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Parasites and their (endo)symbiotic microbes

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Abstract

Thanks to modern molecular biology methods, our understanding of the impact of (endo) symbiotic bacteria on parasitic protists and helminths is growing fast. In this issue, 9 papers have been brought together that describe various facets of the relationships between these microorganisms, reveal their range and high frequency, as well as their capacity to create novel biological complexity. Comparative analyses of these host–endosymbiont interactions indicate that there may be no discrete types of relationships but rather a continuum ranging from a dispensable endosymbiont minimally integrated within the host cell to organelles, such as mitochondria and plastids that evolved into an indispensable, deeply integrated components of the cell. We hope that this series of studies on parasites and (endo)symbiotic bacteria will increase awareness about these relationships and their representation in microbial ecology models.

Introduction

For a long time, protists were known to carry bacterial endosymbionts, yet these intimate relationships, their sophistication and impact on the partners have started to be recognized only recently. The first description of a bacterium in a protist cell is ascribed to Hafkine (1890), who observed *Holospora* in the nucleus of a ciliate (Gromov and Ossipov, 1981). While the ability of some *Paramecium* strains to kill others of its kind was widely known (Sonneborn, 1938), it was only after the advent of electron microscopy when this truly peculiar phenomenon was attributed to the so-called killer strains of bacteria (called kappa bodies) carried by the ciliate protist (Hamilton and Gettner, 1958). By the middle of the 20th century, hundreds of ciliate species were described to harbour bacteria in virtually every cellular compartment. Research into their diversity, adaptation, interactions and modes of infection of the host, as well as their ecological and evolutionary significance received substantial attention (Fokin, 2004). A somewhat reminiscent situation happened with bacterial endosymbionts in trypanosomatid flagellates, which were first documented in *Strigomonas culicis* (as diplosomes) more than a century ago (Novy *et al.*, 1907), yet remained understudied until recently. For a long time, the technology represented substantial limits in dissecting relationships among miniscule cells, their endosymbionts and partners, and consequently, the interest in the subject seemed to vane and remained at the margins of mainstream biology.

However, that has changed in the last decade and we see at least two major reasons for that. The first reason is the sensitivity and affordability of methods for analysing DNA, RNA and proteins, which reached a level at which one can efficiently study the endosymbionts. The second one is the realization that these truly invisible relationships are much more widespread and important than appreciated earlier and that they have a major impact on protists, which in turn have a profound impact on life on this planet (Pawlowski *et al.*, 2012; Bork *et al.*, 2015).

Ecological and evolutionary implications

Symbiotic relationships between (parasitic) protists and bacteria constitute a driver that has so far been under-represented in microbial ecology models. Endosymbionts undoubtedly play roles in the adaptation of their hosts to environmental challenges and likely in the origin of novel biochemical pathways. They have a number of functions in the host cell, some of which remain unknown (Archibald, 2015*b*; O'Malley, 2015). It has been argued that in its extreme form, endosymbionts become completely integrated at the cellular level as organelles (Archibald, 2015*a*). By stretching the imagination, one can even predict that every stably entrenched endosymbiont may enter an (extremely) long evolutionary trajectory to become an organelle. Indeed, only recently commonalities and overlaps have been identified in the fields of symbiosis and organelle origin, leading to a proposal that there may, in fact, be a continuum between what has historically been labelled as 'symbionts', 'parasites', and 'organelles' (Keeling and McCutcheon, 2017). For decades, organelles have been defined as cellular compartments with complex and dedicated targeting machineries embedded in their membranes (Cavalier-Smith, 1987). However, now we know of prokaryotic proteins that are encoded in the protist nucleus and targeted into the endosymbiotic bacterium (Nowack and Grossman, 2012; Morales *et al.*, 2016). Hence, the barrier between endosymbionts and organelles became

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blurred (Keeling *et al*., 2015). Either of them can develop into a complex and versatile version of its initial form or can become subject to rampant simplification and/or degeneration, which we now know can lead, at least in the case of an organelle, to a complete elimination (Karnkowska *et al*., 2016). The same may hold true for extant bacteria-free protists. It is plausible to speculate that they once harboured bacteria, similar to some of their present relatives, as in the case of diplonemids (Tashyreva *et al*., 2018). The reasons for their loss may vary from intolerable selfishness of the endosymbiotic organism to its degeneration to a point when it became fully dispensable for the host cell. The transition between stages may in some cases be surprisingly fast (Boscaro *et al*., 2017).

It is generally accepted that all extant mitochondria arose from an α-proteobacterium sometime before the last common eukaryotic ancestor (Gray, 2012) (although, see Martijn *et al*., 2018 for recent alternative views), whereas the origin of extant plastids was more convoluted. They arose by the acquisition of a cyanobacterium by a cell already containing a mitochondrion and subsequently spread throughout eukaryotic lineages by several rounds of secondary and tertiary symbioses (Archibald, 2015*b*).

Current models

Developing models for the basis of interactions between microbial species and how they affect their hosts is a major challenge and symbiotic interactions provide a wide range of opportunities to dissect the origin and profound impacts of close interactions. Another level of complexity of these interactions is produced by their multilayered nature or frequent transiency. In any case, symbiosis is now regarded as a major force for the creation of new biological complexity. This applies both to the deep evolutionary history when the eukaryotic cell came into being and to the functions and properties, it acquired afterwards thanks to the endosymbiotic systems (Archibald, 2015*a*; Keeling *et al*., 2015).

The papers brought together in this volume provide evidence of the colourful and important roles (endo)symbionts and viruses play in the lives of parasites. Prior to the full entry of the sequencing era into this field, the information available about the bacterial endosymbionts and viruses in parasites was mainly confined to the electron microscopic pictures, in which they look pretty much alike or, in the case of bacteria, to their 16S rRNA sequences. However, now we can assemble the genomes of multiple endosymbionts and viruses at once, as well as dissect their metabolism, their interactions with the host cell metabolism and, in cases were more endosymbionts and viruses share the host cell, we can address mutual interactions among them. As a consequence, it is only now when the available sensitivity and range of methods allow researchers to start dissecting these complexities. This will eventually allow estimating the true impact of these interactions, which is clearly deeper than previously thought. In some cases, the host cell and its (endo)symbionts seem to be entwined as tightly as it gets in biology. Indeed, no eukaryotic organism lives in isolation. Each is influenced by omnipresent microbes and viruses, with their assemblages living inside of a given eukaryote being known as the microbiome and the virome (Angly *et al*., 2006).

Some of these systems are well-studied while others are relatively unknown. They include single- and/or multi-cellular parasites, bacteria and viruses since all of them play major roles in the population dynamics and effects on their hosts. Disentangling interactions among these players is currently easier in model systems and in those where these interactions are non-transient. These models can eventually be used to build a common evolutionary and ecological framework, informative for more complex and less studied systems.

Not surprisingly, the best-studied symbioses are those involving humans and mice and their intestinal microbiome. Intense research in the last few years dramatically reshaped our view on how the microbiome can impact human health and development (Knight *et al*., 2017). Moreover, the complex interplay between the mammalian host and its microbiome is further complicated by eukaryotes inhabiting the host, also called the eukaryome (Lukeš *et al*., 2015; Chabe *et al*., 2017). Mostly due to technical reasons (extremely high sequence diversity), it is much more difficult to study the eukaryome as compared with the microbial community. As a consequence, the eukaryome remains a rather overlooked component of the holobiont which, however, must inevitably play a significant role in its life.

In their study, Jirků-Pomajbíková *et al*. (2018) dissected the complex relationships among the rat, the tapeworm *Hymenolepis diminuta* and the gut microbiome. Similar to several other intestinal parasites, *H. diminuta* is now considered a gut symbiont. It is frequently used by individuals for self-treating of inflammatory bowel disease and other intestinal inflammations and meets criteria for helminth therapy. For this purpose, its life stages are now being produced in semi-industrial amounts (Smyth *et al*., 2017). The authors convincingly demonstrated that a controlled infection with *H. diminuta* indeed protects rats against severe inflammatory colitis by inducing a type 2 immune response, resulting in faster recovery. The gut microbiome is disrupted during the artificially induced colitis in response to colonization by the tapeworm but, somewhat unexpectedly, does not play a major role in the *H. diminuta*-mediated protection.

The work of Rebello *et al*. (2018) investigated the role of the HIV-1 peptidase inhibitor Lopinavir in *Leishmania amazonensis* metabolism and demonstrated that it induces lipid accumulation in promastigotes. Using this broad-specificity inhibitor against viral peptidases, they shed a new light on the first-line defense against viral infection in *Leishmania* and other trypanosomatids. This is particularly important because *Leishmania* treatment remains challenging and new therapies need to be developed (Gillespie *et al*., 2016; Aronson *et al*., 2017). Understanding parasites' response to different drugs is imperative in achieving this goal.

In his review of what is known about symbionts of blood-feeding organisms, Husník (2018) seeks to identify some common trends in the varied landscape they represent. The blood-feeding strategy emerged multiple times independently in the evolution of notorious mosquitoes and tsetse flies, vectors of a range of serious diseases, as well as in the predecessors of less known vampire finches and lampreys. Regardless of their phylogenetic position and because of the highly specialized diet, the blood-feeders rely on either extracellular (usually located in the gut) or intracellular obligate symbionts (found in specialized bacteriocyte cells or even dedicated organs such as bacteriomes) that supply them with vitamins and potentially other compounds. The author also draws attention to the fact that in this complex system, represented here by the endosymbiont-loaded tissues of the tsetse fly, a prokaryote-to-eukaryote horizontal gene transfer event occurs much more frequently than in other systems (Husnik and McCutcheon, 2018).

In their comprehensive review, Telleria *et al*. (2018) summarized current knowledge of the sand fly microbiome. Phlebotomine sand flies are vectors of several etiological agents causing viral encephalitis, bartonellosis and leishmaniasis (Soares and Turco, 2003; Akhoundi *et al*., 2016). The authors reviewed studies on viruses of public health importance, bacteria and fungi present in the sand fly gut and described them in the context of vector development and insect immune response. Finally, they highlighted the cellular mechanisms the insects utilize in order to survive the potential threats posed by viruses, bacteria and *Leishmania* spp. they transmit (Telleria *et al*., 2018).

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Paranaiba *et al.* (2018) evaluated a group of *Leishmania* species non-infective to humans for the presence of double-stranded RNA viruses related to *Leishmaniavirus* belonging to the family *Totiviridae*. Two isolates of *Leishmania enriettii* were analysed and shown to be virus-free. This is an interesting result in the light of a recent broad survey of trypanosomatids, which also failed to document totiviruses outside of the genus *Leishmania* (Grybchuk *et al.*, 2018). Moreover, in these understudied flagellates, Paranaiba *et al.* (2018) elaborated on the structure of glycoconjugates.

Harmer *et al.* (2018) offer a comparative view of endosymbiotic events during kinetoplastid evolution. While trypanosomatid flagellates are mostly known thanks to human pathogenic trypanosomes and leishmanias, their predecessors parasitizing insects occasionally acquired a bacterium, which provides them with essential vitamins and heme. From the comparison of two independently acquired bacteria belonging either to the family Burkholderiaceae or Alkaligenaceae, the authors concluded that these partnerships arrived at quite different outcomes. In the light of a recent finding that the basal trypanosomatid *Paratrypanosoma confusum* retains a cytostome (Skalický *et al.*, 2017), they discuss possible alternative acquisition routes for the bacterium that turned into an endosymbiont. Moreover, Harmer *et al.*, (2018) suggested that the endosymbiotic bacteria may also be found in free-living kinetoplastids (see the cover of this issue).

A particular case of *Candidatus* Kinetoplastibacterium sorsogonicusi, the bacterial endosymbiont of *Kentomonas sorsogonicus* (Trypanosomatidae) was analysed by Silva *et al.* (2018). The authors sequenced the whole genome of the endosymbiont and found it to be completely syntenic with the genomes of other known *Ca.* Kinetoplastibacterium spp., but more reduced in size. The most conspicuous loss is that of the heme synthesis pathway, making the host cell dependent on external heme. It remains to be investigated further why the ability to synthesize such an essential compound was lost in *Ca.* Kinetoplastibacterium sorsogonicusi, while it was retained in all other bacterial endosymbionts of trypanosomatids characterized thus far (Kořený *et al.*, 2013).

Agha *et al.* (2018) investigated the life-history traits of a fungal parasite infecting the planktonic, bloom-forming protist *Planktothrix* spp. and report that the disease outcome is largely modulated by temperature. Yet the establishment of the infection and the exploitation of host resources were modulated differently. The authors conclude that parasite fitness results from the interplay of individual parasite traits that are differentially controlled by the host and external environment (Agha *et al.*, 2018). The importance of this observation cannot be overestimated, as chytrid parasites in nature can inflict mass mortality on hosts, leading to changes in phytoplankton size distributions, and, potentially, the delay or even suppression of bloom events on a global scale (Frenken *et al.*, 2017).

The last paper of this series is devoted to *Blastocystis*, which colonizes the gut of about 1 billion people, yet there is still no consensus whether this stramenopile protist is a commensal or a parasite, linked to several gastrointestinal diseases. In their unique study, Scanlan *et al.* (2018) show that *Blastocystis* is rare in healthy Irish infants, whereas every 2nd adult from the same region is positive. Consequently, the authors suggest that infants obtain *Blastocystis* by horizontal transfer. The microbiome (and likely the eukaryome) of an infant is shaped by many forces and may have a paramount impact on health status in adulthood (Blaser, 2014). Since recent studies have found *Blastocystis* at a higher prevalence in healthy individuals as compared with those with a gastrointestinal ailment (Rossen *et al.*, 2015), the results presented by Scanlan *et al.* (2018) are actually good news, as they show that even in the Westernized world humans can acquire beneficial eukaryotes later in life.

Concluding remarks

Thanks to powerful methods and steadily growing number of model systems, as well as growing awareness of the importance of endosymbiotic relationships and their impact on the biosphere, we believe that their research has exciting times ahead. Furthermore, we predict that as a result of these efforts, the interplay between (endo)symbionts and their hosts will expand into a picture of sophisticated and colourful networks of dynamic interactions that will require another rewriting of the textbooks.

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