Protein control of membrane and organelle dynamics: Insights from the divergent eukaryote Toxoplasma gondii
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Abstract
Integral membrane protein complexes control key cellular functions in eukaryotes by defining membrane-bound spaces within organelles and mediating inter-organelle contacts. Despite the critical role of membrane complexes in cell biology, most of our knowledge is from a handful of model systems, primarily yeast and mammals, while a full functional and evolutionary understanding remains incomplete without the perspective from a broad range of divergent organisms. Apicomplexan parasites are single-cell eukaryotes whose survival depends on organelle compartmentalisation and communication. Studies of a model apicomplexan, Toxoplasma gondii, reveal unexpected divergence in the composition and function of complexes previously considered broadly conserved, such as the mitochondrial ATP synthase and the tethers mediating ER–mitochondria membrane contact sites. Thus, Toxoplasma joins the repertoire of divergent model eukaryotes whose research completes our understanding of fundamental cell biology.

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Introduction
Membrane bound complexes play key roles in the control of organelle function through mediating interactions and exchange between membranes and through defining membrane shape. Some complexes mediate interactions between membranes of the same organelle, intraorganellar contacts, such as the mitochondrial cristae, which creates microenvironments needed for functional control of organelar pathways. Other complexes mediate interactions between membranes of different organelles, enabling organelar cooperation in biosynthetic pathways and in controlling cellular ion and metabolite levels, such as the ER–mitochondrion membrane contact sites (MCS)s. While these roles are fundamental for eukaryotic cell biology, most of our current knowledge is focused on commonly studied organisms that are often closely related to each other in the perspective of the full tree of eukaryotes (Box 1).

For example, yeast and mammals are the subjects of numerous studies; however, both belong to the Opisthokont clade. Here we describe recent progress in our understanding of the role of membrane complexes in mediating membrane interactions, shape, and function, in the divergent eukaryote, Toxoplasma gondii, a model organism for the apicomplexan phylum, of the SAR clade (Box 1).

Intraorganellar contacts: Complexes mediating mitochondrial cristae biogenesis and shape
One of the hallmarks of mitochondria is their double membrane composed of an outer and inner membrane. The latter folds to form cristae, whose shape and dimension control mitochondrial respiration [7], and which provide spatial organisation for the assembly of the mitochondrial electron transport chain complexes into super complexes [8]. Cristae morphology is mediated by three key protein complexes (reviewed here [9] in more details):

(I) Assemblies of the mitochondrial genome maintenance protein (Mgm1 or OPA1 in yeast or metazoans, respectively) stabilise and tighten the cristae neck. No homologs of Mgm1/OPA1 can be found in the Toxoplasma genome [10] and whether this role has been replaced by parasite-specific components remains to be discovered.
Box 1. The importance and benefits of understanding cell biology in divergent Organisms.

The scheme herein is drawn after [1] and shows eukaryotic clades (blue) and model organisms (black and icon) mentioned in this review. The study of divergent organisms is an important goal for a cell biology community that strives to have knowledge of the full repertoire of functions and mechanisms taking place in eukaryotes.

In addition to this critical basic scientific outcome, comparing the cell biology of organisms from different clades is a powerful tool to identify core features of cellular pathways common to all eukaryotes, to discover new functions of known machineries of well-studied organisms, and to obtain understanding of common functional mechanisms.

For example, distant organisms may employ proteins that are unrelated at the sequence level to perform the same cellular function. Comparing the structural feature of such proteins highlights the structural elements required for function, as was shown with the role of the mitochondrial outer membrane importer Mim1/2 from yeast and its functional homolog from trypanosomes pATOM3 [3].

In another example, the study of divergent organisms can demonstrate that a machinery found in yeast but not metazoan, was lost in metazoan rather than being yeast specific. The ER–mitochondria encounter structure (ERMES), was initially considered fungi specific, but then found in *Trypanosoma* and amoeba [4,5]. ERMES’s role in trypanosomes is likely different from yeast [6], and amoeba have highly divergent mitochondria-like organelle, suggesting that there are still functions of ERMES that are unknown.

(II) The F$_{0}$F$_{1}$-ATP synthase typically forms rows of dimers that induce membrane curvature to establish cristae [11]. Interestingly, *Toxoplasma* mitochondria have a bulbous cristae shape, divergent from the predominantly lamellar cristae found in opisthokonts. Moreover, *Toxoplasma* ATP synthase contains numerous novel subunits only found in apicomplexans and other members of the SAR clade [12]). A recent structural study provided answers to both these puzzles. Tomography studies found that the *Toxoplasma* ATP synthase assembles into hexamers, which are the basis for higher order pentagonal pyramids that dictate the unique cristae shape [13]). Single particle analysis via CryoEM further found that this unique hexamerisation is mediated through interactions between some of the new parasite subunits in the lumenal region of the complex [13]. These findings provided an exciting example of how divergent organisms evolve novel solutions to fundamental cell biology needs.

The ATP synthase is not the only *Toxoplasma* mitochondrial complex that has many new subunits not found in opisthokonts. All three others respiratory complexes found in apicomplexans (II, III, and IV) have divergent compositions, and each complex consists of new subunits not seen in organisms from other clades [14]. In opisthokonts, complexes of the electron transport chain assemble into supercomplexes, which enhances the efficiency of electron transfer and the stability of the individual complexes [15]. Evidence for supercomplex formation is emerging for *Toxoplasma* [16] and the related apicomplexan, *Plasmodium falciparum* [17]. It is possible that some of the new subunits are involved in supercomplex formation, much like the role of new ATP synthase subunits in mediating hexamerisation; however, this remains to be experimentally addressed. Revealing the functions of the unique respiratory complex subunits is an outstanding and important task for the field, as a novel function not seen in other eukaryotes may unravel.

(III) The mitochondrial contact site and cristae organisation system (MICOS) is a multi-subunit assembly responsible for cristae junction formation and for contacts with the outer mitochondrial membrane. MICOS is found across the eukaryotic tree with two core subunits found in all studied clades: MIC60 and MIC10 [18]. However, the size and composition of the complex vary. Opisthokont MICOS consist of four to six subunits, while in *Trypanosomes* (from the clade discoba (Box 1)), there are nine MICOS components [19]. With the new components came a new function: *Trypanosomes* MICOS include a thiolredoxin that is hypothesised to take over the role of the missing oxidoreductase, Mia40, essential for protein import into the intermembrane space in opisthokont mitochondria [20]. In *Toxoplasma*, homologs of MIC60/10 are also identifiable in the genome [21], but no experimental work has addressed MICOS composition and function yet. Like *Trypanosomes*, *Toxoplasma* is also missing a Mia40 homolog [22], thus, it is intriguing to find out if the same convergence occurred in both these organisms, which are models of unrelated clades. Moreover, it would be of interest to discover whether other novel *Trypanosomes* MICOS properties are found in *Toxoplasma* or indeed representatives of other clades, that might point to ophistokont being the outlier as proposed by the *Trypanosomes* MICOS study [20].
Proteins complexes control inter-organelle interactions via membrane contact sites

While often cell biology textbooks discuss organelles as separate entities, it is known that contact, communication, and exchange between organelles are required for function. The past decade has seen a growing appreciation of the protein complexes mediating these interactions. These so-called membrane contact sites (MCSs) are defined with four general rules: the membranes are in close proximity (but there is no membrane fusion); the juxtaposed membranes are actively tethered; the contact performs a dedicated cellular function, and the MCS contains a MCS-specific proteome/lipidome [23]. Contacts following these rules have been identified between any two organelles studied to date, and the molecular details of numerous MCS have been elucidated. However, as most MCS studies are performed in opisthokonts, the full functional and evolutionary understanding of the role of MCS in eukaryotic biology remains incomplete without the perspective from a broad range of divergent organisms. Pioneering work focused on less well-studied model systems has exposed novel contacts [24] involving a mitochondria-like organelle and ER [25], acidocalcisome and mitochondria [26], and even a contact between the vacuole surrounding intracellular parasites and host-cell organelles [27]. In this part of the review, we will highlight recent advances on the protein complexes mediating MCS between organelles inside Toxoplasma (Box 2).

Mitochondrion and ER

ER—mitochondria MCSs are the best-studied contacts, with functions including calcium and lipid exchange and control of mitochondrial dynamics, autophagy, and apoptosis [28]. The first tether reported for this contact is the ER—mitochondria encounter structure (ERMES) discovered in yeast [29]. ERMES was subsequently found in various divergent organisms across the eukaryotic tree while, somewhat surprisingly, not conserved among the opisthokonts [44], highlighting an example of the importance of exploring different systems (Box 1). Another one of those MCS is mediated by the ER—localised inositol trisphosphate receptor (IP3R) and the voltage-dependent anion channel (VDAC), which is an outer mitochondrial membrane porin. In this contact, which was first described in mammals [30], the proximity between IP3R and VDAC facilitates calcium mobilisation from the ER into the mitochondrion, where calcium moves through a mitochondrial calcium uniporter (MCU) to the lumen. The VDAC-IP3R contact is further mediated by the proteins Grp75 and DJ-1 [31]. The presence of porins eukaryotes raised the question of whether this contact might be broadly conserved [18], however, a study of the role of Toxoplasma VDAC points to a divergence [32]. On the one hand, the study identifies structures with the features of ER—mitochondrial MCS in Toxoplasma and further shows that these contacts are reduced upon VDAC depletion. On the other hand, however, the depletion and resulting MCS reduction have no effect on calcium homeostasis [32]. Moreover, homologs for IP3R and MCU were lost in apicomplexans [33]. Thus, while a function for VDAC in mediating ER—mitochondrion MCS is supported, a role in calcium homeostasis is not. Furthermore, a partner for this putative tether is yet to be identified. Interestingly, while a VDAC-IP3R MCS is found in Trypanosomes, it mediates exchange between the mitochondrion and acidocalcisome rather than the ER [34]. Therefore, it seems that porin mediated mitochondria contacts may assume different roles in divergent organisms.

Another complex proposed to mediate an ER—mitochondrial MCS in yeast is the ER membrane complex (EMC). The ER resident EMC was suggested to interact with the mitochondrial Tom5, and the resulting MCS is thought to facilitate phospholipid transfer between the two organelles in both yeast [35] and mammals [36]. Like VDAC, most EMC subunits are found in most eukaryotic organisms [37], raising the possibility of a putative common function across eukaryotes. This notion was supported by the report of EMC found in the ER—mitochondrial interface of Trypanosomes, where it plays a role in the control of phospholipid synthesis [38].

Of note, EMC, or some of its subunits, are proposed to perform multiple other roles, including acting as an ER transmembrane protein insertase [39]. EMC is composed of up to nine subunits encoded by up to ten genes in different organisms. In yeast, EMC genes could be individually deleted without a major effect on viability, but deletion of two (or more) subunits results in lipid synthesis defect [35]. Interestingly, in Trypanosomes, individual gene deletion or depletion affects viability, and some have a drastic effect on lipid synthesis [38]. Single-subunit essentiality seems to be the case also in

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Box 2. Toxoplasma gondii as a model apicomplexan.

As the field of eukaryotic cell biology expands the repertoire of model organisms from divergent clades, Toxoplasma is established as an effective model for apicomplexan and other groups within the SAR clade, tools and resources that are the best developed than for any other member of the SAR. This includes the below:

- Wide range of genetic manipulation systems [3].
- Relative high efficiency of genetic manipulation due to high transfection rates, a balanced nucleotide composition, and haploidy of relevant life stages.
- Single copy of many organelles including mitochondrion and Golgi, relative large size and typical signature of organelle morphology.
- Relatively ease, low cost, and safe culture.
- Being clinically relevant.
Toxoplasma, where seven of the eight predicted EMC encoding genes are critical for growth, as predicted by a genome wide CRISPR screen (Table 1) and confirmed in our unpublished work (Ovciarikova et al., in prep). Whether this importance for fitness is linked to a role in ER–mitochondria tethering, ER–insertase, or other roles remains to be studied. Complexome profiling in the apicomplexan P. falciparum found only seven of the eight predicted EMC subunit homologs in a putative EMC complex and further found the subunit EMC5 also in a separate, unknown, complex [17] supporting the hypothesis of multiple roles.

**Table 1.** A summary of the data available for homologs of the EMC components in two model apicomplexans, Toxoplasma gondii, and Plasmodium falciparum. Fitness scores for Toxoplasma are from the study by Sidik et al. [40], and for Plasmodium falciparum are from a PiggyBac screen [41]. In both, lower score reflects higher contribution to fitness. Localisation prediction is based on localisation of organelle proteins by isotope tagging in Toxoplasma [42]. Presence in a complexome analysis provides evidence for belonging to the same complex [17].

| Gene ID          | Localisation prediction | Fitness-conferring score | Gene ID          | Present in complexome analysis | Fitness-conferring score |
|------------------|-------------------------|--------------------------|------------------|-------------------------------|--------------------------|
| TGME49_205740    | ER                      | −5.03                    | PF3D7_0811200    | Yes                           | −3.23                    |
| TGME49_267840    | ER                      | −4.08                    | PF3D7_1410000    | Yes                           | −3.42                    |
| TGME49_230100    | ER                      | −3.02                    | PF3D7_1360200    | Yes                           | −2.81                    |
| TGME49_259000    | ER                      | −4.77                    | PF3D7_1435400    | Yes                           | −3.3                      |
| TGME49_293200    | ER                      | −2.68                    | PF3D7_0306700    | Yes                           | −3.39                    |
| TGME49_239690    | No data                 | −3.57                    | PF3D7_1214400    | No                            | −2.76                    |
| TGME49_243390    | ER                      | −1.71                    | PF3D7_1310500    | Yes                           | −3.08                    |
| TGME49_249310    | ER                      | −4.1                     | PF3D7_1139900    | Yes                           | −2.84                    |

**Nuclear-mitochondrial contacts**
Recent studies have started addressing the composition and function of nuclear—mitochondrial MCSs, where the first characterised contact might be involved in phospholipid homeostasis [43]. Putative mitochondrial—nucleus contacts were observed in the apicomplexans Toxoplasma (Figure 1, and our unpublished work) and Plasmodium [44]. In the latter, nuclear—mitochondrial proximity is enhanced in parasites that survived treatment with artemisinin, a potent anti-malarial drug, and it was proposed that the new contacts may support mitochondrial retrograde signalling, resulting in transcriptional changes in the

*Figure 1.* Putative membrane contact sites observed and described in Toxoplasma gondii. A scheme of a single Toxoplasma tachyzoite (left) showing mitochondrion (green), ER (light purple), nucleus (dark purple), and inner membrane complex (IMC) (pink). Contact sites discussed in the main text are highlighted in circles with identity of proposed components shown: (1) ER–mitochondrion; (2) nucleus–mitochondrion; and (3) mitochondrion–IMC. EM images of Toxoplasma (middle/right) showing examples of organelle proximity that provide evidence for the contacts shown in (A), whereby A and B are colour coordinated, and C has no added colours for clarity.
nucleus as a survival mechanism [44]. Likewise, in cancer cells, these newly described contacts were proposed to promote survival via a mitochondrial retrograde response [45]. Identifying the tethers mediating these contacts in a broad array of divergent organisms is the next step for the field.

Contacts described only in Toxoplasma

Apicomplexans and other alveolates contain a network of membrane sacs likely of ER–Golgi origin, named the inner membrane complex (IMC) in apicomplexans. In Toxoplasma, MCSs are seen between the IMC and the mitochondrion [46]. Microscopy observations showed that mitochondrion–IMC contacts change dynamically during the Toxoplasma lytic cycle, which coincides with mitochondrial morphological changes from its typical lasso shape when the parasites are inside their host into a ball-shape when they are out [46]. A parasite specific protein of the mitochondrial outer membrane, named lasso maintenance factor 1 (LMF1), acts as a bridge between the two organelles, whereby LMF1 depletion results in loss of the lasso shape [47]. This mitochondrion positioning is important for cell division and for parasite proliferation in vitro [47]. Whether LMF1 acts as a direct tether and what might be its partner/s in the IMC remains to be discovered.

Another organelle specific to Apicomplexa and related groups is their secondary plastid, the apicoplast. Proximity between mitochondrion and apicoplast [48] and between the apicoplast and the ER [49] have been detected by electron microscopy, which might indicate contacts. An apicoplast–ER contact was also reported in a study of an apicoplast-localised two-pore channel (TgTPC) [50]. TgTPC depletion reduces these contacts and affects calcium homeostasis, thus this putative contact is proposed to allow calcium exchange between these two organelles [50]. Whether TgTPCs is a tether component and what might be the ER partner remains to be explored.

Conclusions

Like many fundamental cell biology processes, the role of membrane complexes in controlling organelle function is best studied in common model organisms, largely of the opisthokont clade. Often, complex conservation between yeast and mammals is termed ‘evolutionarily conserved’ and even seen as ‘gold standard’ for a given complex’s composition or function. However, studies in divergent organisms reveal divergent traits and define true evolutionary conservation, thus highlighting the importance of stirring away from this simplified view. In this context, research in the divergent Trypanosomatids has provided numerous examples for unexpected divergence in biology. While studies of organisms from other divergent clades are lagging, Toxoplasma is established as an effective and relevant model organism for the SAR clade or for groups within it for addressing questions of divergent cell biology (Box 2).

As the picture widens, we find that single cell parasites do not always have ‘less’ subunits of functional complexes compared to their opisthokont parallel. Rather the parasite complexes may consist of other unrelated proteins, as is likely the case for the VDAC mediated mitochondria–ER MCS in Toxoplasma. Or, the divergent complexes have more subunits, as seen in the respiratory chain enzymes of apicomplexans. This comparison provides powerful insights. For example, the higher number of components of apicomplexan ATP synthase might be linked to the reduction of its mitochondrial genome. In support of this notion, the Toxoplasma ATP synthase structure exposed reduced hydrophobicity of a truncate and nuclear encoded subunit a, enabling its post translational translocation into the mitochondrion and compensated for by interaction generated with novel subunits.

Thus, eukaryotic biology should not be defined in comparison to what has been found first in opisthokont, as often is the case, but rather revised in a broad context, when each complex is studied in a divergent repertoire of systems. This would enhance the ability of the cell biology field to understand the fundamental role of biological processes and their evolution.

Conflicts of interest

Nothing declared.

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References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Burki F, Roger AJ, Brown MW, Simpson AGB: The new tree of eukaryotes. Trends Ecol Evol 2020, 35:43–55.
2. Weiss LM, Kim K: Ebscohost: Toxoplasma gondii: the model apicomplexan, perspectives and methods. edn 2nd. London, UK: Academic Press; 2014.
3. Vitali DG, Kaser S, Kolb A, Dimmer KS, Schneider A, Rapaport D: Independent evolution of functionally exchangeable mitochon- drial outer membrane import complexes. Elife 2018, 7.
4. Wideman JG, Gawryluk RM, Gray MW, Dacks JB: The ancient and widespread nature of the ER-mitochondria encounter structure. Mol Biol Evol 2013, 30:2044–2049.
5. Buczek D, Wojtkowska M, Suzuki Y, Sonobe S, Nishigami Y, Antoniewicz M, Kmita H, Makalowski W: Protein import complexes in the mitochondrial outer membrane of Amoebozoa representatives. BMC Genom 2016, 17:99.
6. Povelones ML, Tiengwe C, Gluenz E, Gull K, Englund PT, Jensen RE: Mitochondrial shape and function in
trypanosomes requires the outer membrane protein, TbLOK1. Mol Microbiol 2013, 87:713–729.

7. Baker N, Patel J, Khacho M: Linking mitochondrial dynamics, cristae remodeling and supercomplex formation: how mitochondrial structure can regulate bioenergetics. Mitochondrion 2019, 49:259–268.

8. Cogliati S, Frezza C, Soriano ME, Varanita T, Quintana-Cabrera R, Corrado M, Cipolat S, Costa V, Casarin A, Gomes LC, et al.: Mitochondrial cristae shape determines respiratory chain supercomplexes assembly and respiratory efficiency. Cell 2013, 155:160–171.

9. Iovine JC, Claypool SM, Alder NN: Mitochondrial compartmentalization: emerging themes in structure and function. Trends Biochem Sci 2021, 46:902–917.

10. Voleman L, Dolezal P: Mitochondrial dynamics in parasitic protists. PLoS Pathog 2019, 15, e1008008.

11. Kuhlbrandt W: Structure and mechanisms of F-type ATP synthases. Annu Rev Biochem 2019, 88:515–549.

12. Huet D, Rajendran E, van Dooren GG, Lourido S: Identification of cryptic subunits from an apicomplexan ATP synthase. Elife 2018, 7.

13. Muheip A, Kock Flygaard R, Ovciarikova J, Lacombe A, et al.: Mitochondrial inner membrane import pathway. Nature 2021, 590:121-125.

14. Seid A, Mullner-Wong LS, Rajendran E, Tjhin ET, Daegle LF, Aw YY, Faou P, Webb AI, Tonkin CJ, van Dooren GG: Eluding the mitochondrial protome of Toxoplasma gondii reveals the presence of a divergent cytochrome c oxidase. Elife 2018, 7.

15. Berndtsson J, Aufschnaiter A, Rathore S, Marin-Buera L, Dawitz H, Diesl J, Kohler V, Barrientos A, Buttner S, Fontanesi F, et al.: Respiratory supercomplexes enhance electron transport by decreasing cytochrome c diffusion distance. EMBO Rep 2020, 21, e101015.

16. Maclean AE, Bridges HR, Silva MF, Ding S, Ovciarikova J, Hirst J, et al.: Complexome profile of Toxoplasma gondii mitochondria identifies divergent subunits of respiratory chain complexes including new subunits of cytochrome bc1 complex. PLoS Pathog 2021, 17, e1009301.

This work mapped the protein composition of all four respiratory complexes in Toxoplasma. It further provides evidence for supercomplex formation in these parasites.

17. Evers F, Cabrera-Orefice A, Elturbe DM, Kea-Te Lindert M, et al.: Complexome profile of Toxoplasma gondii mitochondria identifies divergent subunits of respiratory chain complexes including new subunits of cytochrome bc1 complex. PLoS Pathog 2021, 17, e1009301.

This work mapped the protein composition of all four respiratory complexes in the parasitic causing Plasmodium parasites. It further provides evidence for supercomplex formation in these parasites.

18. Wideman JG, Munoz-Gomez SA: The evolution of ERMIONE in mitochondrial biogenesis and lipid homeostasis: an evolutionary view from comparative cell biology. Biochim Biophys Acta 2016, 1861:900–912.

19. Wideman JG, Munoz-Gomez SA: Cell biology: functional conservation, structural divergence, and surprising convergence in the MICOS complex of trypanosomes. Curr Biol 2018, 28: R1245–R1248.

20. Kaurow J, Vancova M, Schimanski B, Cadena LR, Heller J, Bily T, Potesil D, Eichenberger C, Bruce H, Oeljeklaus S, et al.: The diverged trypanosome MICOS complex as a hub for mitochondrial cristae shaping and protein import. Curr Biol 2018, 28:3393–3407, e3395.

21. Munoz-Gomez SA, Slamovits CH, Dacks JB, Baier KA, Spencer KD, Wideman JG: Ancient homology of the mitochondrial contact site and cristae organizing system points to an endosymbiotic origin of mitochondrial cristae. Curr Biol 2015, 25:1489–1495.

22. Eckers E, Cynklaff M, Simpson L, Deponte M: Mitochondrial protein import pathways are functionally conserved among eukaryotes despite compositional diversity of the import machineries. Biol Chem 2012, 393:513–524.

23. Scorrano L, De Matteis MA, Ems R, Giordano F, Hajnoczky G, Kornmann B, Lackner LL, Levine TP, Pellegrini L, Reinisch K, et al.: Coming together to define membrane contact sites. Nat Commun 2019, 10:1287.

24. Santos HJ, Nozaki T: Interorganelar communication and membrane contact sites in protozoan parasites. Parasitol Int 2021, 83:103272.

25. Voleman L, Najdрова A, Astralidsson A, Tumova P, Einarsson E, Svindrych Z, Hagen GM, Tachezy J, Svard SG, Dolezal P: Giardia intestinalis mitosomes undergo synchronized fission but not fusion and are constitutively associated with the endoplasmic reticulum. BMC Biol 2017, 15:27.

26. Ramakrishnan S, Asady B, Docampo R: Acidocalcisome-mitochondrion membranes contact sites in trypanosoma brucei. Pathogens 2018, 7.

27. Pernas L, Adomako-Ankomah Y, Shastri AJ, Ewald SE, Trecek M, Boyle JP, Boothroyd JC: Toxoplasma effector MAF1 mediates recruitment of host mitochondria and impacts the host response. PLoS Biol 2014, 12, e1001845.

28. Lin S, Meng T, Huang H, Zhuang H, He Z, Yang H, Deng M: Molecular machineries and physiological relevance of ER-mitochondrion membrane contacts. Theranostics 2021, 11:974–995.

29. Kornmann B, Currie E, Collins SR, Schuldiner M, Nunnari J, Weissman JS, Walter P: An ER-mitochondria tethering complex revealed by a synthetic biology screen. Science 2009, 325:477–481.

30. Sazakbaki G, Bianchi K, Varnai P, De Stefani D, Wicckowski MR, Cavagna D, Nagy AI, Balla T, Rizotto R: Chaperone-mediated coupling of endoplasmic reticulum and mitochondrial Ca2+ channels. J Cell Biol 2006, 175:901–911.

31. Liu Y, Ma X, Fujioka H, Liu J, Chen S, Zhu X: DJ-1 regulates the integrity and function of ER-mitochondria association through interaction with IP3R3-Gpr75-VDAC1. Proc Natl Acad Sci U S A 2019, 116:25322–25328.

32. Mallo N, Ovciarikova J, Martins-Duarte ES, Baehr SC, Biddau M, Wilde ML, Uboldi AD, Lemgruber L, Tonkin CJ, Wideman JG, et al.: Depletion of a Toxoplasma porin leads to defects in mitochondrial morphology and contacts with the endoplasmic reticulum. J Cell Sci 2021, 134.

This work demonstrated a divergent role for the universally conserved mitochondrial porin VDAC in parasites and highlight that it may mediate a putative divergent ER-mitochondrion MCS.

33. Bick AG, Calvo SE, Mootha VK: Evolutionary diversity of the mitochondrial calcium uniporter. Science 2012, 336:886.

34. Docampo R, Huang G: The IP3 receptor and Ca(2+)-signaling in trypanosomes. Biochim Biophys Acta Mol Cell Res 2021, 1868:118947.
biosynthesis in *Trypanosoma brucei*. *bioRxiv* 2021. 2021.2006.2017.448810.

39. Guna A, Volkmar N, Christianson JC, Hegde RS: The ER membrane protein complex is a transmembrane domain insertase. *Science* 2018, 359:470–473.

40. Sidik SM, Huet D, Ganesan SM, Huynh MH, Wang T, Nasamu AS, Thiru P, JPJ Saeij, Carruthers VB, Niles JC, et al.: A genome-wide CRISPR screen in Toxoplasma identifies essential apicomplexan genes. *Cell* 2016, 166:1423–1435. e1412.

41. Zhang M, Wang C, Otto TD, Oberstaller J, Liao X, Adapa SR, Udenze K, Bronner IF, Casandra D, Mayho M, et al.: Uncovering the essential genes of the human malaria parasite *Plasmodium falciparum* by saturation mutagenesis. *Science* 2018, 360.

42. Barylyuk K, Koreny L, Ke H, Butterworth S, Crook OM, Lassadi I, Gupta V, Tromer E, Mourer T, Stevens TJ, et al.: A comprehensive subcellular atlas of the Toxoplasma proteome via hyperLOPIT provides spatial context for protein functions. *Cell Host Microbe* 2020, 28:752–766. e759.

43. Eisenberg-Bord M, Zung N, Collado J, Drwesh L, Fenech EJ, Fadel A, Dezorella N, Bykov YS, Rapaport D, Fernandez-Busnadiaigo R, et al.: Cnm1 mediates nucleus-mitochondria contact site formation in response to phospholipid levels. *J Cell Biol* 2021, 220.

This work describes the first tether for the newly describe nuclear-mitochondrial MCS via complementary screens in yeast.

44. Connelly SV, Manzella-Lapeira J, Levine ZC, Brzostowski J, Krymskaya L, Rahman RS, Ellis AC, Amin SN, Sa JM, Wellems TE: Restructured mitochondrial-nuclear interaction in *Plasmodium falciparum* dormancy and persister survival after artemisinin exposure. *mBio* 2021, 12, e007521.

45. Desai R, East DA, Hardy L, Faccenda D, Rigon M, Crosby J, Alvarez MS, Singh A, Mainenti M, Hussey LK, et al.: Mitochondria form contact sites with the nucleus to couple prosurvival retrograde response. *Sci Adv* 2020, 6.

46. Ovciarikova J, Lengrubler L, Stilger KL, Sullivan WJ, Sheiner L: Mitochondrial behaviour throughout the lytic cycle of *Toxoplasma gondii*. *Sci Rep* 2017, 7:42746.

47. Jacobs K, Charvat R, Arrizabalaga G: Identification of Fis1 interactors in *Toxoplasma gondii* reveals a novel protein required for peripheral distribution of the mitochondrion. *mBio* 2020, 11.

48. Nishi M, Hu K, Murray JM, Roos DS: Organellar dynamics during the cell cycle of *Toxoplasma gondii*. *J Cell Sci* 2008, 121:1559–1568.

49. Tomova C, Humbel BM, Geerts WJ, Entzeroth R, Holthuis JC, Verkleij AJ: Membrane contact sites between apicoplast and ER in *Toxoplasma gondii* revealed by electron tomography. *Traffic* 2009, 10:1471–1480.

50. Li ZH, King TP, Ayong L, Asady B, Cai X, Rahman T, Vella SA, Coppens I, Patel S, Moreno SNJ: A plastid two-pore channel essential for inter-organelle communication and growth of *Toxoplasma gondii*. *Nat Commun* 2021, 12:5802.