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Review

MicroRNAs in the interaction between host and bacterial pathogens

Claire Maudet [a], Miguel Mano [b,c], Ana Eulalio [a,*]

[a] Institute for Molecular Infection Biology (IMIB), University of Würzburg, 97080 Würzburg, Germany
[b] International Centre for Genetic Engineering and Biotechnology (ICGEB), 34149 Trieste, Italy
[c] UC-BIOTECH, Center for Neuroscience and Cell Biology (CNC), University of Coimbra, 3004-504 Coimbra, Portugal

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ABSTRACT

MicroRNAs (miRNAs) are small non-coding RNAs with a central role in the post-transcriptional control of gene expression, that have been implicated in a wide-range of biological processes. Regulation of miRNA expression is increasingly recognized as a crucial part of the host response to infection by bacterial pathogens, as well as a novel molecular strategy exploited by bacteria to manipulate host cell pathways. Here, we review the current knowledge of bacterial pathogens that modulate host miRNA expression, focusing on mammalian host cells, and the implications of miRNA regulation on the outcome of infection. The emerging role of commensal bacteria, as part of the gut microbiota, on host miRNA expression in the presence or absence of bacterial pathogens is also discussed.

1. Introduction

MicroRNAs (miRNAs) are a class of genome-encoded small RNAs, typically 20–22 nt in length, which post-transcriptionally repress the expression of cellular mRNAs [1,2]. In the canonical biogenesis pathway, miRNA genes are transcribed by RNA Polymerase II into a primary precursor (pri-miRNA), and then processed in two steps catalyzed by members of the RNAse III family of enzymes, Drosha and Dicer. In the first step, inside the nucleus, the Microprocessor complex (composed of the enzyme Drosha and the RNA-binding protein DGCR8) processes the pri-miRNA into a precursor hairpin of ca. 70 nt (pre-miRNA), which is then exported to the cytoplasm by Exportin-5. The pre-miRNA is subsequently cleaved by Dicer to yield a miRNA duplex of approximately 20 bp.

To perform their regulatory functions, one of the strands of the miRNA duplex assembles with the Argonaute and GW182 (TNRC6 in mammals) proteins into the RNA-induced silencing complex (RISC); miRNA-loaded RISC can also be referred as miRISC. MiRNAs repress the expression of cellular mRNAs containing partially complementary binding sites, present mostly within the 3′-UTR and coding sequence of target transcripts. The 'seed region', a region of 6–8 nt at the 5′-end of miRNAs is the main determinant of target specificity. MiRNA families are defined by a common seed sequence, and are thus expected to regulate a largely overlapping set of targets. Despite the important role of miRNAs as regulators of gene expression, the mechanism by which miRNAs repress target gene expression remains controversial and the subject of extensive research. The emerging consensus in the field is that miRNAs act mainly by repressing translation and promoting deadenylation and subsequent degradation of target mRNAs [3,4].

According to the latest release of miRBase, the public repository of miRNA sequences, 30424 mature miRNAs have been reported in 206 species; 2578 unique mature miRNAs are currently annotated in the human genome, many of which showing a high degree of conservation across species (miRBase 20.0, June 2013, www.mirbase.org). Considering that individual miRNAs can have hundreds of targets, it has been predicted that miRNAs can regulate up to 60% of the human transcriptome [5]. However, pinpointing the relevant targets responsible for a given phenotype remains a difficult challenge. Consistent with a major role in post-transcriptional control of gene expression, miRNAs have been shown to participate in a wide range of biological processes, including development, cellular differentiation, proliferation, apoptosis, metabolism and immune response [6–8]. In recent years, it has become clear that miRNA biogenesis is tightly regulated at the levels of transcription and processing, in a cell type specific and developmentally controlled fashion [2,9]. Early studies of miRNAs described their expression profiles in disease-relevant cells and tissues and revealed a correlation between altered miRNA expression and several pathologies, including cancer,

* Corresponding author. Address: Institute for Molecular Infection Biology (IMIB), University of Würzburg, Josef-Schneider-Str. 2/D15, D-97080 Würzburg, Germany. Fax: +49 931 3182578.
E-mail address: ana.eulalio@uni-wuerzburg.de (A. Eulalio).

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neurodegenerative and cardiovascular diseases [10–12]. This has increased the interest in using miRNA expression profiles for disease diagnosis and prognosis, as well as the use of miRNA mimics or inhibitors as therapeutics.

MiRNAs have also been shown to play crucial roles during infection by diverse pathogens, including viruses, parasites and bacteria (reviewed in [13–15]). Seminal work on the role of miRNAs in host-pathogen interactions focused on virus infections. Initial miRNA profiling experiments revealed that several DNA viruses (herpesvirus, polyomavirus and adenovirus) express multiple miRNAs in infected cells to manipulate the levels of both viral and cellular mRNAs, thereby influencing viral replication and pathogenesis [15]. In addition to virus-encoded miRNAs with roles in infection, several host cell miRNAs that either repress or enhance the replication of different viruses have been identified. Viruses have also been shown to modulate the levels of host miRNAs, mainly through the expression of viral suppressor proteins that antagonize the small RNA-directed immunity of the host [16].

Considerably less is known about the effect of bacterial pathogens on host miRNA expression, as well as the reciprocal effect, i.e. the role of host cell miRNAs on modulating bacterial infections. The first evidence of miRNA regulation as a result of bacterial infection was obtained upon infection of the plant *Arabidopsis thaliana* with the extracellular pathogen *Pseudomonas syringae* [17]. Specifically, infection was shown to induce the transcription of miR-393a, which represses the receptor for the hormone auxin, a negative regulator of plant defense. In this case, up-regulation of miR-393a contributes to the resistance of the plant against the bacterial pathogen. To counteract this antibacterial defense mechanism, *P. syringae* secretes effector proteins into the host cell, which are able to suppress transcription, processing or activity of host miRNAs [18].

The miRNA response of mammalian cells to infection was initially inferred from a pioneering study that investigated the expression of a panel of ca. 200 miRNAs in human monocytes following stimulation with the bacterial wall component lipopolysaccharide (LPS), which is primarily sensed by Toll-like receptor 4 (TLR4) [19]. This analysis revealed the up-regulation of miR-132, miR-146a/b and miR-155. These and subsequent findings showed that both miR-146 and miR-155 targeted mRNAs in the signaling cascade downstream of TLR4, leading to a model in which these two miRNAs facilitate a negative-feedback loop that may protect host cells from an excessive TLR4 response.

This review aims to provide an overview of the current knowledge on the role of miRNAs during infection by different bacterial pathogens and in response to commensal bacteria, focusing on mammalian host cells.

2. MicroRNA responses to pathogenic bacteria

Although the vast majority of bacteria are innocuous or even beneficial to mammalian hosts, bacterial pathogens are the causative agents of several relevant diseases worldwide, such as foodborne illnesses caused by *Listeria monocytogenes* and *Salmonella* species, peptic ulcers and gastric cancers associated with *Helicobacter pylori* and tuberculosis caused by *Mycobacterium tuberculosis*. To ensure their survival and replication, bacterial pathogens manipulate a wide range of host cellular functions/pathways through the delivery of effector proteins into host cells [20,21]. Regulation of miRNA expression upon infection by bacterial pathogens is emerging as a crucial part of the host response to infection, as well as a novel molecular strategy exploited by bacteria to control host cell pathways. The sections below and Fig. 1 review the current literature on the regulation of host miRNAs by different bacterial pathogens.

3. *Listeria monocytogenes*

L. monocytogenes is a Gram-positive facultative intracellular pathogen that can cause gastroenteritis in healthy individuals, and serious illnesses in immunocompromised individuals and pregnant women [22]. Upon entry in phagocytic or non-phagocytic cells, *L. monocytogenes* readily escapes the bacteria-containing vacuole in a process mediated by the pore-forming toxin listeriolysin-O (LLO), moves in the host cell cytoplasm and efficiently spreads to neighboring cells [22]. This foodborne pathogen has been used as a

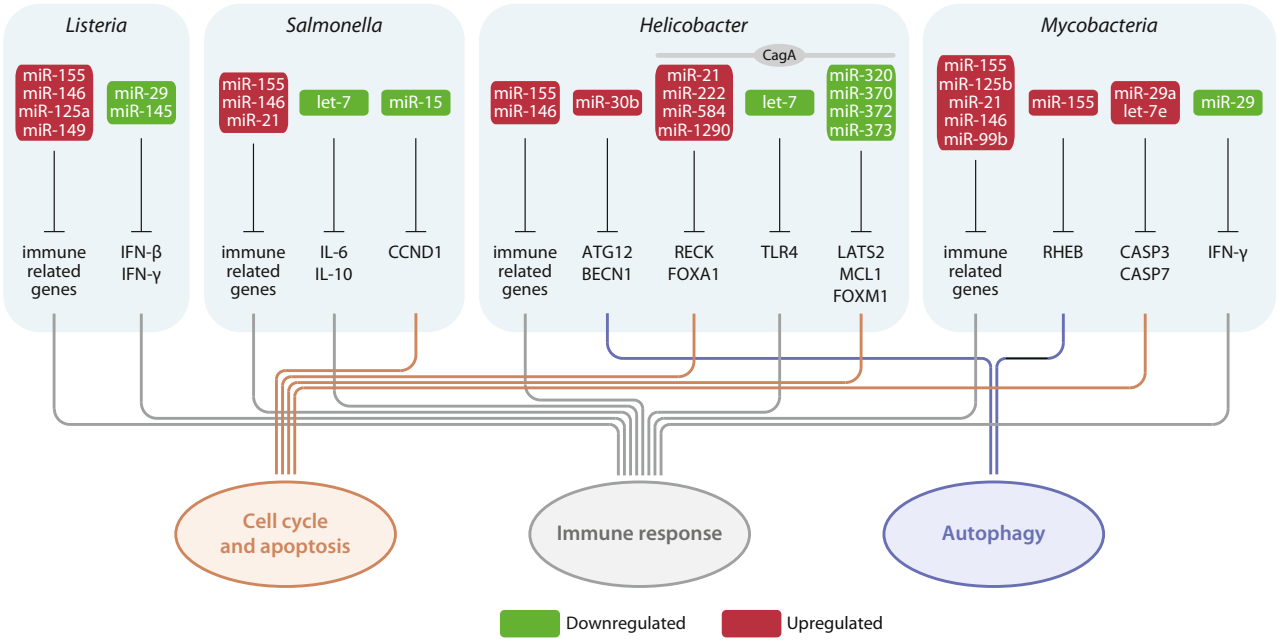

Fig. 1. Overview of selected host mammalian miRNAs regulated by representative bacterial pathogens: *Listeria* (Gram-positive, intracellular), *Salmonella* (Gram-negative, intracellular), *Helicobacter* (Gram-negative, extracellular/intracellular) and *Mycobacteria* (intracellular).

model to understand how Gram-positive bacteria modulate miRNA expression in infected host cells.

Genome-wide miRNA expression profiling in mouse bone marrow derived macrophages infected with *L. monocytogenes* identified the immune-related miRNAs miR-155, miR-146a, miR-125a-3p/5p and miR-149 as being significantly induced upon infection [23]. Interestingly, these five miRNAs were also up-regulated upon infection with the LLO-deficient mutant, suggesting that extracellular sensing of *L. monocytogenes* is sufficient to stimulate miRNA expression. These miRNAs, which target genes involved in the innate immune response such as SHIP1, TRAF6 and IRAK1, are likely to be involved in the regulation of the innate immune response against *L. monocytogenes*.

The effect of *L. monocytogenes* infection on miRNA expression has also been studied in human intestinal epithelial cells (Caco-2 cells) [24]. Similarly to what has been observed in macrophages, the expression of miR-155 was strongly increased in Caco-2 cells; miR-146b and miR-16 were also induced upon infection, albeit to a lesser extent. Strikingly, the up-regulation of these miRNAs could be fully recapitulated upon treatment of epithelial cells with purified LLO. Infection by *L. monocytogenes* also led to the down-regulation of let-7a and miR-145 in Caco-2 cells [24]. MiR-145 has been predicted to target IFN-β, a cytokine with anti-inflammatory properties that was shown to be beneficial to *L. monocytogenes* infection [25,26]. Moreover, inhibition of miR-145 expression was shown to inhibit inflammatory responses, notably the production of the pro-inflammatory cytokines IL-5 and IL-13 [27]. Down-regulation of this miRNA could thus constitute a mechanism by which *L. monocytogenes* dampens the host immune response to facilitate its intracellular survival.

Modulation of miRNA expression in response to *Listeria* infection was also shown to occur in vivo. During systemic infection of mice with *L. monocytogenes*, miR-29 has been shown to be down-regulated in natural killer (NK) cells [28]. Ma et al. hypothesized that inhibition of miR-29 can potentiate the immune response directed against *Listeria*, due to the increased expression of its target IFN-γ, leading to a more effective clearance of the bacteria. *L. monocytogenes* was also shown to modulate the expression of miRNAs in the ileum of orally infected mice, in particular miR-143, miR-148a, miR-200b and miR-200c [29]. Interestingly, this effect was dependent on the gut microbiota (see below).

4. *Salmonella* Typhimurium

Salmonella enterica serovar Typhimurium (*Salmonella*) is an extensively studied Gram-negative, facultative intracellular pathogen, and one of the most common causes of gastroenteritis in humans [30]. *Salmonella* can infect both phagocytic and non-phagocytic cells, where it resides inside the so-called *Salmonella* containing vacuole (SCV). For invasion of host cells and intracellular replication, *Salmonella* secretes more than 30 effector proteins into the host cytoplasm, through two distinct type-III secretion systems (T3SS). These effectors modulate various host cellular pathways necessary for productive infection [31,32].

Modulation of the host miRNA repertoire by *Salmonella* infection was first described in mouse macrophages, where the NF-κB dependent miRNAs miR-155, miR-146a/b and miR-21, were shown to be strongly induced upon infection [33]. These miRNAs were also regulated upon infection with *Salmonella* mutant strains defective in cell invasion (ΔSPI-1) and/or replication (ΔSPI-2), as well as upon treatment of cells with purified *Salmonella* LPS, demonstrating that regulation of these miRNAs is triggered by the sensing of an extracellular stimulus. Up-regulation of these miRNAs was also observed in human monocytes [34]. Members of the let-7 miRNA family were shown to be down-regulated upon *Salmonella* infection both in macrophages and in epithelial cells [33], suggesting that repression of this miRNA family constitutes a common signature of the infection of phagocytic and non-phagocytic cells by *Salmonella*. The let-7 family targets two major immunomodulatory cytokines: the pro-inflammatory cytokine IL-6 and the anti-inflammatory cytokine IL-10 [33,35]. Thus, *Salmonella* infection, through the inhibition of let-7, induces the expression of both cytokines resulting in a balanced inflammatory response.

In recent studies, we observed that *Salmonella* infection also down-regulates the miR-15 family in HeLa cells [36]. Interestingly, overexpression of miR-15 family members inhibits *Salmonella* infection very efficiently, through repression of Cyclin D1 (CCND1), a protein required for G1/S transition. Of note, we showed that down-regulation of miR-15 by *Salmonella* favors cell cycle progression and, ultimately, bacterial replication.

The effect of *Salmonella* infection on miRNA expression has also been studied in vivo, in piglets and zebrafish embryos. In the ileum of infected piglets, *Salmonella* induces miR-29a expression as early as 3 h post-infection [37]. MiR-29a was shown to target Caveolin-2 (CAV2), an inhibitor of the small Rho GTPase CDC42, which plays an essential role in *Salmonella* invasion of epithelial cells [38]. Infection of zebrafish embryos with *Salmonella* induces expression of miR-146a/b, miR-21 and miR-29a [39]. In zebrafish embryos, miR-146a/b down-regulation by *Salmonella* infection had no major effect on canonical pro-inflammatory genes, except for the up-regulation of apolipoprotein genes that have been previously linked to immunoregulation and host defense [40,41].

5. *Helicobacter pylori*

H. pylori, a highly prevalent Gram-negative pathogen estimated to chronically infect the gastric mucosa of roughly half the world's population, is the primary etiological cause of gastritis, peptic ulcer and gastric cancer [42].

The first evidence of mammalian host miRNA regulation during infection by bacterial pathogens was obtained using *H. pylori* infection as a model [43]. In a pioneering study, Zhang et al. revealed that expression of miR-21 is increased upon *H. pylori* infection and provided evidence that miR-21 induces host cell proliferation, suggesting that miR-21 could constitute a link between *H. pylori* infection and gastric cancer progression.

Several studies reported that the immune-related miRNA miR-155 is strongly up-regulated upon *H. pylori* infection, both in vitro (epithelial cells, lymphocytes and macrophages [44–46]) and in vivo (human biopsies of infected gastric mucosa [44,46–48]). Induction of miR-155 was shown to be dependent on LPS sensing by TLR4 and consequent NF-κB pathway activation [45,46], as well as on bacterial toxins secreted through the type-IV secretion system, in particular the vacuolating toxin A (VacA) and the γ-Glutamyl transpeptidase [44]. Interestingly, miR-155 knockout mice fail to control *H. pylori* infection, due to impaired Th1 and Th17 responses, but are less susceptible to *H. pylori*-induced gastritis and gastric cancer [48]. Induction of miR-155 negatively regulates the release of IL-8 triggered by infection, suggesting that miR-155 could function as a negative regulator to fine-tune inflammatory responses towards *H. pylori* [46]. In macrophages, miR-155 was also shown to target genes involved in DNA damage response, and could therefore be involved in enhanced resistance to apoptosis upon DNA damage induced by *H. pylori* [45]. MiR-146a, other NF-κB-dependent miRNA, was also shown to be induced upon *H. pylori* infection both in vitro and in vivo [49–51]. This miRNA was shown to negatively regulate inflammatory responses directed against *H. pylori* through modulation of IRAK1, TRAF6 and PTGS2 [49–51]. Like miR-155, miR-146a

is likely to play a role in a negative-feedback loop to modulate inflammatory responses to *H. pylori*.

In addition to the regulation of miR-155 and miR-146a/b, a common response to Gram-positive and Gram-negative pathogens is the down-regulation of the let-7 miRNA family. *H. pylori* was shown to decrease let-7 expression in vitro and in vivo through its major virulence factor CagA [52,53]. Down-regulation of the let-7 family leads to the de-repression of their target TLR4 and thus potentiates the NF-κB inflammatory response. Balance between the expression of miR-155, miR-146a/b and the let-7 family appears to be therefore critical to control *H. pylori*-induced inflammation.

A distinguishing feature of *H. pylori* is its ability to induce carcinogenesis [42]. Potential roles of miRNAs in this process have been investigated by several groups. *H. pylori* can induce the expression of miR-21 and miR-222, two miRNAs that target RECK, a known tumor suppressor [43,54]. Relevant to the possible contribution of miR-21 and miR-222 to tumor progression, it has been shown that overexpression of these miRNAs induces proliferation of gastric epithelial cells. In addition to down-regulation of the let-7 family, the virulence factor CagA was also shown to down-regulate miR-370 and miR-320, two miRNAs involved in tumor suppression [55,56]. miR-370 inhibits cell proliferation through the down-regulation of FoxM1 and consequent activation of the cell cycle inhibitor $p27^{KIP1}$; down-regulation of miR-370 in infected cells can thus lead to increased cell proliferation [55]. Expression of MCL1, a target of miR-320 and a known anti-apoptotic gene, is increased in *H. pylori*-infected cells, thus preventing apoptosis and potentially increasing the risk of carcinogenesis [56]. CagA was also shown to stimulate the expression of miR-584 and miR-1290, which target FOXA1, a negative regulator of the epithelial-mesenchymal transition (EMT) [57]. These observations linking *H. pylori* infection, in particular the secretion of the CagA virulence factor, with the dedifferentiation of gastric epithelial cells and EMT, constitute another mechanism that can potentially contribute to carcinogenesis induced by *H. pylori*. On the other hand, Belair et al. showed that *H. pylori* can interfere with miRNA expression to block cell cycle progression [58]. These authors showed that *H. pylori* infection of gastric epithelial cells causes down-regulation of miR-372 and miR-373 via CagA leading to the de-repression of their target LATS2, a tumor suppressor, and thus to cell cycle arrest at the G1/S transition. Although in vivo evidence is still lacking, this process might be a mechanism used by *H. pylori* to inhibit gastric epithelium renewal as a host defense mechanism against bacterial infection. Although the processes leading to carcinogenesis and tumor progression upon *H. pylori* infection remain controversial and highly debated, these studies have uncovered several mechanisms by which miRNAs can possibly contribute to these phenomena.

H. pylori has also been shown to manipulate host miRNA expression for its own benefit. *H. pylori* infection induces miR-30b expression in AGS cells and in gastric tissues [59]. This miRNA targets ATG12 and BECN-1, two main players of the autophagy pathway, and miR-30b up-regulation can thus allow *H. pylori* to evade clearance by autophagy in infected cells. Notably, *H. pylori* can also favor its own colonization of the gastric epithelium by inducing a transient hypochlorhydria, i.e. decrease of gastric acidification, through the up-regulation of miR-1289 and the consequent repression of HKα, one of the subunits of the gastric H^+/K^+ ATPase [60].

6. *Mycobacterium* species

Tuberculosis (TB) remains a major public health challenge globally, estimations indicating that roughly one third of the world's population has been infected with *M. tuberculosis*, with 1.5 million associated deaths occurring annually [61]. The genus *Mycobacterium* includes highly pathogenic species such as the etiological agent of TB, *M. tuberculosis*, and *Mycobacterium leprae* (responsible for leprosy), but also opportunistic pathogens such as *Mycobacterium avium*, which can affect immunocompromised individuals [62].

Similarly to other pathogenic bacteria, *Mycobacterium* species can modulate expression of miR-155 upon infection [63–67]. *Mycobacterium bovis* infection was shown to up-regulate miR-155 expression in macrophages, through TLR2 and NF-κB signaling, leading to increased apoptosis of infected cells [63]. In a different study, Rajaram et al. established a link between virulence of *Mycobacterium* species, production of TNF-α and the differential expression of miR-155 and miR-125b [65]. TNF-α mRNA is a direct target of miR-125b, whereas miR-155 can stimulate TNF-α synthesis through its target SHIP1, a negative regulator of the PI3K/AKT pathway. Interestingly, stimulation of cells with lipomannan, a component of the bacterial cell wall, from either a virulent strain (*M. tuberculosis*) or an avirulent strain (*Mycobacterium smegmatis*) leads to opposing effects on TNF-α synthesis: lipomannan from *M. tuberculosis* inihibits TNF-α synthesis, whereas that from *M. smegmatis* induces its expression. This phenomenon is dependent on the balance between miR-155 and miR-125b levels: *M. tuberculosis* induces comparatively high levels of miR-155 and low levels of miR-125b whereas *M. smegmatis* induces low levels of miR-155 and high levels of miR-125b. Whether stimulation of miR-155 expression is favorable or detrimental for *Mycobacterium* infection remains unclear. For instance, Kumar et al. showed that, in mouse macrophages, *M. tuberculosis* can modulate the cell environment for its own benefit via induction of miR-155 expression, through the virulence-associated secreted protein ESAT-6 [64]. Indeed, miR-155 up-regulation can activate the AKT pathway which is required for *M. tuberculosis* survival in macrophages and inhibit the synthesis of the pro-inflammatory cytokine IL-6. In another study, Wang et al. showed that up-regulation of miR-155 can be detrimental for *M. tuberculosis* infection in mouse macrophages, by activating the autophagy pathway [67]. MiR-155 can induce autophagy through the repression of the negative regulator Rheb, and of other components of the mTOR signaling pathway [67,68].

Other miRNAs, such as miR-142-3p, are differently regulated upon infection by different species of *Mycobacterium*. On one hand, this miRNA is strongly up-regulated upon *M. tuberculosis* and *M. smegmatis* infection of macrophages and this limits bacterial phagocytic uptake by targeting N-Wasp, an actin-binding protein required for invasion [69]. On the other hand, expression of miR-142-3p is strongly inhibited upon *M. bovis* infection, resulting in the activation of the NF-κB pathway via the de-repression of the target IRAK1 [70].

Mycobacterium species can also subvert miRNAs to evade host immune responses. As for other pathogenic bacteria, *Mycobacterium* infection leads to the up-regulation of the immune-related miRNAs miR-21 and miR-146a both in vitro and in vivo [66,70–74]. In particular, *M. bovis* was shown to induce miR-21 expression in a NF-κB-dependent manner in T-cells and dendritic cells (DC), leading to inhibition of IL-12 production [74]. Up-regulation of miR-21 was also shown to promote apoptosis in DC, through targeting of Bcl2. *M. bovis* can, by increasing miR-21 expression, impair anti-mycobacterial Th1 response. *M. leprae* can also induce miR-21 expression in infected monocytes and macrophages. These cells rely on the vitamin D-dependent antimicrobial pathway to clear infections and by targeting CYP27B1 and IL-1β, miR-21 can inhibit the synthesis of two vitamin D-dependent antimicrobial peptides and therefore allow *M. leprae* to persist in infected cells [72]. In macrophages and DC, *M. tuberculosis* infection strongly increases miR-99b expression, dampening the inflammatory

response [73]. TNF-α and its receptor TNFRSF-4 are direct targets of miR-99b and inhibition of this miRNA activates the production of TNF-α, and indirectly of other inflammatory cytokines (IL-6, IL-12 and IL-1β).

Similarly to what was observed for *Listeria* infection, expression of miR-29 is down-regulated in IFN-γ-producing NK cells upon *M. bovis* infection [28]. Inhibition of miR-29 was shown to potentiate the immune response directed against *M. bovis* thus enabling a more effective clearance of the bacteria. On the contrary, expression of miR-29a, as well as of let-7e, is induced upon *M. avium* infection of macrophages, resulting in inhibition of the infection-induced apoptosis via targeting of Caspase-3 and Caspase-7 [66].

Overall, and as observed for other pathogens, the T-cell response to pathogenic *Mycobacterium* infection appears significantly different from that of myeloid cells, in terms of miRNA regulation. Indeed, miR-21, miR-29a and miR-142-3p, which are up-regulated by *Mycobacterium* in macrophages [69,71,72,75], are strongly down-regulated upon infection in T-cells [76], further highlighting that miRNA expression and miRNA regulation are highly dependent on the cell context.

7. Other bacterial pathogens

The central role of miR-155 in the immune response triggered by pathogenic bacteria is now well established for *Salmonella*, *Listeria*, *Helicobacter* and *Mycobacterium* and evidence of its regulation by other bacteria is accumulating. For example, miR-155 appears to be a critical player in the host response to *Citrobacter rodentium*, a Gram-negative bacteria that causes enteritis, since miR-155 knockout mice are unable to clear infection by this bacteria [77]. The Staphylococcal enterotoxin B (SEB), an exotoxin secreted by *Staphylococcus aureus*, can cause food poisoning, lung injury and respiratory failure by triggering an acute inflammatory response [78]. This toxin induces a very strong increase of miR-155 expression that leads to accumulation of IFN-γ and abnormal inflammatory responses [79]. Interestingly, miR-155 knockout mice are protected against all deleterious effects of SEB, suggesting a pivotal role of this miRNA in the pathogenicity of SEB and more broadly of *S. aureus*. Differential regulation of miR-155 has also been linked to the virulence properties of two subspecies of *Francisella tularensis*, a Gram-negative bacteria that causes tularemia. The low virulent subspecies, *F. tularensis* subspecies *F. novicida*, strongly increases miR-155 expression, inducing a strong inflammatory response via the activation of the PI3K/AKT pathway, while the highly virulent subspecies, *F. tularensis* subspecies *tularensis*, does not induce miR-155 expression and thus triggers only a mild inflammatory response, possibly explaining its high virulence [80,81].

In addition to the bacterial pathogens described above, systematic profiling of host miRNA expression has been applied to other bacterial pathogens. For example, infection by *Brucella melitensis*, a Gram-negative facultative intracellular bacteria causing brucellosis, induces changes in the expression of several miRNAs (e.g. let-7b, miR-92a, miR-93, miR-181b and miR-1981) [82]. These have putative targets involved in apoptosis, autophagy and immune response. In a different study, miRNA profiling upon infection with the Gram-positive bacteria *Streptococcus uberis* suggested that, besides the NF-κB-associated miRNAs, the miRNA response to Gram-positive bacteria is markedly different to the response to LPS of Gram-negative bacteria [83]. Only 9 of the 21 miRNAs regulated most strongly upon *S. uberis* infection have also been reported to be regulated upon LPS stimulation; with 5 of these 9 miRNAs showing an inverse response to *S. uberis* infection in comparison to LPS. The expression of miRNAs upon infection of mouse alveolar macrophages with the Gram-negative bacteria *Pseudomonas aeruginosa* revealed that 8 miRNAs were up-regulated in macrophages, with miR-302b being the most significantly increased. The up-regulation of miR-302b results in the dampening of the inflammatory response via its target IRAK4, an activator of the NF-κB pathway [84].

Increasing evidence also supports a link between autophagy, miRNA regulation and bacterial infection, as described above for *Helicobacter* and *Mycobacterium*. The Crohn's disease associated adherent-invasive *Escherichia coli* can also interfere with the autophagy pathway through the up-regulation of miR-30c and miR-130a, which target two key regulators of the autophagy pathway – ATG5 and ATG16L1 [85].

8. Interplay between host miRNAs and gut microbiota

The gastrointestinal tract of mammals is colonized by a large microbial population. Increasing evidence suggests an essential role for the gut microbiota in the establishment and maintenance of an appropriate gut mucosa homeostasis. A growing number of disorders have been linked to gut microbiota dysbiosis, including gastrointestinal diseases (e.g. bowel disease), cancer, cardiovascular diseases and obesity. Although it is not surprising that the resident microbiota modulates host gene expression, the role of miRNAs in the interplay between the gut microbiota and the host is just beginning to be explored (reviewed in [86]).

Several studies have tackled this question by performing comparative analysis of miRNA expression of germ-free mice and mice colonized with the microbiota from pathogen-free mice. Dalmasso et al. identified 9 miRNAs differentially expressed in colonized mice relative to germ-free mice: miR-298 (ileum) and miR-128, miR-200c*, miR-342-5p, miR-465c-5p, miR-466d-3p, miR-466d-5p, miR-665 and miR-68 (colon) [87]. One of these miRNAs, miR-665, was shown to target the ATP-binding cassette sub-family C member 3 (Abcc3), which encodes for a protein associated with multidrug resistance, involved in the metabolism of xenobiotic and endogenous toxins. Colonization of mice with microbiota decreases the expression of miR-665 thus increasing Abcc3 expression, with likely consequences in xenobiotic metabolism. A similar study by Singh et al. using germ-free mice and conventionally raised mice, showed that the endogenous microbiota also impacts the expression of miRNAs in the caecum (16 miRNAs differentially regulated) [88]. Various putative target genes of the deregulated miRNAs encode for proteins involved in the regulation of intestinal barrier function and immune regulation. Xue et al. reported that the microbiota negatively regulates miR-10 expression in the intestinal epithelial cells and dendritic cells, through a MyD88-dependent pathway [89]. Taking into consideration that the IL-12/IL-23 p40 subunit, a key regulator of innate immune responses against commensal bacteria, was identified as a target of miR-10, it is likely that this miRNA is involved in the maintenance of host immune homeostasis. Interestingly, a mouse model of colitis also expressed lower levels of miR-10 compared with control mice, and conversely high levels of IL-12/IL-23 p40. Although these studies provide compelling evidence that the intestinal microbiota induces differential expression of miRNAs, further studies are necessary to conclusively address how miRNA modulation impacts the gut homeostasis.

Recently, two reports analyzed if miRNA modulation through the gut microbiota is affected during infection by a bacterial pathogen, and whether this has consequences for the host transcriptome. Treatment with the probiotic *Lactobacilli* species (*Lactobacillus casei* and *Lactobacillus paracasei*) was able to limit *L. monocytogenes* dissemination in a gnotobiotic humanized mouse model [90]. This effect correlated with changes in the transcriptome of the host (in particular IFN-stimulated genes) as well as of *L. monocytogenes* (e.g. propanediol and ethanolamine catabolism

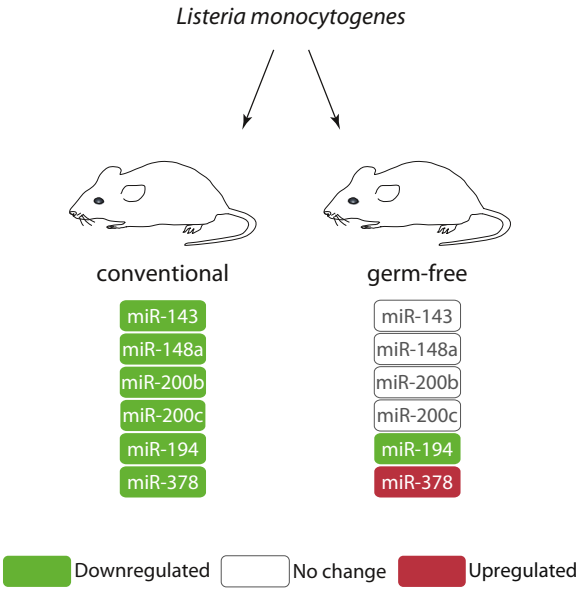

Fig. 2. Role of gut microbiota in the regulation of miRNA expression during *Listeria* infection (cf. Ref. [29]).

and cobalamin biosynthesis genes). Interestingly, treatment with *Lactobacillus* also leads to changes in host miRNAs, with a higher expression of miR-200b, miR-215, and miR-192, which are normally repressed during *L. monocytogenes* infection and to a lower expression of miR-181b, which does not vary during infection [90]. Putative targets of these miRNAs include the NOD-like receptor NLR5, several GTPases and members of the Schlafen family involved in cell growth and T-cell development. The general role of the intestinal microbiota in the regulation of miRNA expression during *Listeria* infection has been also analyzed through the comparison of miRNA signatures in the ileum of conventional and germ-free mice, infected or not with *L. monocytogenes* [29]. Although the intestinal miRNA signature is globally stable in the different conditions, the expression levels of 6 miRNAs (miR-143, miR-148a, miR-194, miR-200b, miR-200c, and miR-378) were significantly decreased upon *L. monocytogenes* infection in conventional mice. The expression of 4 of these miRNAs (miR-143, miR-148a, miR-200b and miR-200c) decreased only in conventional mice, whereas miR-378 was decreased in conventional mice and increased in germ-free mice (Fig. 2). Several candidate target genes whose expression inversely correlated with the expression of these miRNAs were identified; however, their relevance in the context of host-pathogen interplay has yet to be addressed. These results reveal an interesting microbiota-dependent regulation of miRNA expression upon *Listeria* infection, suggesting a role for microbiota in the reprogramming of host gene expression during infection by bacterial pathogens, likely with relevant implications to the success of infection.

9. Perspective

Advancements in RNA-sequencing have enabled the systematic profiling of the miRNome changes upon infection by different bacterial pathogens, as illustrated above for *Listeria*, *Salmonella*, *Helicobacter*, *Mycobacteria* and other pathogens. These analyses have revealed a common set of miRNAs as key players in the host innate immune response against bacteria, specifically miR-146, miR-155, miR-21, miR-125 and let-7. Concurrently, it has also become clear that closely related species such as *M. tuberculosis*, M. *smegmatis*, *M. bovis* and *M. avium* or *F. tularensis* and F. *novicida*, regulate a different subset of host miRNAs. Another very important concept that has emerged from these studies is that changes in miRNA expression following exposure or infection to different bacteria is highly dependent on the cell context, with different cell types responding very differently to the same pathogen. Of note, the mechanisms underlying the vast majority of the bacterial-induced miRNA expression changes described above are not fully understood. In this context, the existence of bacterial effector protein(s) that, once secreted into host cells, might act as RNA silencing suppressors/activators to directly or indirectly manipulate the host miRNA pathway is a fascinating hypothesis. Similar factors have been characterized in viruses and in the bacterial plant pathogen *P. syringae*, however are yet to be identified in bacterial pathogens that infect mammals. Another intriguing hypothesis is that silencing suppressors may derive from RNAs rather than from proteins. Indeed, a mechanism by which viral RNAs can directly mediate the degradation of specific host miRNAs has been reported for Herpesvirus and cytomegalovirus [91–93]. Recently, *E. coli* endogenous non-coding RNAs, OxyS and DsrA, were shown to impact on the physiology of *Caenorhabditis elegans*, through the down-regulation of specific *C. elegans* genes [94].

While the characterization of changes in the expression of host miRNAs during infection by several bacterial pathogens is now well under way, significantly less is known about the role of miRNAs in modulating bacterial infection. Notwithstanding the emergence of congruent regulations, such as those of host inflammatory response, autophagy and cell cycle pathways, a more detailed analysis of the effect of miRNAs in host-pathogen interaction is necessary. Along this line, given the putative involvement of miRNAs in different steps of the host response to bacterial infections, the possibility to manipulate bacterial infections by modulating the levels of host cell miRNAs is an attractive concept, with obvious therapeutic applications. We have recently undertaken a systematic approach to identify miRNAs regulating infection by *Salmonella* Typhimurium, using a fluorescence microscopy-based screening of a library of miRNA mimics [36]. Using this approach, we identified 17 miRNAs that decrease and 11 miRNAs that increase *Salmonella* infection by at least 2-fold, providing evidence that miRNAs can be used to modulate infection by bacterial pathogens. Among the miRNAs that decrease infection more efficiently, we identified the miR-15 family, which is down-regulated upon *Salmonella* infection, thus showing an active role of *Salmonella* in down-regulating host miRNAs that counteract infection, to promote its own survival and replication [36].

The presence of miRNAs in the bloodstream and other body fluids and, most importantly, the recognition that the pool of circulating miRNAs does not mirror the relative abundance of intracellular miRNAs, implying a selective mechanism of miRNA secretion, has raised interest in the use of miRNAs as diagnostic markers. Analysis of circulating miRNAs in the serum of individuals infected with *M. tuberculosis* identified 59 up-regulated miRNAs and 33 down-regulated [75]. Among the up-regulated miRNAs, miR-29a was described as a potential diagnosis marker to discriminate tuberculosis patients from healthy individuals. An intriguing hypothesis is that secreted miRNAs, associated with AGO2 in nuclease-resistant complexes or in small membrane vesicles denominated exosomes, can participate in long-range signaling events [95], preparing the host neighboring tissues to counteract infections or, on the other hand, facilitating the spreading of infections by making neighboring tissues more susceptible. Supporting the first hypothesis, it has been shown that T-cells can transfer miRNAs to antigen-presenting cells via exosomes, suggesting that intercellular miRNA transfer may contribute to coordinate and/or fine-tune gene expression during the onset of the immune response [96]. The

extent and relevance of these miRNA-mediated signaling events to the outcome of different bacterial infections remains, nonetheless, to be fully investigated.

In addition to bacterial pathogens, commensal bacteria as part of the gut microbiota have also been shown to interfere with host miRNA expression both in the absence of a pathogen, as well as upon infection with the foodborne pathogen *L. monocytogenes*. More studies are required to better understand the role of miRNA modulation triggered by microbiota in the reprogramming of host gene expression and their implications to gut homeostasis and/or infections by bacterial pathogens.

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