Trends in Parasitology

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Review

Noncoding RNAs in Parasite–Vector–Host Interactions

Chaima Bensaoud,[1] Michael Hackenberg,[2,3] and Michail Kotsyfakis[1,*]

Systems biology approaches, especially in the big data era, have revolutionized modern parasitology. Of the many different molecules participating in parasite–host interactions, noncoding RNAs (ncRNAs) are now known to be (i) transmitted by the vector to possibly modulate vertebrate host responses and favor vector survival and (ii) regulated in the host by parasites to favor parasite survival. Here we provide an overview of the involvement of ncRNAs in the parasite–vector–host triad and their effect on host homeostasis based on recent advances and accumulating knowledge about the role of endogenous vertebrate noncoding RNAs in vertebrate host physiology.

Highlights

ncRNAs participate at every stage of the parasite–vector–host interaction, subverting vertebrate host responses to vectors and host responses to parasites.

Extracellular vesicles are important ncRNA transporters that contain several ncRNA species, including some with unknown functions, such as transfer RNAs.

Parasite and vector ncRNAs subvert fundamental cellular processes involved in other disease, such as cancer, so may provide important leads for future therapeutic development.

ncRNAs: Universal Participants in Parasite Infection

ncRNAs, originally regarded as genomic garbage, are now known to be diverse and important cellular regulators [1,2]. Functional genomics, together with whole-genome and RNA sequencing (RNA-Seq) of different eukaryotes, including humans, have highlighted the fundamental role played by ncRNAs in health and disease [3]. Many ncRNAs are involved in the processing and regulation of other RNAs, such as mRNA, tRNA, and rRNA [4]. ncRNAs can be divided into two main groups: the **long noncoding RNAs** (**lncRNAs**) (see Glossary) and the **small noncoding RNAs** (**sncRNAs**). lncRNAs are long transcripts [from 200 nucleotides (nt) to 100 kb in length] lacking an open reading frame (ORF) [5,6] and usually transcribed by RNA polymerase II and controlled by the transcriptional activators of the SWI/SNF complex. lncRNAs are involved in the regulation of various cellular processes, including transcription, intracellular trafficking, and chromosome remodeling [7]. By contrast, sncRNAs (18–200 nt) are attracting increasing interest due to their diversity; they include small interfering RNAs (siRNAs), microRNAs (miRNAs), and piwi-interacting RNAs (piRNAs). siRNAs target and degenerate gene transcripts through an RNA-induced silencing complex (RISC)-mediated process and bind specifically to a single gene location [8,9]. piRNAs are 26–31 nt species that regulate gene expression through interactions with P-element-induced wimpy testis (piwi) regulatory proteins to form piRNA-induced silencing complexes, which are responsible for silencing transposons in the germline [10,11]. miRNAs, the most comprehensively characterized small RNA subclass, participate in various biological processes through post-transcriptional regulation of specific target genes [12] and are also present in the **extracellular vesicles** (**EVs**) secreted by most cells [13]. miRNAs post-transcriptionally regulate gene expression by silencing protein expression through cleavage and degradation of the mRNA transcript or by inhibiting translation [14].

Recent studies, supported by deep-sequencing efforts, have shed light on the role of ncRNAs in the tripartite parasite–vertebrate host–disease vector interaction [15,16]. It is becoming clear that ncRNAs are not only produced by the vector to eventually subvert vertebrate host responses but that parasites regulate host ncRNA expression to favor parasite survival. Here we review current knowledge about ncRNAs in parasite–host–vector interactions in order to establish future research directions.

[1]Biology Center of the Czech Academy of Sciences, Branisovska 31, 37005 Budweis, (Ceske Budejovice), Czechia
[2]Dpto. de Genética, Facultad de Ciencias, Universidad de Granada, Campus de Fuentenueva s/n, 18071, Granada, Spain
[3]Lab. de Bioinformática, Centro de Investigación Biomédica (CIBM), PTS, Avda. del Conocimiento s/n, 18100, Granada, Spain

*Correspondence:
kotsyfakis@paru.cas.cz (M. Kotsyfakis).

sncRNAs in Vector–Host Interactions

As they take their blood meal, hematophagous ectoparasites face several barriers in the vertebrate, including the host's hemostatic, inflammatory, and immune responses [17]. Nevertheless, some ectoparasites are able to overcome these host responses and remain attached to the host during feeding [18]. Each ectoparasite has evolved different strategies depending on its lifestyle, and it is becoming increasingly clear that sncRNAs are among the key players in ectoparasite–host interactions. Particular attention has been given to miRNAs, which have been studied in several arthropods and of which some examples are detailed below. miRNAs recognize target mRNAs via a small sequence at the 5′ end of the miRNA called the seed region [19]. A single miRNA usually interacts with many transcripts, and a single transcript can be targeted by many miRNAs [20]. miRNAs are usually predicted in next-generation sequencing (NGS) data using *in silico* tools such as miRDeep2, mireap, and miRanalyzer (where a genome sequence is required) or by mapping against a reference library of homologous sequences [21]. Many miRNAs are highly conserved and only very rarely are they lost within a lineage [22], with functional validation provided through knockdown experiments in model organisms [22].

sncRNAs in Ticks

Ticks must mount particularly robust host defense reactions as their feeding attachment takes days or even weeks compared to the few seconds or minutes taken by other hematophagous ectoparasites like mosquitoes or fleas [23]. Once on their host, ticks use their mouth parts to dilacerate host skin and introduce their hypostome, essential for attachment [24]. Tick bites therefore trigger complex host immune system cascades to eliminate and kill the ectoparasite [25]. In response, ticks secrete a huge arsenal of complex molecules in their saliva.

There has been considerable interest in the identification and validation of tick salivary components over the past three decades facilitated by advances in molecular biology. Salivary molecules are pluripotent, show considerable redundancy, and belong to different protein families including metalloproteases, Kunitz domain-containing proteins, cystatins, ixodegrins, and lipocalins [26]. Modern technologies and high-throughput approaches, especially when considered from the systems biology perspective, have filled in many of the 'missing pieces of the puzzle' by enabling the characterization of tick salivary molecules and gene expression dynamics throughout tick feeding [27]. Thanks to transcriptome and proteome projects, several tick ORFs have now been shown to encode potential regulators of vertebrate host physiology [28]. These projects have also revealed a significant number of sncRNAs in tick saliva, which themselves are now suggested to participate in the parasite–host interaction [28].

miRNAs produced by ticks participate in the tick–host interaction in different tick species. For example, Barrero and colleagues sequenced small RNA transcriptomes derived from various life stages and organs of the cattle tick *Rhipicephalus* (*Boophilus*) *microplus* [28], *which* infests a single host during its life cycle and consequently relies on bovine phenolic compounds to recognize its preferred and optimal host [29]. Tick miRNA expression was related to host odor recognition by ticks, as their expression changed according to tick exposure to their host [29]. In another example, Hackenberg and colleagues performed *in silico* analysis to show that the saliva-specific miRNAs of *Ixodes ricinus* might have combinatorial effects on the host targetome, that is, many tick miRNAs may target vertebrate host genes in the same host homeostatic pathway, and the expression of a given host gene may also be regulated by more than one saliva-specific tick miRNA [30]. The authors suggested combinatorial effects of vector miRNAs on host target genes, which may be of importance in evolutionary terms to maintain robust regulation of certain host genes and pathways important in the tick–host interaction. miR-8-3p, bantam-3p, mir-317-3p, and miR-279a-3p were predicted to target host KEGG pathways such as 'gap

Glossary

Epitranscriptomics: the biochemical modification of RNA affecting its fate and influencing the way genes are expressed.
Extracellular vesicles (EVs): vesicles released into the extracellular fluid. They can be classified as exosomes, microvesicles (MVs), and apoptotic bodies according to their cellular origin.
Long noncoding RNAs (lncRNAs): RNA fragments (>200 nucleotides) that are not translated to protein.
miRNA sponges: artificial long RNAs used to sequester miRNAs *in vivo*. They can be used to validate target predictions and suppress specific miRNA expression.
Small noncoding RNAs (sncRNAs): small RNA fragments (1–200 nucleotides) that are not translated to protein.
Transfer RNAs (tRNAs): RNA adaptors acting as a physical link between mRNA and the amino acid sequence of proteins.

junction' and 'inflammatory mediator regulation of TRP channels', which play a role in the host homeostatic response (Figure 1A) [30].

There are now numerous studies on miRNAs in tick–host interactions. However, these studies are mostly *in silico* analyses, and more functional validation is required to better understand the *in vivo* roles of sncRNAs. Further studies are also needed to delineate sncRNA dynamics during feeding and the full arsenal of tick salivary gland molecules targeting host defenses.

sncRNAs in Mosquitoes

sncRNAs have also been studied in other important pathogen-bearing arthropods such as mosquitos (Figure 1B). In a recent systematic review of miRNAs in mosquitoes, miRNAs were found to be dysregulated in a species-, sex-, stage-, and tissue/organ-specific manner [31]. Further, aberrant miRNA expression was observed in development, metabolism, and insecticide resistance [31]. Lei and colleagues measured miRNA expression in pyrethroid-resistant and susceptible strains of laboratory populations of *Culex pipiens* and showed that miR-278-3p was upregulated in susceptible strains [32]. In another study by Hong and colleagues, cpi-miR-71 was significantly downregulated in female adults from a deltamethrin-resistant strain, indicating that cpi-miR-71 may contribute to deltamethrin resistance [33].

Mosquito miRNAs might also be involved in host–vector interactions. In a small RNA-seq study of adult female salivary glands and saliva of the African malaria vector *Anopheles coluzzii,* Arcà and

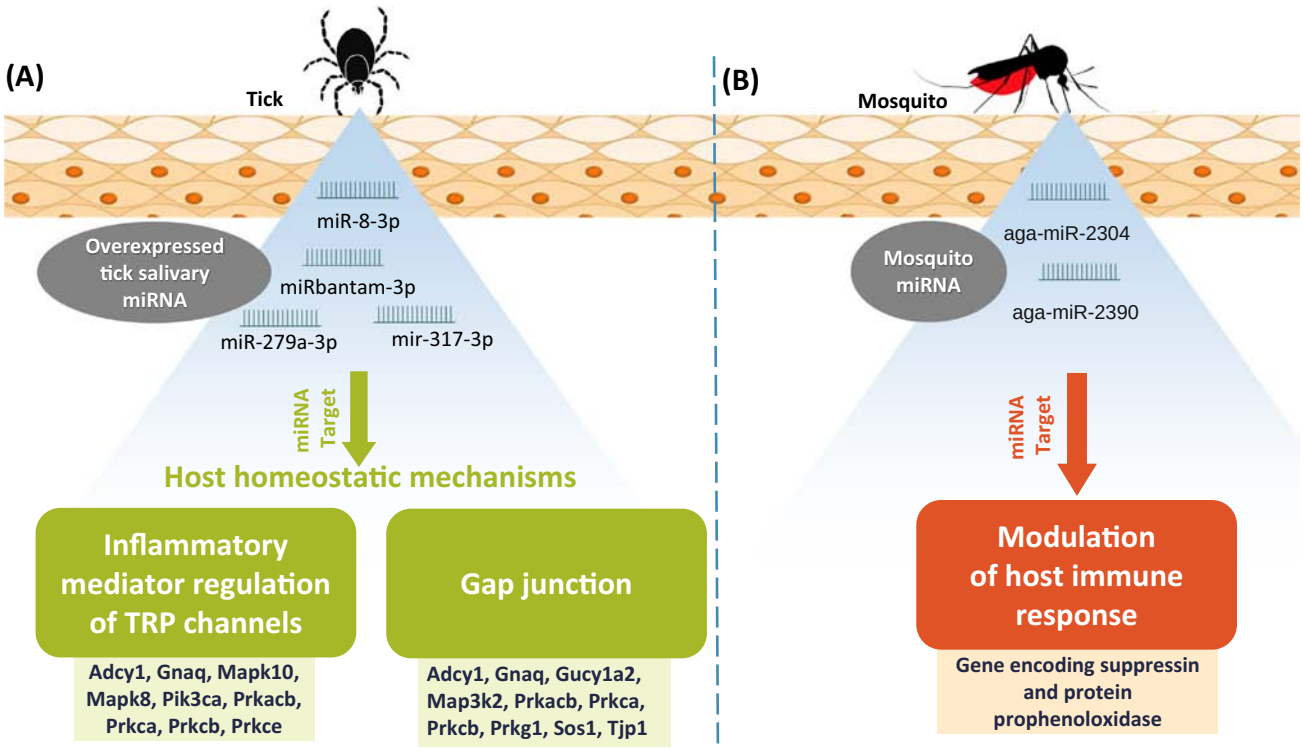

Figure 1. Hematophagous Arthropods Secrete Several Molecules through Their Saliva to Affect Different Host Responses. (A) miR-8-3p, bantam-3p, mir-317-3p, and miR-279a-3p from the tick *Ixodes ricinus* were predicted to target gap junctions and inflammatory mediator regulation of TRP channels, which play a role in the host homeostatic responses. (B) aga-miR-2304 and aga-miR-2390 from *Anopheles gambiae* might modulate immune responses by targeting suppressin and prophenoloxidase, which enhance natural killer cell activity and cellular and humoral defenses, respectively.

colleagues examined anopheline salivary miRNA composition and verified whether saliva-enriched miRNAs could regulate host responses [34]. Ten miRNAs enriched in salivary glands were abundant in saliva (among the top 30) and, therefore, likely to be injected into the vertebrate skin during blood feeding with the potential to target host genes at the biting site [34]. Using a computational approach, Thirugnanasambantham and colleagues showed that aga-miR-2304 and aga-miR-2390 from *Anopheles gambiae* might modulate immune responses [35]. Aga-miR-2304 and aga-miR-2390 target the gene encoding suppressin (Figure 1B), which enhances natural killer cell activity by eliciting interferon-α and -β synthesis and secretion [36], and the gene of the protein prophenoloxidase involved in cellular and humoral defenses [37].

Other classes of sncRNAs, such as small siRNAs and piRNAs, may also be relevant to host–parasite interactions. Hess and colleagues tried to identify critical features of viral infection in dengue serotype 2 (DENV2)-infected *Aedes aegypti* by deep-sequencing sncRNAs. They showed that piRNAs (24–30 nt) and unusually small RNAs (usRNAs) (13–19 nt) were produced in DENV-infected mosquitoes, implicating the piwi pathway in antiviral defenses [38]. Schnettler *et al.* showed that piRNA-like molecules are produced following infection with the mosquito-borne Semliki Forest virus in mosquito cell lines and that knockdown of piRNA pathway proteins enhanced the replication of this arbovirus and defined the contribution of piRNA pathway effectors [39]. Several studies have also shown that all siRNA pathway components are highly expressed in the midgut of *Aedes aegypti*, and knockdown of key siRNA pathway members, such as Ago2 and Dicer2, markedly increases the replication of Alphavirus and Flavivirus [40–42].

Overall, mosquito miRNAs are thought to interact with multiple target genes to elicit biological functions [31]. Nevertheless, individual miRNAs and their potential targets and function, have yet to be fully determined and require further investigation. Also, research in this area might provide avenues for the use of miRNAs as vector-control tools, by allowing setting of criteria for target prediction using machine-learning algorithms and exploration of miRNA:mRNA networks at the post-transcriptional level [31].

sncRNAs in Parasite–Host Interactions

sncRNAs are thought to participate in the interaction between the vertebrate host and ectoparasites to facilitate parasite feeding or survival. Parasites transmitted by vectors may also use epitranscriptomic mechanisms to manipulate the transcriptional programs of their ectoparasite 'host'[43].

sncRNAs in Protozoan Apicomplexa

Parasites transmitted by arthropods must survive both their vector and the vertebrate host. This '*bellum omnium contra omnes*', or 'war of all against all' [27], also suggests that these parasites manipulate the vertebrate host through epigenetic factors such as sncRNAs. There is now evidence that protozoan parasites might modulate the expression of host miRNAs in order to survive in the vertebrate intracellular environment [15]. Martin-Alonso and colleagues [44] conducted one of the first large-scale analyses of miRNA expression in mice infected with malaria. Malaria is mainly caused by *Plasmodium falciparum* transmitted to humans through the bite of a female *Anopheles* mosquito. Martin-Alonso *et al*. showed that *Plasmodium* infection dysregulated a specific set of brain miRNAs in mice, among which several were more abundant in cerebral malaria (CM) compared with noncerebral malaria (NCM) mice. This group of miRNAs was postulated to play an important role in CM and included several molecules widely associated with inflammatory responses. Of these, it was hypothesized that miR-223 actively participates in infection, regulating the differentiation of several key players of the innate immune response to modulate the early stages of infection [44].

Other medically important apicomplexans. such as *Trypanosoma* and *Cryptosporidium,* have also been reported to alter host cell miRNA expression [45]. These parasites use different mechanisms to reorganize host cell functions to allow their survival and replication inside host cells, as they are obligate intracellular parasites [46]. With regard to host miRNA expression, Ferreira and colleagues performed pathway, functional enrichment, and upstream regulator analysis of differentially expressed genes targeted by differentially expressed miRNAs in *Trypanosoma cruzi* [47]. They showed that miRNA target-enriched biological processes and pathways were associated with immune response and metabolism such as IFN-γ, TNF-α, NF-κB signaling signatures, cytotoxic T lymphocyte-mediated apoptosis, mitochondrial dysfunction, and Nrf2-modulated antioxidative responses [47]. Thus, miRNAs may be implicated in the pathophysiology of acute *T. cruzi infection* [47].

Leishmania, another parasite of the order Trypanosomatida is spread by *Phlebotomus* and *Lutzomyia* sandflies [48,49]. *Leishmania* sp. causes leishmaniasis, which affects about 12 million people worldwide and is considered a global health problem due to its widespread global distribution [48,49]. There have been several studies on the altered expression of host miRNAs in human and/or murine macrophages infected with *L.* (*L.*) *major, L.* (*L.*) *donovani*, or *L.* (*L.*) *amazonensis* [50]. Also, other studies showed host miRNA dysregulation in human host cells infected with *L.* (*L.*) *infantum* or *L.* (*Viannia*) sp. [51–53]. Several MHC- or interferon-associated genes were among the targets of the host miRNAs perturbed upon parasite infection [53].

It is worth mentioning that even parasites not transmitted by ectoparasite vectors can manipulate vertebrate host responses. Zhou and colleagues demonstrated significant alterations in miRNA expression in gastrointestinal epithelial cells in response to *Cryptosporidium parvum* infection [54]. At the molecular level, activation of the TLR/NF-κB signaling pathway in host cells upon infection may trigger the transcriptional regulation of miRNA genes similar to the protein-coding genes controlled by the pathway [55]. Other studies have shown that specific miRNAs may modulate epithelial immune responses to *C. parvum* infection at every step of the innate immune network, including production of antimicrobial molecules, expression of cytokines/chemokines, release of epithelial cell-derived exosomes, and feedback regulation of immune homeostasis [54,55].

Overall, there have been considerable efforts to identify sncRNA profiles, and particularly miRNA profiles, whether using cloning, microarrays, or direct sequencing. Nevertheless, miRNA target analysis and the gene regulatory networks of miRNA families require further investigation, since only a few studies have performed functional validation, limiting our understanding of miRNA repertoires and functions in the mosquito.

sncRNAs in Helminths

Helminths are also medically important multicellular parasites [56]. They do not usually transmit microbial infections, instead invading host tissues or starving the host of nutrients [57]. Helminths secrete miRNAs into host blood, and these miRNAs were shown to represent promising candidate diagnostic biomarkers of helminthic infection [58]. Thanks to deep-sequencing approaches, several miRNAs have been discovered from trematodes, nematodes, and cestodes, and these were associated with TGF-β, MAPK, Toll-like receptor, and PI3K/AKT signaling pathways and insulin growth factor regulation [59]. Cai and colleagues observed changes in over 130 miRNAs after *Schistosoma* infection in a mouse model in comparison to before infection [60]. Of these miRNAs, miR-155, miR-223, and miR-146 are already known to suppress Toll-like receptor and cytokine signaling via a negative feedback loop involving downregulation of IL-1 receptor-associated kinase 1 (IRAK1) and TNF receptor-associated factor 6 (TRAF6) proteins [60].

miRNAs were also identified in adult *Clonorchiasis sinensis* by applying deep sequencing and bioinformatics extrapolations combined with stem–loop real-time PCR analysis. Hepatic miRNA expression profiles from *C. sinensis*-infected mice were compared at 2, 8, and 16 weeks after infection using miRNA microarrays and validated by quantitative real-time PCR (qRT-PCR) [61]. During infection, 349 miRNAs were differentially expressed, of which 143 were downregulated and 206 were upregulated. All dysregulated miRNAs were potentially involved in clonorchiasis by regulating various signaling pathways including cancer-related, TGF-β, MAPK, TLR, and PI3K/AKT signaling pathways. Interestingly, 169 of these dysregulated miRNAs were predicted to be involved in the TGF/Smad signaling pathway, which plays an important role in the biliary fibrosis caused by *C. sinensis* [61].

Potential Effect of Host sncRNAs on Parasites

Host cells might also release ncRNAs on invading parasites as a defense mechanism (Figure 2). Lamonte and colleagues showed that miRNAs play a role in establishing *P. falciparum* resistance in mutant sickle cell (HbS) erythrocytes by demonstrating that a unique, noncanonical miRNA activity modulated parasite protein translation and was a major determinant of malaria resistance in HbAS and HbSS erythrocytes [62]. In another study, also involving *P. falciparum*, circulating microparticles abundantly released from red blood cells during the blood stage of malaria infection were shown to be able to transfer hAgo2-miRNA complexes into the parasites within infected red blood cells. Some of these miRNAs (miR-451 and miR-140) targeted and downregulated a critical parasite antigen, *P. falciparum* erythrocyte membrane protein-1 (PfEMP1) [63].

EVs and sncRNA Transporters in Host–Parasite Interactions

EVs in Host–Parasite Interactions

EVs are small membrane vesicles derived from the endocytic compartment of different kinds of cells [64]. EVs carry a series of bioactive cargos consisting of lipids, proteins, metabolites,

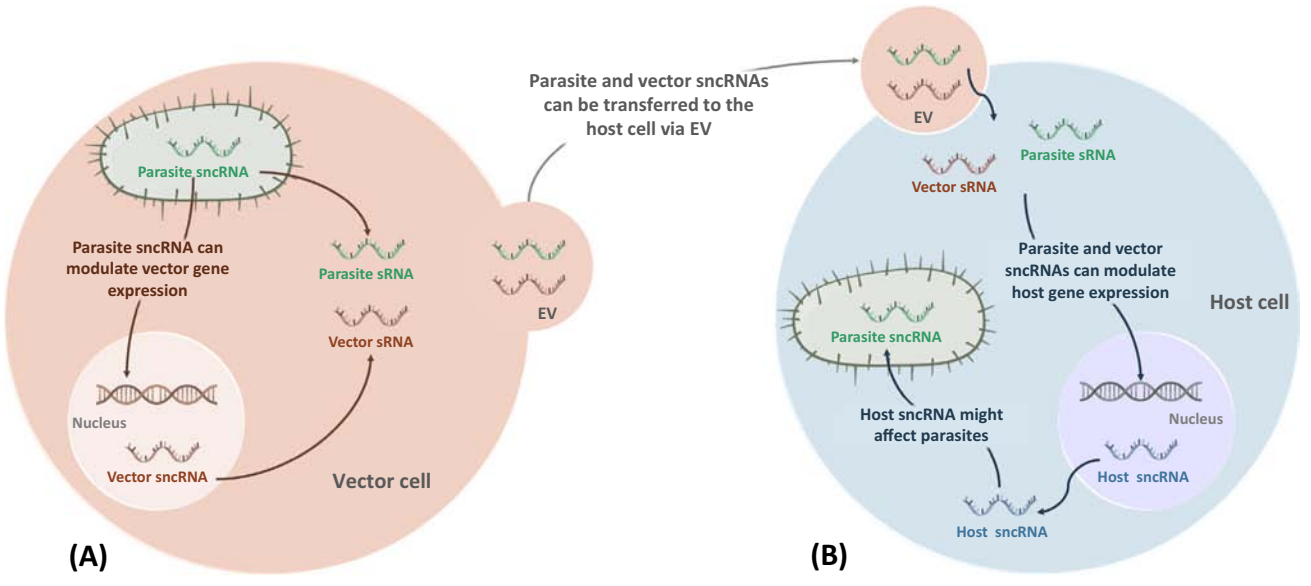

Figure 2. Representation of Possible Vector–Parasite, Host–Parasite, and Vector–Host Interactions through Small Noncoding RNAs (sncRNAs). (A) sncRNAs from parasites might modulate gene expression in vector cells; sncRNAs in vectors/parasites can be transferred to the mammalian host by means of extracellular vesicles (EVs). (B) In the host cell, sncRNAs in vectors/parasites might modulate gene expression; by contrast, hosts release sncRNAs as a defense against parasitic infection.

DNA, and RNA (mRNA, miRNA, and ncRNA). EVs can be divided into four important types according to their mechanism of generation and size: exosomes, microvesicles, apoptotic bodies, and oncosomes [65]. Exosomes play a crucial role in infections by directly transmitting pathogen-related molecules and also indirectly influence infection by modulating other processes such as immune evasion and apoptosis [66]. The past decade has seen considerable interest in elucidating the role of parasitic EVs in parasite–parasite communication as well as in parasite–host interactions. miRNAs are enriched in EVs, which are secreted by most cells (Figure 2) [16]. Potential immunoregulatory miRNAs with targets in the host have been identified in the EVs of the trematodes *Dicrocoelium dendriticum* and *Fasciola hepatica* [16]. Zhu and colleagues characterized *Schistosoma japonicum* exosome-like vesicles (*S. japonicum* EVs), and a specific miRNA ocu-miR-191-5p, most likely originating from the final host, was shown to be associated with *S. japonicum* EVs [67]. The gastrointestinal nematode *Heligmosomoides polygyrus,* which infects mice, secretes vesicles containing miRNAs, Y RNAs (RNA fragments mapping to nematode stem-bulge RNAs), and nematode Argonaute protein [68]. These vesicles were of intestinal origin and were enriched for mammalian exosome protein homologs. Administration of the nematode exosomes into mice suppressed type 2 antihelminthic immune responses and eosinophilia induced by the allergen *Alternaria*. Microarray analysis of mouse cells incubated with nematode exosomes *in vitro* identified Il33r and Dusp1 as suppressed genes, with Dusp1 repressed by nematode miRNAs based on a reporter assay [68].

Depending on the parasite, the released EVs can modulate the host immune response or mediate cellular communication between parasites (Figure 2) [69]. For example, Silva and colleagues showed that *Toxoplasma gondii* secretes/excretes EVs (microvesicles and exosomes) that were recognized by host immune response and contain miRNAs [70].

tRNA in Host–Parasite Interactions

Through specific selection mechanisms, **transfer RNA** (**tRNA**) fragments and miRNAs occur more frequently in exosomes than do other RNAs such as messenger RNAs (mRNAs) and ribosomal RNAs (rRNAs) [71,72]. tRNAs represent sncRNAs involved in the translation of mRNA into protein [73]. tRNA structure is evolutionarily conserved to regulate specific interactions between tRNA, amino acids, and coding RNAs [74]. However, the first studies focused on the role of EV-secreted miRNAs in different processes and especially the immune system [75].

For decades, this specific structure–function relationship of tRNAs constrained the idea that tRNAs or parts of tRNAs participated in other biological processes. However, more recent studies have shown that premature and mature tRNAs can be cleaved by different ribonucleases, thus generating tRNA fragments which have since been implicated in many biological and cellular processes and various pathophysiological mechanisms [76]. Fricker and colleagues showed that the $tRNA^{Thr}$ 3′ half is produced during nutrient deprivation and becomes one of the most abundant tRNA-derived RNA fragments (tdRs). $tRNA^{Thr}$ halves associated with ribosomes and polysomes stimulated translation by facilitating mRNA loading during stress recovery once starvation conditions ceased. Blocking or depleting the endogenous $tRNA^{Thr}$ halves mitigated this stimulatory effect both *in vivo* and *in vitro*. *T. brucei* and its close relatives lack the well-described mammalian enzymes for tRNA half processing, thus hinting at a unique tdR biogenesis in these parasites [77]. tRNA fragments have been found in parasites such as *T. gondii* and *Plasmodium berghei* and might be involved in pathogenic processes in parasites; however, their exact role and function remain elusive [78].

ncRNA Sponges: A Step Further in Parasite–Host Targetomes

miRNAs are not only involved in parasite–host interactions but also in development, health, and disease [79,80]. miRNAs can be modulated by lncRNAs behaving as 'sponges' that sequester their miRNA target [81]. As with most miRNA target genes, a sponge's binding sites are specific to the miRNA seed region, which allows them to block a whole family of related miRNAs [81].

lncRNAs acting as '**miRNA sponges'** under the 'target mimicry' mechanism were first identified in *Arabidopsis thaliana* [82], and this discovery influenced the design and effective delivery of miRNA inhibitors and mimics the development of an artificial approach to determining the effects of miRNA depletion [83]. In this approach, an expression vector carrying multiple binding sites to an miRNA is introduced into cells [83]; following vector gene transcription, the overexpressed synthetic binding sequences occupy the endogenous miRNA in the cells with high affinity, blocking miRNA regulation of its target genes [83]. In this way, miRNA sponges represent a good alternative to injecting synthetic targets and can provide a means of sequestering miRNAs *in vivo*. Therefore, they could be excellent tools to study the miRNA–parasite–host targetome.

There is also emerging evidence indicating that lncRNAs significantly impact host immunity and the ability to respond to infection [84–86]. In a comprehensive survey of host lncRNAs regulated during *Toxoplasma* infection, *T. gondii* was found to be a strong stimulator of the lncRNA transcriptome in host cells, and the parasite exerted an important influence on host responses in a strain-specific manner [87]. Also, in a genomic study, most lncRNAs of *Schistosoma mansoni* were upregulated, suggesting their involvement in the regulation of the rapid adaptation of schistosomula after transition from free-living larvae to the early mammal parasitic stage [88].

Concluding Remarks

The study of host–parasite interactions has been facilitated over recent decades by newer epigenetics and **epitranscriptomics** approaches. In the case of the host–vector–pathogen triad, the specific interactions between the different actors are complex, but ncRNAs appear to participate at every level of the interaction. Sequencing studies have highlighted that sncRNAs might manipulate their vertebrate host and, furthermore, that pathogens also modulate host sncRNA expression to escape the defense pathways that might lead to their degradation. The vertebrate host also tries to overcome these threats. The very rapid adaptation of ectoparasites via the regulation of the molecules they secrete, as well as the development of several pathogen strategies to adapt to their host and their vector, are ongoing research challenges. Although there is ongoing research in this area, several areas require further investigation. Future *in silico* studies should shed light on sncRNA targets in the host, while functional studies might elucidate host gene regulation by parasitic sncRNAs. sncRNAs seem to rely on EVs for shuttling to eventually impact exogenous gene expression, but the full complement of sncRNAs enriched in EVs and their predicted targets needs to be established (see Outstanding Questions). Methods for sncRNA loss of function such as genetic knockouts, antisense oligonucleotide inhibitors, and miRNA sponges could pave the way for a more thorough understanding of the role played by sncRNAs in host–parasite interactions. Further studies are needed to clarify the details of all the interactions to help develop new vector-control strategies and drugs.

Outstanding Questions

What are the roles of sncRNAs in host–parasite interactions?

How are sncRNAs transported into host cells, is their transport specific, and how do parasitic noncoding RNAs hijack the host vesicle transportation system?

What are the mechanisms enabling host gene regulation by parasitic 'noncoding RNAs', and how do they manage to affect gene expression in the nuclei of the vertebrate host cells?

What are the potential defense pathways by which the host targets and controls parasitic sncRNAs?

Author Contributions

All authors searched and read the literature; C.B. and M.K. wrote the manuscript; M.H. and M.K. edited the manuscript.

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