure of AMA1 with a RON2 peptide**. *Science* 2011, **333**:463-467.

36. Vulliez-Le Normand B, Tonkin ML, Lamarque MH, Langer S, Hoos S, Roques M, Saul FA, Faber BW, Bentley GA, Boulanger MJ *et al.*: **Structural and functional insights into the malaria parasite moving junction complex**. *PLoS Pathog* 2012, **8**:e1002755.

37. Heintzelman MB: **Gliding motility in apicomplexan parasites**. *Semin Cell Dev Biol* 2015, **46**:135-142.

38. Tardieux I, Baum J: **Reassessing the mechanics of parasite motility and host-cell invasion**. *J Cell Biol* 2016, **214**:507-515.

39. Whitelaw JA, Latorre-Barragan F, Gras S, Pall GS, Leung JM, Heaslip A, Egarter S, Andenmatten N, Nelson SR, Warshaw DM *et al.*: **Surface attachment, promoted by the actomyosin system of *Toxoplasma gondii* is important for efficient gliding motility and invasion**. *BMC Biol* 2017, **15**:1.

40. Bichet M, Touquet B, Gonzalez V, Florent I, Meissner M, Tardieux I: **Genetic impairment of parasite myosin motors uncovers the contribution of host cell membrane dynamics to Toxoplasma invasion forces**. *BMC Biol* 2016, **14**:97.

41. Periz J, Whitelaw J, Harding C, Gras S, Del Rosario Minina MI, Latorre-Barragan F, Lemgruber L, Reimer MA, Insall R, Heaslip A *et al.*: ***Toxoplasma gondii* F-actin forms an extensive filamentous network required for material exchange and parasite maturation**. *eLife* 2017, **6**.

42. Quadt KA, Streichfuss M, Moreau CA, Spatz JP, Frischknecht F: **Coupling of retrograde flow to force production during malaria parasite migration**. *ACS Nano* 2016, **10**:2091-2102.

43. Stadler RV, White LA, Hu K, Helmke BP, Guilford WH: **Direct measurement of cortical force generation and polarization in a living parasite**. *Mol Biol Cell* 2017, **28**:1912-1923.

44. Straub KW, Peng ED, Hajagos BE, Tyler JS, Bradley PJ: **The moving junction protein RON8 facilitates firm attachment and host cell invasion in *Toxoplasma gondii***. *PLoS Pathog* 2011, **7**: e1002007.

45. Takemae H, Sugi T, Kobayashi K, Gong H, Ishiwa A, Recuenco FC, Murakoshi F, Iwanaga T, Inomata A, Horimoto T *et al.*: **Characterization of the interaction between *Toxoplasma gondii* rhoptry neck protein 4 and host cellular β-tubulin**. *Sci Rep* 2013, **3**.

46. Gonzalez V, Combe A, David V, Malmquist NA, Delorme V, Leroy C, Blazquez S, Ménard R, Tardieux I: **Host cell entry by apicomplexa parasites requires actin polymerization in the host cell**. *Cell Host Microbe* 2009, **5**:259-272.

47. Guérin A, Corrales RM, Parker ML, Lamarque MH, Jacot D, El Hajj H, Soldati-Favre D, Boulanger MJ, Lebrun M: **Efficient invasion by Toxoplasma depends on the subversion of host protein networks**. *Nat Microbiol* 2017, **2**:1358-1366.

48. Poupel O, Boleti H, Axisa S, Couture-Tosi E, Tardieux I: **Toxofilin, a novel actin-binding protein from *Toxoplasma gondii*, sequesters actin monomers and caps actin filaments**. *Mol Biol Cell* 2000, **11**:355-368.

49. Lodoen MB, Gerke C, Boothroyd JC: **A highly sensitive FRET-based approach reveals secretion of the actin-binding protein toxofilin during *Toxoplasma gondii* infection**. *Cell Microbiol* 2010, **12**:55-66.

50. Delorme-Walker V, Abrivard M, Lagal V, Anderson K, Perazzi A, Gonzalez V, Page C, Chauvet J, Ochoa W, Volkmann N *et al.*: **Toxofilin upregulates the host cortical actin cytoskeleton dynamics, facilitating Toxoplasma invasion**. *J Cell Sci* 2012, **125**:4333-4342.

51. Charron AJ, Sibley LD: **Molecular partitioning during host cell penetration by *Toxoplasma gondii***. *Traffic* 2004, **5**:855-867.