Review

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Tick microbiome: the force within

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Ticks are obligate blood-feeders and serve as vectors of human and livestock pathogens worldwide. Defining the tick microbiome and deciphering the interactions between the tick and its symbiotic bacteria in the context of tick development and pathogen transmission will likely reveal new insights and spawn new paradigms to control tick-borne diseases. Descriptive observations on the tick microbiome that began almost a century ago serve as forerunners to the gathering momentum to define the tick microbiome in greater detail. This review will focus on the current efforts to address the microbiomes of diverse ticks, and the evolving understanding of tick microbiomes. There is hope that these efforts will bring a holistic understanding of pathogen transmission by ticks.

Tick microbiome: old players hold new hope

The collection of commensal, symbiotic, and pathogenic microorganisms that occupy various niches of our body is called the microbiome – a term coined originally by Joshua Lederberg [1]. Our perception of the microbiome has undergone a radical change in the past decade or so, humbled by the understanding that our phenome is really shaped by our microbiome [2]. The major focus of the microbiome is currently on its Eubacterial members, but the microbiome is also composed of Archaea, virus, and eukaryotic microbes such as protozoa, nematodes, and fungi, and their interactions both within and across kingdoms might additionally modulate human health [3]. While the lack of standard marker genes and reference database makes it more laborious to define the identities of the non-bacterial members of the microbiome at this juncture, it is expected that rapidly-evolving molecular techniques will help to realize this understanding.

All metazoans have partnered with small or large consortia of microbes to enhance health and survival on this planet. Arthropods are no exception, and the literature is rich with examples of various arthropod–microbiota associations that modulate essential aspects of the arthropod life cycle, including reproductive fitness, survival, and vectorial competence [4–6]. Arthropods vector human, livestock, and plant pathogens worldwide and pose a tremendous health and economic burden [7,8]. It is anticipated that understanding the arthropod microbiome in the context of arthropod survival and pathogen transmission might spur a new generation of arthropod and arthropod-borne pathogen-control strategies. This review will focus on ticks that vector human and livestock pathogens worldwide [9] and summarize our current understanding of the microbiome of ticks. Throughout this review we will use the term 'microbiome' to indicate the bacterial members of the microbial consortia and emphasis will be on bacteria that are not established vertebrate pathogens.

Ticks: vectors of mammalian pathogens

Ticks belong to the order parasitiformes and are divided into four families, the *Nuttalliellidea* and *Laelaptidae* (that each comprises of one single species), *Ixodidae* or hard tick (that includes about 700 species), and the *Argasidae* or soft tick (that includes about 200 species) [10,11]. Ticks are distributed across the world from tropics to subarctic regions, with greatest species diversity in the tropics and subtropics [11]. Several of these serve as vectors of pathogens, and up to 28 species transmit human pathogens worldwide [11]. Detailed geographic distributions of the species and their role in pathogen transmission to humans and livestock are published [9,12] (www.ct.gov/caes). The list of pathogens transmitted by ticks to mammalian hosts is increasing [13–15], owing in part to climatic changes [16], and there is an urgency to develop new strategies to control ticks, prevent infection prevalence, and impair tick-borne pathogen transmission. Ticks are obligate blood-feeders: they have three life stages (larvae, nymphs, and adults) and whereas hard ticks require one blood meal at each stage for development, soft ticks require multiple blood meals at each stage [11]. The tick stages thus have 'on-host' and 'off-host' phases in their life stages, and off-host phases often impose inhospitable conditions of temperature and humidity. Intertwined in this somewhat challenging lifestyle is the pathogen that cycles between the tick and the vertebrate host. The pathogen enters the tick gut when the larval tick takes a blood meal on an infected vertebrate host, and then colonizes the tick gut, as in the case of *Borrelia burgdorferi*, the agent of Lyme disease [17]; or exits the gut and infects the tick salivary glands, as in the cases of *Anaplasma phagocytophilum*, that causes anaplasmosis [18] and *Borrelia hermsii*, the causative agent of tick-borne relapsing fever [19]. Once the larval tick is infected, the pathogen is maintained through subsequent developmental stages (nymph and adult) of the tick, and the tick essentially remains infected for life [20]. Unlike soft ticks that feed multiple times in a given developmental stage, and thus have the potential to transmit and acquire

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harbored pathogens to and from multiple hosts in each developmental stage, hard ticks feed only once at each developmental stage, and thus have only a limited opportunity to transmit and acquire pathogens in each stage. In addition, the success of pathogen transmission and acquisition at each stage are likely influenced significantly by the health of the host. Further, the molting success of each developmental stage, and maintenance of the pathogen during molting, would determine the continuing cycle of vector pathogen burden in the enzootic cycle. Molting is a complex process involving tissue autolysis and regeneration [20,21]. How the pathogen is maintained in the tick through the developmental stages, and how it evades tick immune responses during colonization of the gut and migration to salivary glands, remain to be fully understood. There is increasing interest in determining whether members of the tick microbiome play a key role in these events that are central to vectorial competence.

Intracellular endosymbionts of ticks

The associations of ticks with non-pathogenic bacteria was recognized definitively by Cowdry at the beginning of the 20th century [22] who showed, using light microscopy, that a wide variety of ticks (at least 16 species of ticks, including both hard and soft ticks) harbored Gram-negative *Rickettsia*-like bacteria in various tissues including eggs, ovaries, Malpighian tubules, and intestinal epithelial cells of unfed larvae, with slight differences in the morphology of the bacteria among the tick species. Cowdry also noted that, in some instances, the morphology of the bacteria correlated with tick species, presciently suggesting the occurrence of tick-specific microbiomes. Interestingly, Cowdry also noticed different species of bacteria within the same cell that did not associate with each other, but tended to form distinct clumps [22], and their presence did not appear to have any deleterious effects on the cells. These early findings have been corroborated since, and several tick bacterial endosymbionts with commensal, mutualistic, or parasitic interactions have been identified [23–26]. Noda *et al.* [24] exploited bacterial species-specific 16S ribosomal DNA (rDNA) primers and showed that only bacteria closely related to *Rickettsia rickettsii* of the spotted fever group, members of the class α-Proteobacteria, were associated with the ovaries in *Ixodes scapularis*. Although PCR amplicons specific to *Rickettsia* species were detected in all *I. scapularis* larvae examined, only 50% of the nymphal stage retained the rickettsial bacteria, suggesting that the endosymbiont was either cleared or diminished in male nymphs during molting. The pathogenic potential of these rickettsial endosymbionts remains unknown [27]. Rickettsial endosymbionts are thought to alter tick physiology and transmission of other rickettsial pathogens – as seen by the inverse relationship between the infection prevalence of *R. rickettsii* (pathogenic) and *Rickettsia peacockii* (symbiotic) in *Dermacentor andersoni* [28,29]. These provocative observations allude to the possibility that the presence of specific endosymbionts might modulate the vectorial capacity of the tick. Field and laboratory studies focused on deciphering these correlations would doubtless help to define 'biomarkers' of infection prevalence and transmission in endemic areas, and will be one of the goals of this field.

Noda *et al.* [24] also showed, using 16S rDNA amplicon sequencing, that in *Ornithodoros moubata, Rhipicephalus sanguineus*, and *Hemaphysalis longicornis* the bacterial symbiont found in ovaries and Malpighian tubules were closely related to *Coxiella burnetii*, a mammalian pathogen. In addition, *O. moubata* ovaries and Malpighian tubules also harbored an endosymbiont closely related to *Francisella tularensis*, a mammalian pathogen [30] of the class γ-Proteobacteria. This was consistent with an earlier study [31] that observed two kinds of bacteria in the ovaries and Malpighian tubules of *O. moubata*. *Francisella*-like endosymbionts have also been identified in several *Dermacentor* spp. [26]. Although these bacteria are transovarially transmitted and potentially obligate endosymbionts, the functional consequence of these on the tick vector is not fully understood. Zhong *et al.* [32] showed, using antibiotics to cure the tick of endosymbionts, that *Coxiella* endosymbionts of *Amblyomma americanum* were likely crucial for the survival and fitness of the tick. Further, the presence of the *Coxiella* endosymbionts in the salivary glands of *A. americanum* was suggested to impair the transmission of horizontally acquired pathogens such as *Ehrlichia chaffiensis* [33]. Phylogenetic analysis of the *Coxiella* spp. isolated from different ticks showed distinct phylogenetic clades of *Coxiella* spp., with each clade being specific to the tick species regardless of the geographic location of the tick [29].

In *Ixodes ricinus*, bacteria were observed in the mitochondria of ovaries [34]. These bacteria of the class α-Proteobacteria named *Candidatus Midichloria mitochondrii* [35] entered the mitochondria of cells lining the oocysts, propagated within the inner and outer membranes of the mitochondria, and apparently consumed the organelle [25]. About 94–100% of field-collected adult female *I. ricinus* were infected with this bacterium [34,36], and showed a 100% transovarial transmission rate. Despite the apparent parasitism, the oocysts developed normally in *M. mitochondrii*-infected ticks. The distribution, prevalence, and transovarial transmission of this endosymbiont in *I. ricinus* suggested that this association might be obligate and have a potential role in the fitness of the vector. Interestingly, in laboratory-raised colonies of *I. ricinus*, the prevalence of this mitochondrial endosymbiont was considerably decreased [36], suggesting that the advantage to the tick vector might be revealed only in a field setting.

Ticks of the genera *Ixodes, Rhipicephalus, Amblyomma*, and *Dermacentor* [37] have also been shown to harbor related endosymbiotic bacteria that infect the ovarian mitochondria. More importantly, the endosymbiont previously thought to be restricted to the ovaries was also observed in the salivary glands of some *I. ricinus* ticks. Moreover, humans and animals bitten by these ticks were seropositive for the endosymbiont bacterial antigens [38,39]. Further, Budachtri *et al.* [40] showed that *Amblyomma maculatum* were predominantly infected with *Francisella*, *Wolbachia*, and rickettsial spp., and sequences corresponding to rickettsial outer membrane protein-encoding genes (*ompA* and *ompB*) were also observed in salivary glands, suggesting that these endosymbionts had the potential for transmission to the vertebrate host. While most tick-borne endosymbionts were thought to be innocuous passengers [22,41], these observations

question that presumption. Another endosymbiont frequently identified in hard ticks including *I. ricinus* [42] and *Amblyomma* and *Dermacentor* spp. are *Arsenophonus*-like symbionts [43,44]. *Arsenophonus* spp. belong to the γ-Proteobacteria class, are widely distributed in insects [45], and are presumed to be involved in sex-ratio distortion [46]. Recent studies also demonstrate the presence of *Wolbachia* spp. in *I. ricinus* [47], *I. scapularis* [48], and *A. americanum* [49].

Many vertically, potentially transovarially-transmitted endosymbionts of ticks are very similar to tick-transmitted pathogens (*Coxiella*-like, *Rickettsia*-like, or *Francisella*-like), suggesting that the ancestral origin of these endosymbionts might have been vertebrate pathogens acquired by the tick while feeding on an infected host. These pathogens are suggested to have evolved along two lines – one that adapted specifically to the tick environment and became confined to tick tissues, and a second that adapted to both tick and vertebrate host and became pathogenic [24]. We must also consider the possibility that endosymbionts might have evolved to become virulent mammalian pathogens [50]. Under conditions that remain to be understood, commensal endosymbionts might emerge as vertebrate pathogens. This would suggest that these apparently benign endosymbionts must really be regarded as potential pathobionts awaiting a molecular trigger to realize their pathogenic potential. The presence of commensal endosymbionts closely related to pathogenic bacteria has also been proposed to serve as a barrier to infections by related pathogenic bacteria [29,51,52]. Understanding the mechanisms by which endosymbionts might offer this privilege to the tick could reveal new strategies to control tick-transmitted pathogens.

Beyond the intracellular endosymbionts

Advances in DNA and RNA sequencing platforms and data analysis tools [53,54] have served as key drivers in our ability to realize the full depth of the composition of the tick microbiome in great detail. The unfolding picture of the tick microbiome suggests that it is complex and is composed of more than a handful of intracellular symbionts. Sanger sequencing of full-length 16S rDNA clones, 454-pyrosequencing, Ion torrent, or Illumina based sequencing of 16S rDNA hypervariable regions, as well as a whole genome shotgun approach in conjunction with a novel data analysis approach [55], have been utilized to define the microbiomes of various tick species. Focus, understandably, has been on the hard tick because a larger number of genera in this family (*Ixodidae*) transmit diverse pathogens to humans and live stock [9]. Different studies have used different hypervariable regions of the 16S rDNA [56] (V1–V3, V4, V5, or V6 regions) and examined different tick stages from diverse geographic locations in the world (Table 1). Further, while most studies have utilized whole ticks, only a handful have utilized specific tissues dissected from the ticks [40,57–59]. The limitation of using whole ticks is that it does not allow one to define the tissue-specific microbiome [60], and this understanding might be

Table 1. A brief overview of tick microbiomes assessed in the past 10 years[a]

Genus/species	Target gene	Approach	Geographic location of ticks	Developmental stage	Tissue assessed	Refs
Ixodes ricinus	16S	Sanger sequencing of DGGE fragments[b]	Austria	Adults	Whole ticks	[60]
Ixodes scapularis	16S	Sanger sequencing of clones	USA	Nymphs	Whole ticks	[47]
Ixodes scapularis	16S	Sanger sequencing, TGGE[c]	USA	Adult and nymphs	Whole ticks	[65]
Ixodes ricinus	16S	Sanger sequencing of clones	Netherlands	Nymphs	Whole ticks	[67]
Amblyomma americanum	16S	Sanger sequencing of clones	USA	Adults, larvae and eggs	Whole ticks	[44]
Rhipiciphalus microplus	16S	454 pyrosequencing	USA	Adults	Whole ticks, midguts, ovaries, and eggs	[57]
Ixodes ricinus	16S	454 pyrosequencing	Italy	Nymphs and adults	Whole ticks	[66]
Amblyomma americanum	16S	Ion torrent	USA	Nymphs and adults	Whole ticks	[70]
Amblyomma americanum	16S	454 pyrosequencing	USA	Adults and nymphs	Whole ticks	[63]
Amblyomma, Ixodes, Haemaphysalis[d]	Whole genome	454 pyrosequencing	Japan, Netherlands	Adults and nymphs	Whole ticks	[55]
Ixodes ricinus	Whole transcriptome	Illumina	France	Nymphs	Whole ticks	[68]
Ixodes scapularis	16S	454 pyrosequencing	USA	Larvae, and nymphs	Whole ticks and guts	[58]
Ixodes Haemaphysalis[b]	16S	454 pyrosequencing	Japan	Adults	Salivary glands	[59]
Amblyomma maculatum	16S	454 pyrosequencing	USA	Adult ticks	Guts, salivary glands, and saliva	[40]
Ixodes persulcatus	16S	Illumina sequencing	China	Adult ticks	Flat and fed whole ticks	[72]

[a]Studies focused on specific bacterial genera in ticks are not listed here.

[b]DGGE, denaturing gradient gel electrophoresis.

[c]TGGE, temperature-gradient gel electrophoresis.

[d]When several tick species were assessed in a single study, only the tick genus is listed.

pertinent to derive functional inferences in the context of the biology of the tick and its interactions with tick-borne pathogens. In addition, when whole ticks are assessed, one cannot discern exoskeleton-associated environmental contaminants from *bona fide* tick tissue-associated bacteria. Exoskeleton-associated bacteria should, however, not be dismissed because these might provide additional barrier surveillance strategies – as seen with the microbiome of mammalian skin [61]. While a diverse variety of bacterial genera have been identified in each of these studies, and the observations are potentially influenced by parameters specific to each study, some unifying patterns emerge. Across all genospecies of hard ticks, bacteria of the phylum *Proteobacteria* predominate, followed by *Actinobacteria, Firmicutes*, and *Bacteroidetes*. Bacterial members of the phyla *Acidobacteria, Cyanobacteria, Fusobacteria*, and TM7 are also observed at lower levels in some studies [40,58]. Both aerobic and anaerobic bacteria were observed, and Gram-negative bacteria were predominant [48]. At the genus level, some members were representative of tick species regardless of their geographic locations – including *Rickettsia* and *Coxiella* in *Amblyomma americanum* [44,62,63] and *Rhipicephalus* spp. [64]; *Rickettsia* in almost all *Ixodes* spp. [48,55,58,59,65–68]; and *Wolbachia* in *Ixodes ricinus* [66,67], *Amblyomma maculatum*, and *Rhipicephalus microplus* [40,57], suggesting that these might be obligate endosymbionts of tick species. Narasimhan *et al.* [58] and Moreno *et al.* [65] have examined *I. scapularis* nymphs (laboratory colonies and wild-caught ticks from Connecticut and New York, respectively) and, despite the use of very different techniques to address the microbiome (454 pyrosequencing and temporal temperature gradient gel electrophoresis respectively), they identified many common genera such as *Stenotrophomonas*, *Sphingobacterium*, *Pseudomonas*, and *Acinetobacter* in nymphal and adult stages. Importantly, Narasimhan *et al.* [58] utilized dissected gut tissues whereas Moreno *et al.* [65] utilized whole ticks. Therefore, the identification of several common bacterial genera in both studies suggests that these bacteria are likely *bona fide* tick gut residents. Overall, in addition to obligate intracellular endosymbionts, several bacterial genera are observed more frequently across several hard tick species, and include *Pseudomonas*, *Sphingobacterium*, *Acinetobacter*, *Enterobacter*, and *Stenotrophomonas*. These might represent bacterial genera with a greater propensity to colonize tick species (Figure 1).

A non-traditional approach to the identification of tick-associated bacteria comes from a study by Villar *et al.* [69]. Using a combination of transcriptomic analysis by paired-end RNA sequencing and proteomic analysis by reverse phase liquid chromatography coupled with tandem mass spectroscopy, this study assessed stress response genes and proteins in unfed *Dermacentor reticulatus* larvae. Interestingly, together with increases in *D. reticulatus* stress response genes and proteins, the study also identified 16 transcripts and 14 proteins that corresponded to *Rickettsia* spp. [69]. Thus, in addition to genomic approaches, this approach offers an opportunity to define tick-associated pathogenic and commensal bacteria at the transcriptome and proteome level.

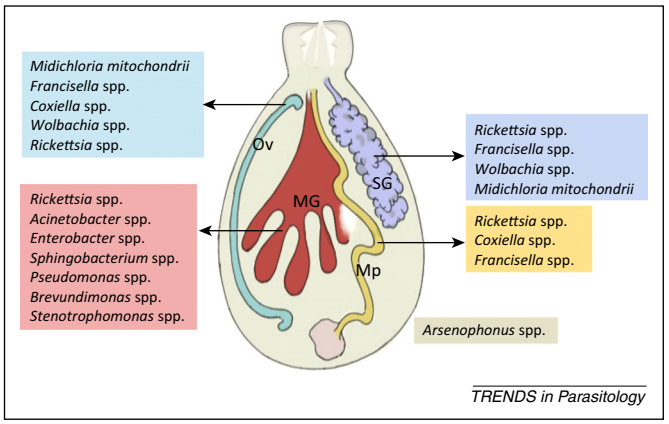

Figure 1. Predominant bacterial members of the tick microbiome. A schematic representation of bacterial genera frequently observed in the salivary glands (SG), midgut (MG), ovary (Ov), and Malphigian tubules (Mp) of ticks. *Arsenophonus* spp. have been frequently identified in ticks, but their tissue distribution has not been determined.

Laboratory and wild-caught ticks showed similarities as well as differences in microbiome composition, potentially as a result of environmental factors including temperature, light–dark cycles, host availability, and vegetation, as well as development stage and feeding/nutritional status of the tick [44,65–67,70,71]. Several studies suggest that feeding increased the bacterial diversity of the tick microbiome [58,65,72]. In a study of the microbiome of *Ixodes persulcatus* from woodland areas of China [73], up to 200 genera in different stages of *I. persulcatus* were observed. When the blood of rats on which these ticks fed was assessed, several bacterial genera found in ticks were also found in rat blood, indicating that at least some of these bacterial members of the tick microbiome were also likely transmitted to the mammalian host [73]. It is likely that only some bacterial taxa are *bona fide* members of the tick microbial consortium and that many are environmental contaminants. We must bear in mind that increased sensitivity of the sequencing platforms comes with the pitfalls of identifying minor environmental contaminants. It will become important to adhere to protocols that would minimize errors both in DNA preparations and analysis [3,74], and it will also be crucial to outline the experimental procedures that would facilitate the research community to glean and define core microbiomes representative of the tick species. Comparisons of the microbiome of field-collected ticks to that of laboratory-reared ticks might additionally serve to highlight core microbiota inherent in specific tick species, and changes in specific bacterial taxa and in proportions of core members between field and lab-reared ticks might provide the context to infer the functional roles of specific members. Age, tick stage, feeding status, rearing procedures of laboratory colonies, as well as geography, time of year and day of field-collected ticks, will all likely influence the microbiome profile, and recording all covariates would be more conducive to a unified understanding of the core microbiome of ticks.

The spatial organization

Several questions arise from these tick microbiome studies. Are the consortia of bacteria on the epithelial cell

surface, are they intracellular, or are they in the lumen of the gut and salivary glands? Of special interest is the gut microbiota because the gut is the site of the first encounter between the tick and the incoming pathogen. Ticks have a unique mode of blood-meal digestion. The gut lumen is alkaline, and enzyme-mediated lysis of red blood cells and release of hemoglobin takes place in the gut lumen. Hemoglobin is taken up by receptors on digestive cells of the tick gut, and is broken down intracellularly by a series of hemoglobinases in the acidic environment (pH 3.5–4.5) of the digestive vesicles [75]. The lumen serves as a 'holding place' for the blood meal as the tick engorges, and the uptake of nutrients commences during engorgement and proceeds through the molting process. Digestion products including heme are transported back into the lumen and defecated. Therefore, if the gut lumen contains extracellular symbionts as observed in mammals [76], the resident bacteria must be capable of surviving the heme-filled lumen, the toxic reactive oxygen species (ROS) from neutrophils and macrophages, and complement proteins and proteases in the bloodmeal of the host. Indeed, Budachetri *et al.* [40] observed that the guts of *A. maculatum* predominantly harbored enterobacterial species that are known to generate ROS and survive ROS-mediated killing [77]. As digestion proceeds through the molt, the lumen of the gut might become congested with digested peptides and debris, and undergo structural and functional changes [78]. The profiles of the gut bacteria, either in the lumen or in the epithelial cells, must therefore undergo dramatic shifts in composition to take advantage of the changing milieu. Consistent with this assumption, Heise *et al.* [72] observed in *A. americanum* females that, upon feeding, proportions of *Coxiella* spp. decreased, *Rickettsia* spp. increased, and genera not detectable in the unfed tick such as *Pseudomonas* increased. Do luminal bacteria come into contact with the epithelial cells of the gut, or are they separated by the peritrophic matrix (PM) that separates the lumen from the digestive cells of the gut [79]? A visible PM is shown to be formed around 6–12 h after tick feeding [80] – are the luminal bacteria therefore in brief contact with the epithelial cells during the pre-PM formation stage? If they are, then the immunome of the tick gut [81,82] must respond to this contact, and the resulting immune milieu must influence the ability of the incoming pathogens to colonize the tick. The microbiome composition might determine the potency of the immune response, and this could potentially be detrimental to incoming pathogens. Microbiome profiles might possibly serve as biomarkers of infection prevalence in ticks in endemic areas. The tick gut is also unique in that, in contrast to other structures of the tick that are formed *de novo* at each ontogenic stage, the gut epithelial cells do not undergo histolysis and are retained from the previous developmental stage [21]. This must have a crucial impact both on the survival and stability of gut microbiota and gut-colonizing pathogens such as *B. burgdorferi* and *Babesia microti*. Detailed ultrastructural examination of the tissue-specific tick microbiome at various stages of tick feeding and development will enhance our functional understanding of tick microbiome.

The functional role of tick microbiome

Understanding the functional consequence of tick–microbiome interactions is fundamental to developing new paradigms based on the microbiome to control ticks and tick-transmitted pathogens. Zhong *et al.* [32] showed that curing *Amblyomma* ticks of their endosymbiont resulted in prolonged time to oviposition, decreased hatching of the eggs, and decreased larval survival. By contrast, curing *Ixodes pacificus* of its rickettsial endosymbiont had no apparent impact on oviposition or tick survival [83]. In the study by Narasimhan *et al.* [58] the tick microbiota, and predominantly the gut microbiota, was shown to play a role in maintaining the integrity of the PM. When ticks were treated with gentamicin, or when ticks were raised in a sterile environment that did not allow a normal tick microbiome to develop, the integrity of the PM was compromised and *B. burgdorferi* colonization was impaired. *Borrelia* colonizes the gut by adhering tightly to the gut epithelial cells [84,85] and is likely shielded from the deleterious elements of the gut lumen by the PM that forms at the apical side of the epithelial cell [80] during tick feeding. The PM thus served a non-canonical role by facilitating *B. burgdorferi* colonization of the tick gut [58], in contrast to the traditional barrier role to preclude pathogen entry that has been suggested for the PM in *Drosophila* [86] and for the mucus layer in the mammalian gut [87]. Alterations in the microbiome composition might also result in differences in the levels of activation of immune response molecules such as Toll and Immune deficiency (IMD) in the gut epithelium [88], and thereby influence pathogen survival and infection (Figure 2). Effector molecules including ROS generated upon immune activation maintain bacterial homeostasis, but also impose collateral damage on the gut epithelium [89]. At least in *Drosophila*, epithelial damage has been shown to signal the secretion of Unpaired 3 (Upd3), a cytokine-like molecule that activates the Janus kinase (JAK)–Signal transducer and activator of transcription (STAT) pathway [90]. Functional orthologs of Upd3-like molecules in the tick gut might be regulated as part of normal gut microbiota–tick interactions to ensure bacterial homeostasis and the maintenance of a viable epithelial barrier offered by the PM (Figure 2). Tick-borne pathogens such as *B. burgdorferi* might have evolved to exploit this normal friction that accompanies the tick–microbiota dialogue (Figure 2). It will be important to define the gut microbial profiles that favor or impair pathogen colonization, as shown for *Plasmodium* survival in the mosquito gut [77]. This understanding is paramount to fully exploit the microbiome to control ticks and tick-transmitted pathogens (Box 1), and remains one of many challenges that tick microbiome research faces (Box 2).

Origin of the tick microbiome

In humans and mice it has been shown that the maternal microbiota (in the birth canal) is inoculated into the offspring and lays the foundation for a healthy microbiome [91]. In addition to the transovarially transmitted endosymbionts of ticks, it is conceivable that the maternal microbiota might serve as the first inoculum in eggs and the developing larvae. The observation by Narasimhan *et al.*

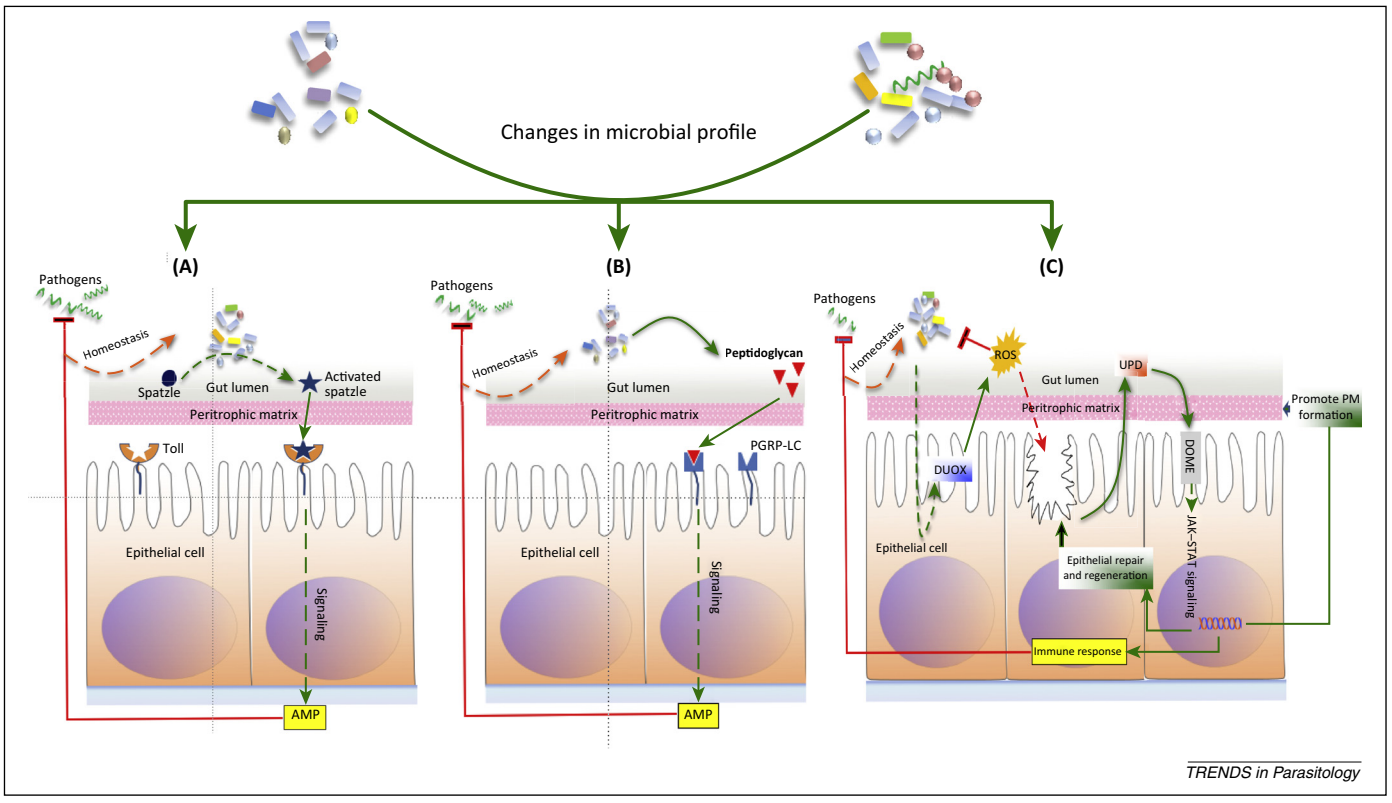

Figure 2. Changes in the microbial composition and the consequent immune responses in the tick gut. Alterations in the gut microbiome associated with feeding, development, and infection could modulate the following immune response pathways in ticks. **(A)** Toll pathway. Microbiota-induced activation of Spatzle, enabling Spatzle–Toll interaction and initiation of the signaling cascade resulting in the production of antimicrobial peptides (AMPs). **(B)** Immune deficiency (IMD) pathway. Sensing of Gram-negative peptidoglycan by the peptidoglycan recognition protein (PGRP-LC) to activate the signaling cascade leading to AMP production. **(C)** JAK–STAT (Janus kinase/Signal transducer and activator of transcription) pathway. Microbiota and pathogen-induced activation of dual oxidase (DUOX) results in reactive oxygen species (ROS) production to control bacteria. ROS-mediated collateral damage to the gut epithelial cells initiates the release of cytokine-like molecule Unpaired 3 (Upd3) that engages with its receptor, DOME, a signal transducing transmembrane protein receptor, to activate the JAK–STAT signaling pathway. STAT transcriptionally regulates pathways leading to immune responses, epithelial regeneration and repair, and peritrophic membrane integrity. AMPs and immune responses generated by Toll, IMD, and JAK–STAT pathways influence pathogen survival and also facilitate bacterial homeostasis (Based on citations [58,87–89]).

[58] that larvae hatched in a sterile environment harbored a significantly different microbiome composition would suggest that *I. scapularis* larvae, at least partially, acquire their microbiota from the environment, including bacteria entering through openings such as the spiracles, mouth, or the anal pore. Copulation can also serve as an additional route to augment bacterial inoculation (paternal transmission) of the tick microbiome [92]. Ticks are obligate hematophagous arthropods, and microbiota on the host skin might also contribute to the microbial diversity of the tick gut. Nevertheless, of all the diverse environmental microbiota that might gain access into the tick, only a few become *bona fide* members of the tick microbiome. Because diet plays a central role in shaping the composition of the mammalian microbiome [93], it is likely that hematophagy might select for certain bacterial genera. Bacteria that can either supplement the deficiencies of mammalian blood, such as the tsetse endosymbiont that provides vitamin B to the tsetse fly [94], or those that encode functions to lyse red blood cells or metabolize nutrients in the blood, might be selected for [95]. If diet were the only deciding factor, then we must observe a similar microbiome in all hematophagous arthropods. A dedicated meta-analysis might help address this possibility. Interestingly, the microbiome of *Drosophila melanogaster*, despite feeding on a variety of decaying matter with ample opportunity to harbor a complex microbiome, has a very low diversity, and the core members are *Acetobacter*, *Gluconobacter*, and *Lactobacillus* spp. [96]. By contrast, despite a restricted diet, the microbiome of the tick appears complex [55], suggesting that the arthropod taxonomy must additionally impose genetic constraints on who stays and who does not. Indeed, data supporting this possibility are presented by Hawlena *et al.* [97] who examined the microbiota of several vectors including *Dermacentor variabilis* and *I. scapularis* from about 200 rodents in southern Indiana. Meta sequence analysis revealed that *Rickettsia* phylotype 1 was always observed in *I. scapularis*, and *Francisella* phylotypes were seen only in *D. variabilis*. Understanding how the tick acquires its microbiota, and how the microbiome composition is shaped by various environmental and genetic factors, will be essential

Box 1. Outstanding questions

- What is the origin of the tick microbiome?
- What external factors shape the tick microbiome composition?
- Can we define specific tick endosymbionts that correlate directly or inversely with infection and transmission of specific pathogens?

Answers to these nested questions are pivotal to transforming our mechanistic understanding of the tick microbiome into strategies to control ticks and tick-transmitted pathogens.

Box 2. Tick microbiome – challenges ahead

Germ-free ticks: an understanding of the functional consequence of core microbial members of the microbial consortium would require that we generate ticks that harbor no bacteria – in other words, germ-free ticks. The ability to generate germ-free mice has contributed greatly to an overall understanding of how members of the microbial consortia might regulate host biology [1,75,86]. Such an opportunity remains to be developed for ticks. Current strategies utilize antibiotics [32,58], but the introduction of antibiotics into ticks is tedious, transient, and subject to potential side-effects of the antibiotic. We would require germ-free facilities to hatch eggs, and then feed the subsequent stages on germ-free mice. While the operational logistics is overwhelming, developing such a protocol would enable great strides in our understanding of tick–microbiome interactions.

Gnotobiotic ticks: once germ-free ticks become available, the ability to generate gnotobiotic ticks (i.e., ticks that harbor one or more specific bacteria) would be the next goal. While it is possible to artificially feed ticks (references cited in [58]) and potentially introduce specific bacteria into the tick, this requires further optimization to feed ticks to repletion. Essential requisites to achieve this goal are the ability to fully define tick microbiomes at the species level, and the ability to grow axenic cultures of the core bacteria. Integrated into these achievements would be the futuristic hope of paratransgenesis: in other words, the potential use of these core bacteria to deliver anti-tick or anti-pathogenic gene products.

Genetically engineered ticks: the ability to generate stable knockout and knock-in ticks is currently not available for tick research. RNA interference-mediated knockdown of gene expression [58] is handicapped by the transient nature of the silencing. As we begin to uncover molecular interactions between the tick and its microbiome, the ability to generate germline knockouts of specific tick genes would help to better understand how tick gene products shape the tick microbiome.

to begin to exploit the microbiota of the tick to control the prevalence of ticks and derail transmission of pathogens by ticks (Box 1).

Concluding remarks

Ticks have evolved over the past 5–65 million years to circumvent the many challenges they had to encounter [98]. A recent study [99] showed that *I. scapularis* has colluded with its microbial partners and borrowed the *tae* (type VI amidase effector) genes, that encode enzymes to degrade cell walls of competing bacteria, in a *trans*-kingdom transfer event several million years ago, and exploits it to control the growth of pathogens like *B. burgdorferi*. On the down-side, the prevalence of antibiotic-resistance genes encoded by several members of the tick microbiome [55] presents a potential opportunity for tick-borne pathogens to pilfer these genes by horizontal gene transfer [100], leading to the emergence of antibiotic-resistant tick-borne pathogens. It is therefore important to address and comprehend the 'Yin and Yang' of the tick microbiome – those that might confound pathogen transmission and those that might enhance pathogen transmission. As the tick microbiome research comes to center-stage poised with powerful molecular and technological advancements, it offers a new vantage point to understand the biology of the tick and its interactions with the microbes it harbors, and this really is the exciting promise of the tick microbiome.

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