Trends in Microbiology

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REVIEWS

Review

MicroRNAs at the Host–Bacteria Interface: Host Defense or Bacterial Offense

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MicroRNAs are a class of small noncoding RNAs that act as major post-transcriptional regulators of gene expression. They are currently recognized for their important role in the intricate interaction between host and bacterial pathogens, either as part of the host immune response to neutralize infection, or as a molecular strategy employed by bacteria to hijack host pathways for their own benefit. Here, we summarize recent advances on the function of miRNAs during infection of mammalian hosts by bacterial pathogens, highlighting key cellular pathways. In addition, we discuss emerging themes in this field, including the participation of miRNAs in host–microbiota crosstalk and cell-to-cell communication.

Highlights

MiRNAs have recently emerged as major players in the interactions between host and bacterial pathogens.

Several host functions have been shown to be regulated by miRNAs during infection, including cell cycle, cytoskeleton organization, autophagy, cell death, and survival.

MiRNAs are an integral part of the host immune response to bacterial infection.

Bacterial pathogens subvert host miRNA expression for their own benefit, promoting survival, replication, and persistence.

A growing number of studies indicate that gut microbiota can influence the miRNome, and vice-versa.

MicroRNAs as Major Regulators of Gene Expression

MicroRNAs (miRNAs) are a class of single-stranded small noncoding RNAs (ca. 20–22 nt in length) that play authoritative roles in post-transcriptional gene regulation in a wide range of organisms, including animals, plants, and viruses [1]. In humans, there are currently more than 2500 mature miRNAs annotated in miRBase, the depository for miRNA sequences. Roughly 60% of the human transcriptome is estimated to be regulated by miRNAs [2]. The canonical miRNA biogenesis pathway entails two steps of processing by RNAse III enzymes, originating a 20–22 bp miRNA duplex. To accomplish the regulatory functions, one of the strands of the duplex (aka guide strand) is incorporated into the miRNA-induced silencing complex (miRISC); the loaded miRISC represses target mRNAs by a mechanism that combines translation repression and mRNA degradation [3].

Cumulating evidence over recent years has shown that miRNAs are major regulators in numerous biological processes (e.g., cell proliferation, apoptosis [4,5]) and that miRNA dysregulation correlates with disease (e.g., cancer, cardiovascular diseases [6,7]). In addition, the involvement of miRNAs during infection by various pathogens, including viruses, parasites, and bacteria, is now a well established concept [8–10]. Particularly in the case of bacterial pathogens, it has become clear that miRNAs are an integral part of the host immune response to efficiently fight infection. Importantly, emerging evidence also demonstrates that bacteria actively harness host miRNAs to promote pathogenicity. Adding to the already intricate role of miRNAs at the host–pathogen interface, recent reports have suggested that miRNAs can be transmitted between cells/tissues, and potentially even between host and bacteria, participating in signaling events relevant to infection.

In this review, we provide a nonexhaustive description of the role of miRNAs in the context of infection by bacterial pathogens, highlighting recurrent themes underlying their action (Figure 1), specifically the regulation of the immune response, cell cycle, cell death/survival, autophagy, and cytoskeleton organization. Additionally, we discuss emerging concepts in the field, namely the role of miRNAs in host–microbiota interplay and cell-to-cell communication. Although

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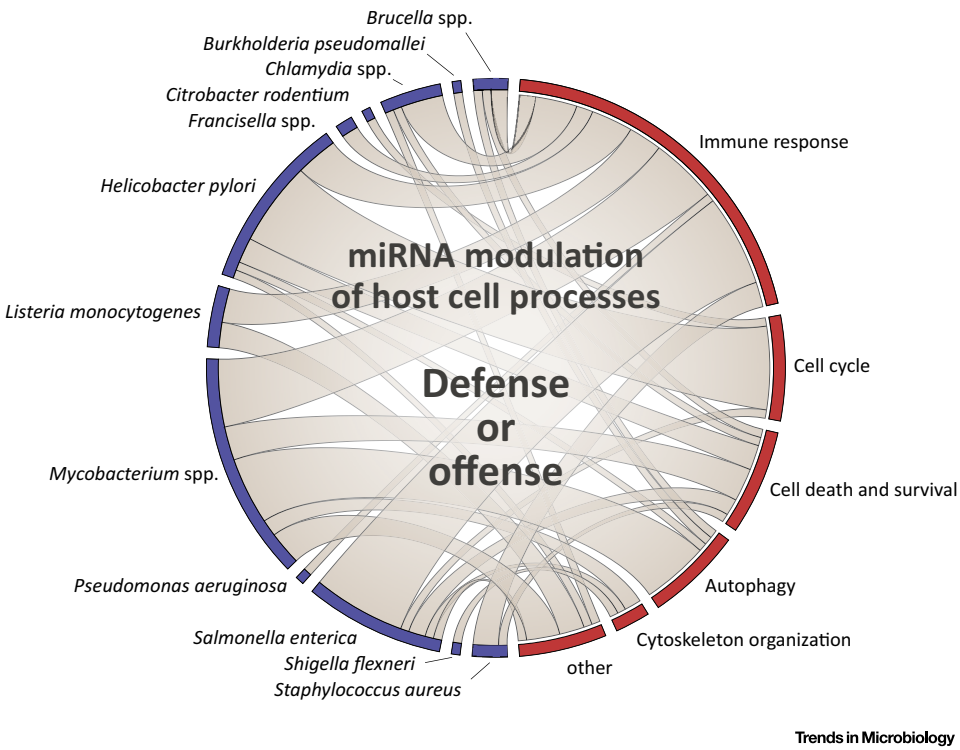

Figure 1. MiRNAs Play Multifaceted Roles during Infection, Controlling Multiple Host Cell Functions. MiRNA changes can reflect host responses to fight infection or can be a strategy devised by bacterial pathogens to subvert critical host functions. Ribbon width represents roughly the number of miRNAs described for each relationship.

important work on the role of miRNAs during bacterial infection has been performed in plants and invertebrates, in this review we focus on studies in mammals (mostly human and mouse).

First Evidence of miRNA Regulation upon Bacterial Infection

MiRNAs were first implicated in bacterial infection in a pioneer work by Navarro *et al*. showing that the recognition of a flagellin-derived peptide from *Pseudomonas syringae* by *Arabidopsis thaliana* induces miR-393a transcription [11]. This miRNA was shown to repress the receptor for auxin, a negative regulator of the plant immune system, ultimately enhancing the plant resistance to *P. syringae* infection. These findings were closely followed by a study in human monocytes revealing that miRNome changes are an integral part of the host response to bacterial infection, specifically in the innate immune response to bacterial lipopolysaccharide (LPS) [12]. This work characterized miR-146 as an anti-inflammatory miRNA contributing to the fine-tuning of the host immune response, via the regulation of Toll-like receptor (TLR-4) and cytokine signaling.

Following these seminal studies, miRNA regulation in the setting of bacterial infection and its consequences to host cell functions have been profusely demonstrated in different model systems using a plethora of pathogens.

Targeting of the Immune Response

Numerous studies have explored the role of miRNA regulation in the immune response against bacteria. MiR-146a/b, miR-155, miR-21, and the let-7 family are the most extensively studied

miRNAs in innate immune response, regulating multiple steps of the associated pathways [9,13,14]. These miRNAs are regulated upon activation of Toll-like receptors (TLRs), key cellular sensors for the recognition of pathogen-associated molecular patterns (PAMPs) derived from various microbes. Interestingly, these and other miRNAs regulate several components of TLR signaling, thus regulating the extent and timing of the inflammatory response [15]. The upregulation of miR-146 and miR-155 upon infection has been shown to be required to mount a proper host immune response. While miR-146 acts mainly as an anti-inflammatory miRNA [12,16], miR-155 functions as a potent proinflammatory modulator [16–22], although it has also been reported to limit the proinflammatory response [16,23,24]. MiR-21 induction by inflammatory stimuli is well described, and its key function in the anti-inflammatory response has emerged [14,25]. Interestingly, miR-21 upregulation during *Mycobacterium leprae* infection has also been described as a bacterial strategy to escape the vitamin D-dependent induction of antimicrobial peptides [26]. Downregulation of let-7 family members is also a recurring feature of the response to bacterial infection. Interestingly, the immunomodulatory effect of let-7 miRNAs varies, depending on the cell context and pathogen [27–34].

Recent studies have uncovered a role for other miRNAs in immune function. For example, miR-302b, similarly to miR-146a/b, is involved in orchestrating a balanced immune response during infection by *Pseudomonas aeruginosa* [35]. MiR-302b expression is induced upon *P. aeruginosa* infection via TLR/NF-κB-dependent pathways. In turn, miR-302b targets IRAK4, a protein required for the activation of NF-κB through TLR receptors, preventing exacerbated inflammation. Importantly, expression of miR-302b and IRAK4 knockdown decreased mortality in a mouse model of *P. aeruginosa* infection. Increased miR-301b expression upon *P. aeruginosa* infection has also been shown to modulate inflammation [36]. MiR-301b targets c-Myb, which decreases the anti-inflammatory cytokines IL-4 and TGF-β1, leading to excessive inflammation and to an impaired host defense. In the context of *Neisseria gonorrhoeae* infection, miR-718 acts as a negative regulator of inflammation in macrophages. MiR-718 regulates PI3K/AKT signaling via direct targeting of PTEN, ultimately reducing proinflammatory cytokine production and increasing bacterial burden [37].

MiRNA-mediated repression of the NF-κB pathway constitutes a strategy for increasing host cell survival by preventing exacerbated immune responses to infection, but it can also be exploited by pathogens to thrive. Examples of the latter include *Brucella abortus* and *Mycobacterium tuberculosis*, which promote their survival in infected macrophages by increasing the expression of the A20 deubiquitinase, a negative regulator of the NF-κB pathway, through downregulation of miR-125b-5p and let-7f, respectively [30,38].

Macrophage polarization resulting in M1 (classically activated macrophages, proinflammatory state) or M2 (alternatively activated macrophages, anti-inflammatory state) phenotypes, a molecular event crucial for inflammation, is also modulated by interfering with miRNA expression. *M. tuberculosis* infection decreases miR-26a-5p, consequently derepressing the transcription factor KLF4, favoring M2 macrophage polarization, a friendly niche for this bacterium [39]. In contrast, miR-20b is downregulated in *M. tuberculosis*-infected macrophages, favoring M1 polarization through activation of the NLRP3/IL-1β/caspase-1 axis [40]. Along the same lines, *Staphylococcus aureus* infection ensures M1 polarization through the reduction of miR-24, and consequent activation of the CHI3L1-mediated MAPK pathway [41]. Recently, a new immune-related miRNA has been discovered in the context of *Helicobacter pylori* infection [42]. *H. pylori* reduces miR-4270 expression, which, in turn, increases the levels of the immune receptor CD300E, compromising the ability of the infected macrophages to express and expose MHC class II molecules and, consequently, their activation by effector T cells.

MiRNAs have also been implicated in phagocyte recruitment to sites of infection. For example, infection with *Salmonella enterica* serovar Enteritidis leads to an increase in miR-128, which, via direct targeting of macrophage colony-stimulating factor (M-CSF), impairs macrophage recruitment to infection sites [43]. During *M. tuberculosis* infection, upregulation of miR-223 likewise decreases the expression of the cytokine IL-6 and chemokines CXCL2 and CCL3, thus inhibiting a proper recruitment of neutrophils to infected tissues [44].

Control of the Cell Cycle

The cell cycle is arguably the cellular process that has the strongest impact on cell morphology, metabolism, and homeostasis, constituting, not surprisingly, an important target for pathogen interference. Due to the link established between *H. pylori* persistent colonization and gastric carcinogenesis [45], the regulation of the cell cycle associated miRNAs by this pathogen has been the subject of extensive research (Figure 2). Increased levels of miR-21 in *H. pylori*-infected cells and tissues promotes cellular proliferation and invasion by targeting the known tumor suppressor RECK [46]. MiR-222 was also shown to be upregulated in *H. pylori*-infected gastric tissues and to repress the expression of RECK and of the homeodomain-interacting protein kinase-2 (HIPK2), thus promoting proliferation and invasion of gastric cancer cells [47,48]. Contributing to an effect of *H. pylori* in promoting cell cycle progression, down-regulation of miR-370 and miR-101 upon infection leads to the derepression of the transcription factor FoxM1 and the oncogene SOCS2, respectively [49,50]. An additional observation in the context of *H. pylori* infection concerns the epigenetic silencing of miR-210 [51]. DNA methylation of the miR-210 gene promoter was shown to be increased in *H. pylori*-positive human gastric biopsies, when compared with negative controls, leading to the derepression of STMN1 and DIMT1, both regulators of gastric epithelial cell proliferation. Along the same line, aberrant methylation of the let-7 promoter can occur, through a mechanism dependent on the *H. pylori* virulence factor CagA, leading to Ras pathway activation [33]. Several other miRNAs, including

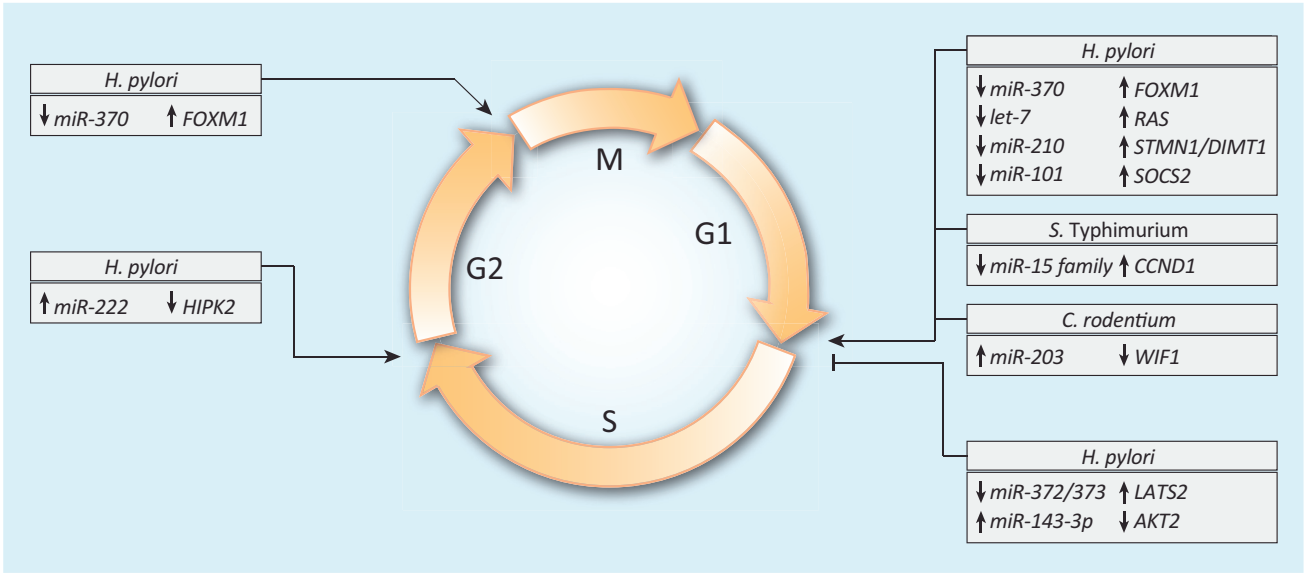

Figure 2. MiRNA Modulation upon Infection by Bacterial Pathogens Induces Alterations of the Host Cell Cycle. Perturbations of the host cell cycle might contribute to bacterial survival and/or proliferation by maintaining their proliferative niche. *H. pylori*, *Helicobacter pylori*; *S.* Typhimurium, *Salmonella enterica* serovar Typhimurium; *C. rodentium*, *Citrobacter rodentium*.

miR-203, miR-204, miR-212-3p, miR-320, miR-361-3p, miR-375, miR-584, and miR-1290, have also been implicated in *H. pylori*-dependent cell proliferation and tumorigenesis [52–57]. These studies show that dysregulation of these miRNAs during *H. pylori* infection consistently favor cell proliferation and, ultimately, tumorigenesis. Nonetheless, it should be noted that *H. pylori* has also been shown to block cell cycle progression of gastric epithelial cells, through miRNA regulation [58,59]. Specifically, downregulation of miR-372 and miR-373 in *H. pylori* infected cells derepressed the tumor suppressor LATS2, thus arresting cells at the G1/S transition [58]. In a recent study, Wang *et al*. analyzed miRNA expression in gastric cancer patients, comparing *H. pylori*-positive and -negative subgroups. Among the 53 miRNAs differentially expressed, miR-143-3p was the most upregulated miRNA in *H. pylori*-positive gastric cancer tissues. Dysregulation of miR-143-3p dampened cell growth, apoptosis, migration, and invasion through the direct targeting of the kinase AKT2 [59]. Although it appears difficult to conciliate the contradictory results concerning miRNA-mediated cell cycle phenotypes elicited during *H. pylori* infection, it is conceivable that a fine balance between cell proliferation and inhibition of epithelial cell renewal is required by this bacterial pathogen to maintain its intracellular niche.

In addition to *H. pylori*, other bacterial pathogens regulate host cell cycle via miRNA modulation (Figure 2). The expression of the miR-15 family, known to block host G1/S cell cycle transition through the repression of Cyclin D1 [60], is downregulated upon *Salmonella enterica* serovar Typhimurium infection [61]. Interestingly, the miR-15 family, as well as other miRNAs that hinder G1/S progression, strongly inhibit *S.* Typhimurium replication. By decreasing the expression of the miR-15 family, *S.* Typhimurium favors cell cycle progression and, ultimately, bacterial replication. An additional example is the increased miR-203 expression upon *Citrobacter rodentium* infection, which deregulates Wnt/β-catenin signaling through the direct repression of the Wnt antagonist WIF1, thereby increasing cell proliferation and crypt hyperplasia [62].

Modulation of Cell Death/Survival Pathways

Host cell death is often triggered as part of the immune defense response, but pathogens employ diverse strategies to regulate host cell death for their own benefit. Among others, miRNA regulation is emerging as a relevant theme in the regulation of host cell death. For example, an increase in miR-582-5p and miR-155 expression in *M. tuberculosis*-infected monocytes impairs apoptosis by decreasing expression of the transcription factors FOXO1 and FOXO3, respectively [63,64]. Incubation with *M. tuberculosis* early secreted antigenic target 6-kDa protein (ESAT-6) was also shown to induce miR-155 expression in macrophages. Interestingly, in this case, upregulation of miR-155 promoted apoptosis by decreasing SOCS1 in a TLR-2/NF-κB-dependent manner [65]. The role of miR-155 in the context of mycobacterial infection as a positive or negative regulator of apoptosis and autophagy (see below) is still controversial, and it likely depends on the cell context and bacterial strain. Indeed, miR-155 via the regulation of SHIP1/Akt pathway was shown to have opposite effects on the control of *M. tuberculosis* infection by innate and adaptive immune cells [18]. In macrophages, miR-155 promotes cell survival, providing a niche for bacterial replication. On the other hand, miR-155 favors the long-term survival of T cells that secrete cytokines required to control infection. Another example of a miRNA with a dual role in infection is miR-27b, recently shown to be induced in murine macrophages as a response to *M. tuberculosis* infection [66]. Interestingly, miR-27b directly targets the Bcl-2-associated athanogene 2 protein (Bag2): on the one hand, Bag2 decrease inhibits the production of proinflammatory factors by blunting NF-κB activity, while on the other hand it increases p53-dependent apoptosis and reactive oxygen species (ROS) production, thus decreasing bacterial burden. Besides *M. tuberculosis*, other pathogens have been shown to manipulate host cell survival through modulation of host miRNA

expression. The strong induction of miR-30c-5p expression upon *Chlamydia trachomatis* infection is an additional example of this phenomenon. An increase in miR-30c-5p during infection ensures stable p53-mediated downregulation of Drp1, a master regulator of mitochondrial fission, preserving mitochondrial integrity and promoting host cell survival during infection [67].

Disruption of Autophagy

Autophagy is a fundamental process responsible for the recycling of macromolecules and damaged cytosolic organelles, particularly relevant under a variety of stress stimuli, and an important player of host innate immunity responsible for capturing and degrading intracellular bacteria. Not surprisingly, bacterial pathogens have evolved multiple strategies to counteract autophagy, including modulation of host miRNAs (Figure 3). Illustrating the importance of this regulatory mechanism, different *Mycobacterium* species, namely *M. tuberculosis* and *Mycobacterium bovis* Bacillus Calmette–Guérin (BCG), blunt host autophagy through regulation of host miRNAs. MiR-17 is reduced upon *M. tuberculosis* infection, leading to derepression of the autophagy regulator MCL1 and its transcriptional regulator STAT3 [68]. Conversely, BCG infection increases miR-17 expression, repressing ULK1, an essential autophagy-initiation protein [69]. In both cases, miR-17 regulation favors mycobacterial survival by impairing host autophagy. Reduction of miR-26a levels upon *M. tuberculosis* infection also enhances the expression of MCL1, in this case through derepression of its transcriptional factor KLF4 [39]. MiRNAs favor mycobacterial survival in macrophages by interfering with autophagosome formation and vesicle maturation. Indeed, increased levels of miR-20a upon BCG infection repress ATG7 and ATG16L1, both necessary for phagosomal maturation [70]. In addition, upregulation of miR-144-3p expression upon BCG infection compromises autophagosome formation by decreasing ATG4a protein levels [71], while an increase in miR-144-5p upon *M. tuberculosis* infection suppresses autophagy by targeting the autophagy regulator DRAM2 [72]. *M. tuberculosis* can also target the initial steps of autophagy through inhibition of Beclin-1 and UVRAG, via increased miR-30a and miR-125a-3p expression, respectively [73,74]. *M. tuberculosis* also induces the expression of miR-33 and miR-33*, which target multiple effectors (ATG5, ATG12, LC3B, LAMP1), activators (AMPK), and transcriptional regulators (FOXO3, TFEB) of the autophagy and lysosome pathways [75].

Notwithstanding its crucial involvement in immune response, miR-155 has recently been shown to contribute to autophagy regulation following *M. tuberculosis* infection. Increased miR-155 in *M. tuberculosis*-infected dendritic cells compromises the autophagy pathway by targeting ATG3 [76], an E2-ubiquitin-like conjugating enzyme critical for LC3 lipidation during autophagosomal formation. Contrasting with these findings, a previous study reported that miR-155 induction in *M. tuberculosis*-infected macrophages elicits a proautophagic outcome through the repression of the negative regulator of autophagy RHEB, ultimately compromising bacterial intracellular survival [77].

Regulation of autophagy by miRNAs has also been studied for other bacterial pathogens. Upregulation of miR-30b upon *H. pylori* infection decreases Beclin-1 and ATG12, proteins necessary for the formation and maturation of autophagosomes, ultimately contributing to the prevention of bacterial clearance [78]. Beclin-1 and ATG12 (along with ATG2B, ATG5, and BNIP3L) are also targeted by miR-30d, which is induced by *H. pylori* infection [79]. Infection of lung epithelial cells by *Burkholderia pseudomallei* increases the expression of miR-4458, miR-4667-5p, and miR-4668-5p. These three miRNAs repress the expression of ATG10, which is essential for autophagosomal maturation, ultimately favoring bacterial intracellular survival [80]. The miR-196 family was also shown to inhibit the autophagy pathway by modulating the levels

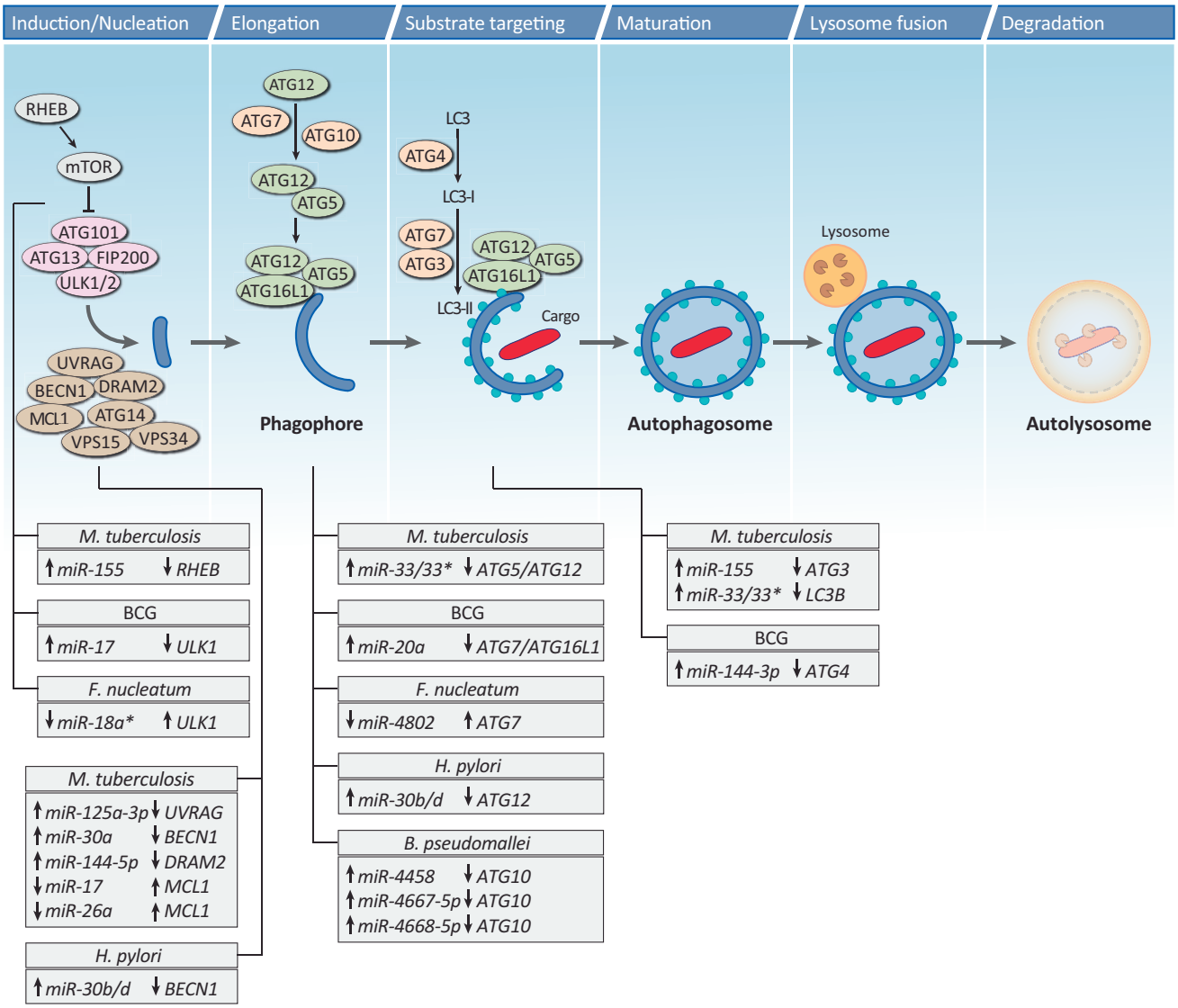

Figure 3. MiRNome Changes upon Bacterial Infection Have a Strong Impact on Autophagy. Infection by multiple bacterial pathogens leads to alterations of miRNA expression, which regulate multiple steps of autophagy. A recurring theme underlying miRNA modulation during infection is the inhibition of autophagy and, hence, of bacterial degradation. *M. tuberculosis*, *Mycobacterium tuberculosis*; *F. nucleatum*, *Fusobacterium nucleatum*; *H. pylori*, *Helicobacter pylori*; *B. pseudomallei*, *Burkholderia pseudomallei*; BCG, Bacillus Calmette–Guérin.

of IRGM, an autophagy regulator, and thus increase intracellular levels of adherent invasive *Escherichia coli* (AEIC) [81]. The same study described a synonymous variant of IRGM implicated in Crohn's disease, which, due to an altered miR-196 binding site, likely changes xenophagy efficacy.

Targeting of Cytoskeleton Organization

The ability to interfere with the host cytoskeleton is a recurring feature amongst intracellular bacterial pathogens. Notwithstanding other well described and highly efficient strategies to interfere with the host cytoskeleton, namely those orchestrated by effector proteins delivered

into the host cytosol by specialized bacterial secretion systems, miRNA regulation is also emerging as an important mechanism in this context. For example, miR-29a upregulation in ileal samples of *S.* Typhimurium-infected piglets was shown to directly target Caveolin-2, an inhibitor of the small Rho GTPase Cdc42, thus favoring bacterial uptake by epithelial cells [82]. In contrast, miRNAs negatively regulate bacterial uptake and/or invasion, most likely as part of the host response to counteract infection. MiR-142-3p, for example, is induced upon *M. tuberculosis* infection of macrophages and, by targeting N-WASP, an actin binding protein essential for phagocytosis, this miRNA diminishes mycobacterium uptake [83]. Induction of miR-21 in macrophages infected by *Listeria monocytogenes* was shown to repress the actin modulating proteins RhoB and MARCKS, decreasing bacterial phagocytosis [84]. Along the same line, upregulation of miR-331-3p upon *S.* Typhimurium infection observed in piglet whole-blood samples was suggested to impair bacterial uptake by targeting VAV2, an activator of several Rho GTPases (e.g., Cdc42, Rac1, and RhoA) [85]. Recently, the miRNA miR-29b-2-5p was found to modulate infection by *Shigella flexneri* [86]. Interestingly, miR-29b-2-5p has a dual regulatory role during infection, enhancing both bacterial binding to host cells and intracellular replication. In particular, miR-29b-2-5p enhances *S. flexneri* binding to the host cells through the direct regulation of the UNC5C protein, ultimately promoting filopodia formation and bacterial capture. Interestingly, miR-29b-2-5p is decreased by *S. flexneri* intracellular replication, through degradation by the exonuclease PNPT1, which could constitute a bacterial strategy to control replication and avoid premature host cell death, thus favoring dissemination to adjacent cells; alternatively, the decrease in miR-29b-2-5p can reflect a host response to counteract *S. flexneri* infection.

Crosstalk between the Gut Microbiota and miRNAs

While the analysis of the impact of bacterial pathogens on host miRNA expression is well underway, significantly less is known regarding the effect of commensal bacteria on the host miRNome. The resident intestinal microbiota plays an essential role in gut homeostasis and, accordingly, gut dysbiosis has been increasingly linked to disease. Interestingly, a growing number of studies indicate that the gut microbiota may influence the miRNome of different tissues, and that miRNAs might contribute to shape the interaction between the host and gut microbiota. A seminal study on this topic compared the miRNome of germ-free mice with that of mice colonized with microbiota from pathogen-free mice, revealing nine miRNAs differentially expressed in the ileum and colon [87]. MiR-665, which is decreased in colonized mice, was shown to target the ATP-binding cassette transporter Abbc3, involved in multidrug resistance via the metabolism of xenobiotic and endogenous toxins. In a similar study, Singh *et al*. reported the altered expression of 16 miRNAs in the caecum of conventionally raised versus germ-free mice [88]. Computational analysis revealed that various predicted targets of the differentially expressed miRNAs are involved in the regulation of intestinal barrier function and immune regulation. A recent study compared the miRNome of intestinal epithelial cells from conventional and germ-free mice, and demonstrated that miR-21-5p is induced by commensal bacteria, with implications to the regulation of intestinal epithelial permeability [89].

Interestingly, it was recently shown that the gut microbiota, in particular *Fusobacterium nucleatum*, contributes to chemoresistance of colorectal cancer patients through a mechanism that is, at least partially, mediated by host miRNA regulation [90]. Specifically, *F. nucleatum* downregulates miR-4802 and miR-18a*, leading to derepression of the autophagy proteins ATG7 and ULK1, respectively, ultimately activating autophagy.

Somehow surprisingly, the gut microbiota can also impact host miRNA expression in organs well beyond the gut. For example, two recent studies have identified differential miRNA

expression in aorta [91] and hippocampus [92] of germ-free versus conventional mice, opening novel and interesting avenues for future research.

Conversely, recent findings indicate that host miRNAs contribute to the regulation of the gut microbiome. Indeed, mice in which the intestinal epithelial cells lacked an enzyme required for miRNA processing (Dicer1), and thus had reduced miRNA levels in intestinal contents and feces, presented gut microbiota dysbiosis and aggravated colitis [93].

The regulation of miRNA expression in response to bacterial pathogen infection, specifically *L. monocytogenes*, has also been reported to be influenced by the gut microbiota [94]. Analysis of miRNA expression profiles of the ileum of conventional and germ-free mice infected, or not, with *L. monocytogenes*, revealed four miRNAs (miR-143, miR-148a, miR-200b, and miR-200c) significantly decreased upon infection in conventional mice, but not altered in germ-free mice, and one miRNA (miR-378) decreased in conventional mice and increased in germ-free mice. Another study showed that the oral pretreatment of germ-free mice with the probiotics *Lactobacillus casei* and *Lactobacillus paracasei* significantly restricts *L. monocytogenes* dissemination and reshapes the bacterial and host transcriptomes [95]. Interestingly, lactobacilli pretreatment also led to changes in host miRNAs, specifically counteracting the downregulation of miRNAs typically induced by *L. monocytogenes* infection (miR-200b, miR-215, and miR-192). Although these reports reveal microbiota-dependent regulation of miRNA expression in different scenarios, including in the context of infection by bacterial pathogens, further studies are necessary to understand how this interplay maintains homeostasis and shapes the host response to infection.

Extracellular miRNAs and Cell-to-Cell Communication

The potential exploitation of differential miRNA expression patterns as biomarkers of infectious diseases constitutes an exciting line of research. This has been fostered by the discovery of miRNAs in various body fluids, including plasma, urine, saliva, tears, and breast milk [96]. In this context, infection by *M. tuberculosis* constitutes a major target for the development of miRNA-based diagnostic approaches relying on the detection of miRNAs differentially present in patient's serum and sputum [97].

In addition to their prospective role in diagnostics, growing evidence indicates that miRNAs released in the extracellular milieu, associated with RNA-binding proteins, lipoprotein particles, or lipid vesicles, might act as mediators in cell-to-cell communication [98]. Along this line, it was shown that miR-155 and miR-146a are released from immune cells within exosomes and can be successfully transferred to recipient cells, both *in vitro* and *in vivo* [99]. Upon uptake by recipient cells, these miRNAs reprogram the response to LPS, miR-155 increasing and miR-146a dampening inflammatory gene expression. In light of these findings, it is thus conceivable that, during infection, transfer of miRNAs might also occur between cells, with consequences for infection and pathogenesis.

Recently, Liu *et al*. reported that synthetic miRNA mimics can penetrate *E. coli* and *F. nucleatum*, thereby regulating the expression of bacterial genes, with consequences for bacterial growth [93]. Although the possibility that host miRNAs can directly manipulate bacteria is fascinating, further work is necessary to determine the *in vivo* relevance of these observations at physiological miRNA concentrations, as well as the molecular players and mechanisms mediating the eventual uptake and regulatory action of the miRNAs once inside bacteria.

Seminal studies showed that DNA viruses encode miRNAs which facilitate viral replication, at least in part by repressing host antiviral immune responses [8]. Although bacteria do express small noncoding RNAs (sRNAs; 50–250 nt in length) with important regulatory functions [100], it is

generally assumed that bacteria do not express RNAs similar to metazoan miRNAs. Indeed, recent work failed to detect bacterial sRNAs with miRNA characteristics for four bacterial species (*Legionella pneumophila*, *C. trachomatis*, *M. tuberculosis*, and *Mycobacterium smegmatis*), while a single candidate miRNA of bacterial origin was identified in *Mycobacterium marinum* [101]. Moreover, it was recently reported that *S. enterica* serovar Enteritidis can release sRNAs into the cytosol of infected cells, and that some sRNAs could enter the host miRNA pathway and play regulatory roles in the host, promoting bacterial intracellular survival [102]. The emerging data, suggesting that host miRNAs might directly regulate bacterial expression and that bacterial regulatory RNAs might modulate the host transcriptome, suggest that the role of miRNAs at the host–pathogen interface might be more intricate than previously anticipated.

Concluding Remarks and Future Perspectives

RNA sequencing has largely contributed to the extensive characterization of host miRNome changes upon infection with an increasing number of bacterial pathogens. However, studies performed so far have been restricted to the analysis of bulk cell populations, and thus the heterogeneity of individual cell miRNA responses to infection, and their dependence on the extent of bacterial internalization, replication, or persistence has not been captured using these approaches. With the continuous improvement of single-cell RNA sequencing, this analysis will likely be attainable in the near future (see Outstanding Questions).

In the context of bacterial pathogen infection, miRNAs impact a range of host cell functions, most prominently immune response, cell cycle progression, cytoskeleton organization, cell death/survival, and autophagy. Given the number of annotated miRNAs and the high number of predicted and experimentally validated miRNA targets, a wealth of information is yet to be uncovered concerning the role of miRNAs at the host–pathogen interface. Application of systematic approaches to decipher the function of miRNAs will certainly contribute to further developments in the field. Along this line, we have recently pioneered the application of fluorescence microscopy-based high-throughput screenings of synthetic miRNA libraries to identify miRNAs controlling infection by bacterial pathogens, including *S.* Typhimurium and *S. flexneri* [61,86,103]. The application of CRISPR-based screenings to infection biology will certainly contribute to this analysis. An additional aspect that deserves further attention concerns the identification of the targets of the miRNAs already known to modulate infection. Though the identification of miRNA targets relevant for a given process is, arguably, the most cumbersome aspect of miRNA biology [104,105], this will contribute decisively to the identification of previously unknown factors/pathways and provide exciting insights on the complex interaction between bacteria pathogens and the host.

Over the past decade, it has become established that miRNAs play a crucial role in the interplay between bacterial pathogens and the host, both as part of the host defense against invading pathogens and as part of the bacterial arsenal to subvert host cell functions to their own benefit. In our opinion, the study of miRNAs and their targets will continue to provide important insights into different facets of the host–pathogen interplay, and to reveal the importance of miRNA regulation in the crosstalk with the microbiota.

Outstanding Questions

What is the global contribution of miRNAs to the outcome of infection by bacterial pathogens?

How heterogeneous is the miRNA response to infection, at the single cell level?

Does miRNA transfer between host cells contribute to shape infection?

What is the impact of microbiota-dependent miRNA regulation in host homeostasis and in the response to infection?

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