Contact and competition between mitochondria and microbes

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Highlights:

Contact sites between eukaryotic pathogens and mitochondria are prevalent

Host and parasite factors that mediate contact sites are tools to interrogate their role

Interactions between host mitochondria and parasites can shape each other's metabolism

Contact sites can promote metabolic exchanges between host mitochondria and parasites

Abstract:

Invading microbes occupy the host cytosol and take up nutrients on which host organelles are equally dependent. Thus, host organelles are poised to interact with intracellular microbes. Despite the essential role of host mitochondria in cellular metabolic homeostasis and in mediating cellular responses to microbial infection, we know little of how these organelles interact with intracellular pathogens, and how such interactions affect disease pathogenesis. Here, we give an overview of the different classes of physical and metabolic interactions reported to occur between mitochondria and eukaryotic pathogens. Investigating the underlying molecular mechanisms and functions of such interactions will reveal novel aspects of infection biology.

Introduction:

Unlike other organelles, mitochondria possess their own DNA and are descendant from an alphaproteobacterial ancestor [1]. These uniquely compartmentalized organelles also have a remarkable capacity to change in number, morphology, interacting organellar partners and subcellular location in response to cellular cues [2]. Such changes tailor mitochondrial function to meet cellular needs, and coordinate many cellular functions essential for life, including diverse metabolic processes, cell differentiation, and immune signaling [3,4]. It is therefore no surprise that microbial infection—which undoubtedly exerts a profound stress on diverse aspects of cellular function—leads to alterations in mitochondrial behavior, and in several cases, contact between mitochondria and pathogens [5].

Close encounters with the parasite-kind

One of the earliest described interactions between mitochondria and intracellular microbes is the encasement of parasitic vacuoles in which certain parasites reside and replicate, by host mitochondria [6]. First discovered in murine macrophages infected with the intracellular Apicomplexan parasite *Toxoplasma gondii* in 1972, this interaction has since been shown to occur in cell lines of diverse mammalian origin, as well as in zebrafish in vivo [6-8]. More recently, mitochondria have also been shown to encase the vacuoles of other apicomplexan parasites such as *Hammondia hammondi* and *Neospora caninum,* and the microsporidian *Encephalitozoon sp* (Fig.1)[9-11]. Thus, this phenomenon appears to be a more generalized consequence of infection with eukaryotic (and even prokaryotic) pathogens [12]. Intriguingly, naturally occurring strains of *Toxoplasma* and *Neospora* exist that are not encased by host mitochondria, raising the question of whether this host-pathogen interaction evolved in a specific environment [9,13,14].

A hallmark of this encasement is the formation of regions of close membrane apposition between the host outer mitochondrial membrane (OMM) and the parasite vacuole membrane (PVM)[7]. These regions are less than 30 nm in distance, between 2-10 μm in length and can be referred to as trans-kingdom ‘contact sites,’ a term coined to describe homotypic or heterotypic regions of membrane association between organelles [7,15-17]. The most well-studied contact site is that between *Toxoplasma* and host mitochondria, previously termed host mitochondrial association (HMA)(Fig. 1)[14]. HMA is completely dependent on the secreted *Toxoplasma* effector protein MAF1 (mitochondrial association factor 1) that is anchored in the PVM (Fig. 1)[14,18]. Recent evidence supports that the host mitochondrial import receptor TOM70 (translocase of the outer membrane 70) and/or components of the MIB (mitochondria intermembrane space bridging) complex interact with the MAF1 carboxy terminus to mediate HMA [19,20]. Although MAF1 has been implicated in regulating host immune responses in vitro and in vivo, how its OMM-tethering function

contributes to such changes in the host has remained elusive [14,18]. In the case of hepatocytes infected with the apicomplexan *Plasmodium berghei* that is a near-relative of *Toxoplasma,* mitochondria appear in contact with unique PVM protrusions that extrude from the parasite vacuole (PV) into the host cell cytosol, or aligned along the PVM perimeter (Fig. 1). These observations suggest the existence of host factors that position mitochondria peripheral to the *Plasmodium* PVM but that remain as of yet unidentified.

Some progress has been made toward resolving the factors that mediate the formation of contact sites between mitochondria and the vacuolar niche of *Encephalitozoon hellem* (E. hellem) meronts. The *E. hellem* SSP1 (sporoplasm surface protein 1) that is exposed to the host cell cytosol and localizes to mitochondria when added to permeabilized fibroblasts was found to interact with host mitochondrial VDAC (voltage-dependent anion channel) in vitro (Fig. 1)[21]. Although the loss of host VDAC1 and its homologue VDAC3 diminished contact sites between the *E. hellem* vacuole and mitochondria, whether EhSSP1 binding to VDAC1 is required for their formation needs further evaluation [21].

The parasite vacuole membrane is not the only reported interface between mitochondria and eukaryotic pathogens. Intriguingly, direct contact has been reported between the kinetoplastid parasite *Trypanosoma cruzi* (*T. cruzi*) and host mitochondria. Unlike *Toxoplasma* and *Encephalitozoon sp, T. cruzi* proliferates freely in the host cell cytosol [22]. The rearrangement of mitochondria in close proximity to *T. cruzi* parasites occurs shortly after infection and is posited to result from the close contacts formed between the parasite flagellum and host mitochondria [23]. This unique interaction is less than 10 nm in distance and appears to be only 1-2 μm in length, but persists from early to late stages of infection [23]. It is also maintained following cell detachment and reattachment, suggesting that a high-affinity, potentially protein-protein interaction between host mitochondria and the *T. cruzi* flagellum [23]. Investigating the factors that mediate this interaction and whether it is

more broadly conserved among cytosolic kinetoplastids will be an interesting topic for future studies.

Friend or foe?

An obvious question arises from the mitochondria-parasite interactions discussed above: who makes first contact and why? Contact sites promote the exchange of ions, proteins, and metabolites between the apposed membranes [16]. Mitochondria are anabolic hubs and intracellular parasites depend on their hosts for nutrients [24,25]. Therefore, an expectation is that eukaryotic pathogens such as *Toxoplasma, Plasmodium*, and *Microsporidian* hijack mitochondrial metabolites through the formation of contact sites with mitochondria to create a viable niche for themselves.

Microsporidian parasites have a limited capacity for ATP synthesis and thus are under pressure to salvage this energy unit from their host [21,26]. The contact sites formed between the microsporidian meront vacuole and host mitochondria provide a potential route by which *E. hellem* may scavenge host ATP (Fig. 1). Indeed, the host-mitochondrial voltage-dependent anion channel (VDAC), known to transport ATP, is enriched at mitochondria-PV contact sites formed during *E. cuniculi* and *E. hellem* infection [21,26]. It remains unclear, however, whether EhSSP1 binding to VDAC directly enables ATP uptake or whether this interaction is required for microsporidia-mitochondria contact sites.

Recent work suggests that cardiolipin, a lipid unique to mitochondria, may be scavenged by *Toxoplasma* at contact sites [27]. Parasites lacking ACBP2 (acyl coenzyme A (CoA)-binding protein) exhibit decreased cardiolipin levels that is complemented by the expression of MAF1 [27]. How cardiolipin traverses four membranes—the host OMM, parasite PVM, parasite PM, and presumably the parasite OMM—remains a mystery. Both *Toxoplasma* and *Plasmodium* scavenge the antioxidant lipoic acid from host mitochondria despite its de novo synthesis in these apicomplexan parasites [28,29]. Although the molecular factors involved

in lipoate salvage have not yet been identified, it is plausible that, similar to the case of cardiolipin, such exchange is mediated by the extensive contact sites formed between host mitochondria and *Toxoplasma.* Liver-stage *Plasmodium* however form contact sites to a much lesser extent, suggesting these parasites either import less host lipoic acid or utilize other modes for its scavenging [29]. Of note, red blood cells lack mitochondria, and thus blood-stage parasites appear to be well-adapted to an apparent lack of host lipoic acid.

Host fatty acid oxidation (FAO) fuels *T. cruzi* growth [30]. Potential benefits for the parasite may be derived from the use of FAO intermediates generated in host peroxisomal or mitochondrial oxidative pathways, or indirectly from the ostensible increase in glucose availability. In either scenario, contact between host mitochondria and the parasite flagellum, which plays a sensory role in the parasite life cycle, may enable the receipt of mitochondria-derived signals or FAO intermediates that promote parasite adaptation within host cells [23].

While the above studies point to a pro-parasite role for contact sites, emerging evidence suggests they may also facilitate mitochondrial antimicrobial defenses, for example through enabling nutrient competition. During *Toxoplasma* infection, mitochondria fuse around the PV and enhance fatty acid (FA) uptake to enact an innate-type defense that restrict FAs available to *Toxoplasma*, thereby limiting its growth (Fig. 2)[31]. This defense appears to be counteracted by parasite-induction of host mitochondrial fragmentation that occurs at later stages (Fig. 1) [31]. Further work is needed to define the signaling inputs that enhance mitochondrial FA uptake and fusion during infection, and determine the mitochondrial populations that are most required for this defense (i.e. in association with or distant from the PV)[31].

Mitochondria are also large consumers of nutrients for which intracellular microbes are auxotrophic, raising the question of whether mitochondria exploit contact sites to sequester essential metabolites from microbes. For example, mitochondria require a significant amount of ‘host’ purines and deoxynucleotide triphosphates (dNTPs) to replicate their DNA (mtDNA).

All parasitic protozoa characterized to date, including *Toxoplasma, T. cruzi*, and *Plasmodium*, are auxotrophic for purines and have evolved a unique set of purine transporters and salvage enzymes to scavenge these essential nutrients from their host [32-35]. Thus, to compete with parasites for dNTPs, mitochondria may increase purine- or dNTP-uptake during infection. Such a defense may be enacted through an increase in expression or activity of the relevant mitochondrial transporters at contact sites, as in the case of host VDAC during *Microsporidia* infection [21,26](Fig. 1). The amino acid glutamine is similarly required by both mitochondria and parasites to meet the increased demand for ATP and biosynthetic precursors, and thus may also be competed for during infection (Fig. 2) [36-39].

Beyond nutrient competition, mitochondria may deliver molecules that decrease parasite viability, either at contact sites or through vesicle-mediated transport of the relevant enzymes [40,41]. In support of this possibility, mice fed a high-fat diet produce more mitochondrial reactive oxygen species (ROS) and exhibit decreased *Plasmodium berghei* parasite burden [42]. It is important to note however, that mitochondrial ROS can be a double-edged sword and also trigger the differentiation of parasites into more virulent forms, as occurs during *Leishmania* infection [43]. Alternatively, mitochondria may exploit contact sites to supply metabolites that modulate the parasite epigenome so as to slow growth; for example citrate that is required for acetylation, or α-ketoglutarate that serves as a cofactor for epigenetic modifying enzymes (Fig. 2)[44,45]. Interestingly, *Plasmodium sp* differentiates into a chronic stage in hepatocytes while *Toxoplasma* does so in neural and muscle tissues—could this reflect epigenetic consequences of tissue-specific mitochondrial metabolites [46,47]?

Viewing mitochondria-microbe interactions solely from an antagonistic lens neglects the fact that communication between parasites and their hosts is constant and bidirectional, and thus in certain scenarios, may have evolved into (or remained) a mutualistic interaction(s). Such a possibility is not unprecedented; eukaryotes have repeatedly gained complex metabolic

capabilities by establishing symbioses with other organisms [48,49] as evident in the mosaic assembly of a peptidoglycan biosynthetic route through the cooperation of the mealybug *Planococcus citri*, and the two gammaproteobacterial endosymbionts *Candidatus Tremlaya* and *C. Moranella* [50]. Intriguingly, mammals are auxotrophic for certain essential metabolites that some Apicomplexan parasites can synthesize like Vitamin B5, also known as pantothenate (PAN)[51,52]. Vitamin B5 is the precursor for coenzyme A (CoA), a cofactor required for the generation of key metabolites including lipoic acid, fatty acids, and heme, and for a broad range of metabolic processes including the tricarboxylic acid cycle [53]. Thus, the close association of mitochondria to the vacuole of apicomplexan parasites may enable the establishment of a mutualistic metabolic exchange in which mitochondria profit from parasite-derived vitamin B5 and promote the synthesis of lipoic acid that is scavenged by *Toxoplasma* and *Plasmodium* (Fig. 2)[28,29].

Conclusions

Here, we have briefly summarized the current state of knowledge on physical and metabolic interactions between mitochondria and parasites. In most cases, neither the host proteins nor the parasite effectors involved are known, and several questions unanswered. For example, how do mitochondria and parasites sense and contact each other? Do microbes attract and tether mitochondria to scavenge their nutrients, or do mitochondria actively traffic to the parasite to execute host defenses? It is hard to imagine that there is a clear winner. Hosts are under pressure to develop mechanisms to prevent the exploitation of nutrients by microbes, and that would be expected to lead to the emergence of microbial resistance mechanisms. A more holistic approach to define the signals and machinery that drive interactions between parasites and mitochondria will broaden our current understanding of infection biology.

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Declaration of Interest: None.

Figure 1

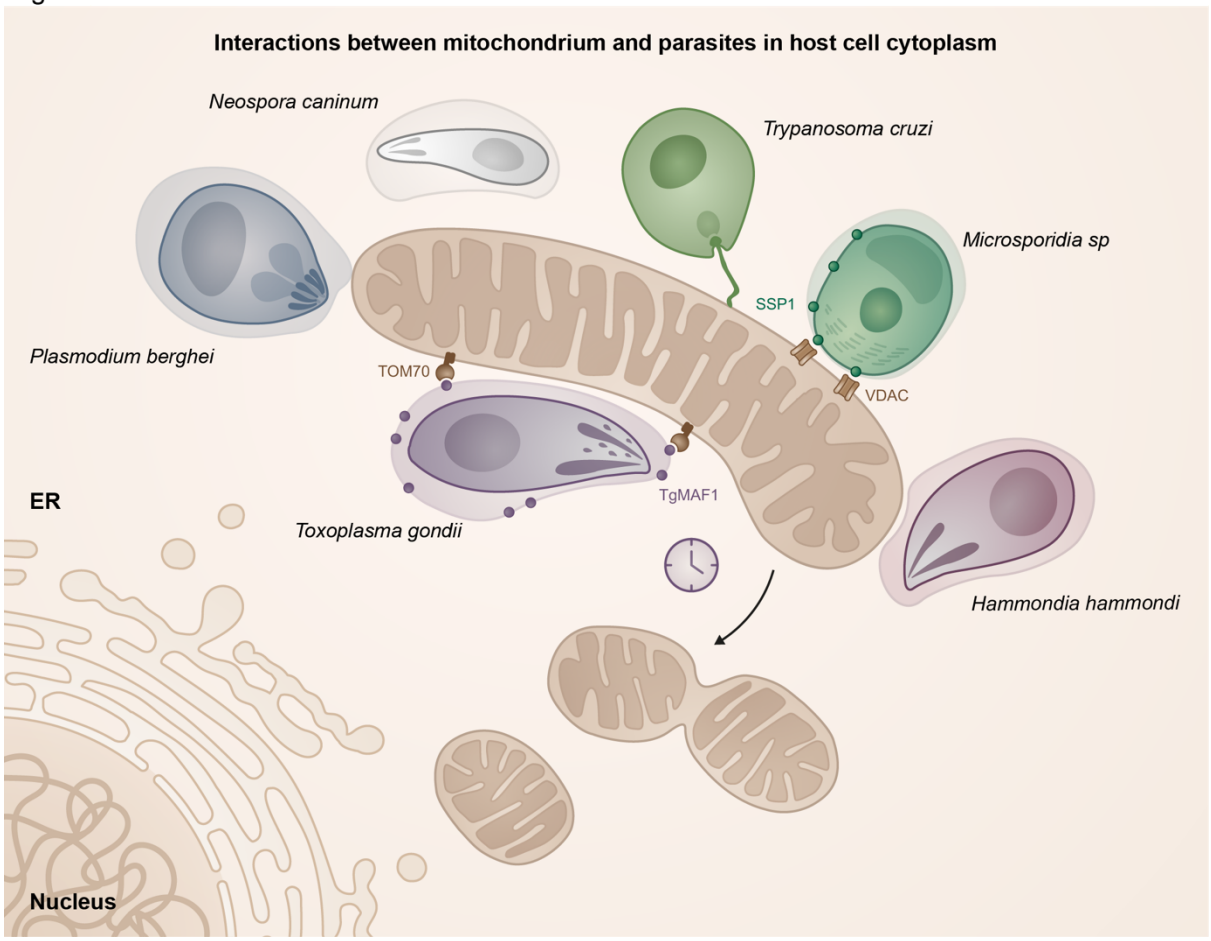

Figure 1: Types of physical contact between eukaryotic pathogens and host mitochondria. Schematic of contact sites between host mitochondria and vacuoles containing the parasites *Toxoplasma gondii, Plasmodium berghei*, *Hammondia hammondi*, *Neospora caninum*, and *Encephalitozoon sp,* as well as the *Trypanosoma cruzi* flagellum. Host proteins and parasite effectors mediating these interactions include *E. hellem* SSP1 (sporoplasm surface protein 1) and host VDAC (voltage dependant anion-selective channel),

and *Toxoplasma* MAF1 and host TOM70 (translocase of the outer membrane 70). Clock indicates fragmentation of host mitochondria at late stages of *Toxoplasma* infection.

Figure 2

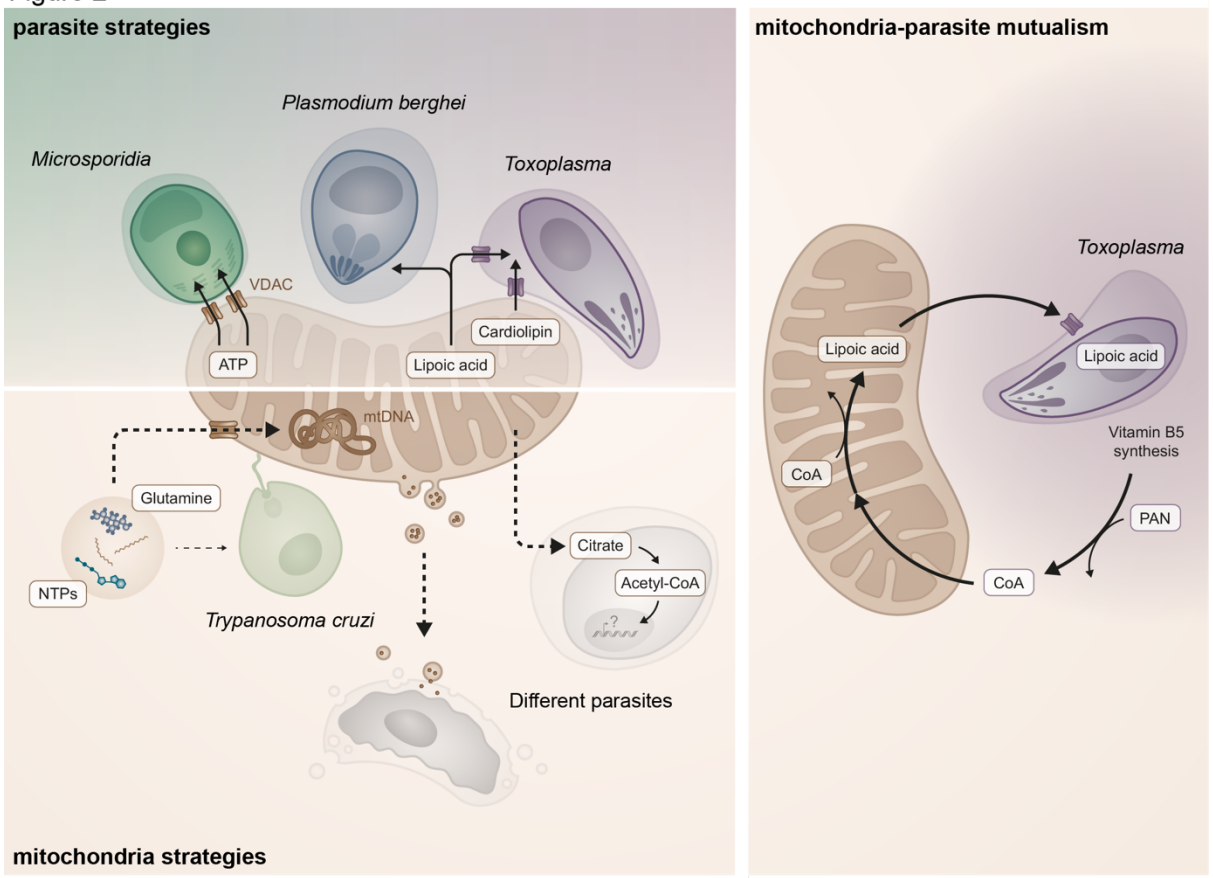

Figure 2: Metabolic competition and cooperation between mitochondria and eukaryotic pathogens. A. Top left panel**:** parasite strategies to access mitochondrial nutrients. *Toxoplasma, Plasmodium and Microsporidian* acquisition of mitochondrial metabolites is mediated by contact sites with mitochondria. Bottom left panel: hypothetical strategies (indicated with dotted line-arrows) by which mitochondria can reduce parasite viability including (from left to right): limiting access of pathogens to host nutrients through increased uptake mitochondrial uptake of glutamine and NTPs, vesicle-mediated delivery of anti-microbial molecules such as reactive oxygen species (ROS), or modulating levels of metabolites such as citrate that can be used for acetylation of the parasite epigenome**.** ATP; adenosine triphosphate; NTPs, nucleotide triphosphates. B. Hypothetical metabolic mutualism between host mitochondria and a parasite. Apicomplexan parasite derived

243 Vitamin B5 (also pantothenate, PAN) enables host synthesis of coenzyme A (CoA).

244 Mitochondrial use of CoA fuels the synthesis of lipoic acid that is scavenged by *Toxoplasma*.

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Outstanding interest:

14. This work identifies *Toxoplasma* MAF1 as the parasite protein that tethers host mitochondria to the PV.

19. This work identifies mitofilin and SAM50 as host mitochondrial proteins important for contact sites between mitochondria and the *Toxoplasma* PV.

20. In this paper, TOM70 is identified as a host mitochondrial protein important for contact sites between mitochondria and the *Toxoplasma* PV.

23. This paper demonstrates direct contact between mitochondria and a eukaryotic pathogen for the first time

50. This work demonstrates peptidoglycan synthesis through cooperation between mealybugs and bacterial endosymbionts.

Special interest:

27. This work provides evidence that host cardiolipin may be scavenged at mitochondria *Toxoplasma* contact sites

31. This paper illustrates that mitochondria can restrict *Toxoplasma* growth through compete for fatty acids

21. This paper identifies *E. hellem* SSP1 as a candidate mediator of microsporidian PV-mitochondria contact sites