Mammalian microRNAs and long noncoding RNAs in the host-bacterial pathogen crosstalk

Mélodie Duval\textsuperscript{a,b,c}, Pascale Cossart\textsuperscript{a,b,c,*} and Alice Lebreton\textsuperscript{d,e,*}

a. Institut Pasteur, Unité des Interactions Bactéries-Cellules, 75015 Paris, France;

b. Inserm, U604, 75015 Paris, France;

c. INRA, USC2020, 75015 Paris, France

d. École Normale Supérieure, PSL Research University, CNRS, Inserm, Institut de Biologie de l’École Normale Supérieure (IBENS), Équipe Infection et Devenir de l’ARN, 75005 Paris, France;

e. INRA, IBENS, 75005 Paris, France.

* Correspondence to: Pascale Cossart; Email pascale.cossart@pasteur.fr

or Alice Lebreton; Email alice.lebreton@ens.fr

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Abstract

Gene expression regulation is a critical question in host-pathogen interactions, and RNAs act as key players in this process. In this review, we focus on the mammalian RNA response to bacterial infection, with a special interest on microRNAs and long non-coding RNAs. We discuss the role of cellular miRNAs in immunity, the implication of circulating miRNAs as well as the influence of the microbiome on the miRNA response. We also review how pathogens counteract the host miRNA expression. Interestingly, bacterial non-coding RNAs regulate host gene expression and conversely eukaryotic miRNAs may regulate bacterial gene expression. Overall, the characterization of RNA regulatory networks represents an emerging theme in the field of host pathogen interactions.

Introduction

During infection, both bacteria and host undergo significant changes of environmental or physiological conditions, to which they can adapt or react. Extra- and intra-cellular pathogenic bacteria can adjust their metabolism and trigger the expression of virulence genes, which allow them to benefit from their host resources. Likewise, infected organisms are capable of sensing the intrusion by bacterial pathogens, and react by triggering innate or adaptive immune defences. The complex interplay of actions/counter-actions occurring between a bacterial invader and an infected organism is referred to as host-pathogen crosstalk. This crosstalk, on the bacterial or on the host
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side, involves the coordinated regulation of gene expression programmes with the phase of infection, controlled at the transcriptional or post-transcriptional level. For instance, the transcriptome of cells infected by intracellular bacteria is completely reshaped, as a result of pathogen detection by specific cellular sensors (reviewed in [1]), and of specific modulation by bacterial effectors (reviewed in [2]).

RNA itself represents a key regulatory molecule. Indeed, its physicochemical properties make non-coding RNAs (ncRNA) versatile tools for interfering with gene expression, independently of the coding function played by messenger RNAs (mRNA). Recognition by base-pairing allows one single ncRNA to bind multiple targets, and thereby to regulate several pathways simultaneously [3]. Conversely, a single gene can be regulated by a variety of ncRNAs, allowing its control in a broad range of conditions.

Over the past 10 years, mechanisms and effects of RNA-mediated regulation on gene expression have been broadly investigated in prokaryotes and eukaryotes. Various examples of RNA-mediated regulation in pathogenic bacteria have been recently reviewed [4–7]. Here, we focus on regulation involving mammalian ncRNAs during host-pathogen interaction, and more precisely on two classes of ncRNAs: microRNAs (miRNA) and long non-coding RNAs (lncRNA). These ncRNAs act at the transcriptional and post-transcriptional levels in the control of host gene expression, and are themselves the object of regulations.

The name lncRNA is given to a class of eukaryotic ncRNAs of more than 200 nucleotides transcribed by RNA polymerase II (PolII). Like messenger RNAs, most of these transcripts are poly-adenylated and spliced [8]. Even though the first mammalian lncRNA, XIST (X-inactive specific transcript), was discovered 25 years ago [9,10], the identification and functional characterization of these noncoding transcripts remain an emerging field. Several lncRNAs have been shown to regulate gene expression at the level of transcription or translation (for reviews see [11–13]). The expression of most lncRNAs is tissue-specific, or restricted to precise developmental stages; in addition, some lncRNAs are involved in host response against viral infection (reviewed in [8]). Very recent studies show that lncRNAs are also involved in the response against pathogenic bacteria, which we will describe below.

miRNAs are a class of PolII-dependent transcripts that are processed into a short mature form (21–24 nucleotides). To date, more than 35,000 distinct microRNAs have been described in 223 organisms (miRBase, release 21), and are predicted to regulate 60% of human protein-coding genes [14]. They are involved in multiple processes including cell proliferation, differentiation, inflammation, etc. [15–17]. After being transcribed, the canonical biogenesis of miRNAs involves the processing of a hairpin precursor into a double stranded duplex by two major nucleases, Drosha and Dicer (reviewed in [15]). After export to the cytoplasm, one strand of the duplex is loaded into an effector complex called RISC (RNA-induced silencing complex), which drives the microRNA to its target mRNA and mediates its function, usually by promoting RNA degradation or impeding translation, even though a few activation mechanisms have also been described [18]. In addition to transcription, the amount of available functional miRNAs in the cytoplasm can be regulated via their sequestration by binding proteins, or by a class of ncRNAs called miRNA sponges. Their deregulation was shown in some instances to promote diseases, such as cancer, auto-immunity or cardiovascular disorders [19–21].

To counteract bacterial infection, host cells adjust their gene expression programme, among other pathways by using miRNA and lncRNA as regulatory molecules. Reciprocally, pathogens can escape host defence mechanisms using a variety of strategies, and in particular by targeting miRNA-mediated regulation. Here, we review the role and subversion of mammalian ncRNAs during host pathogen crosstalk.
1. Host cell microRNA response to bacterial infection

Discovery of microRNA response against pathogens

In 2006, the first studies describing a host miRNA response to challenges with bacterial components were published. Recognition of \emph{Pseudomonas syringae} flagellin by \emph{Arabidopsis thaliana} was shown to induce the expression of \( \text{miR-393a} \) and the subsequent inhibition of the expression of three F-box auxin receptors. This, in turn, decreases signalling by auxin, a plant hormone that modulates immune response, and limits bacterial spread within tissue [22]. In human monocytes, the involvement of miRNAs in innate immune response to lipopolysaccharide (LPS, the major component of the outer membrane of Gram negative bacteria) stimulation was investigated. This work has led to the characterization of \( \text{miR-146} \) as an anti-inflammatory miRNA [23].

Following these two studies, the miRNA response upon bacterial infection in mammals has been widely investigated. Infection by different pathogens leads to the expression of specific microRNA sets that are regulated in a time-dependent manner [24]. Among them, \( \text{miR-155, miR-146, let-7} \) and \( \text{miR-29} \) play important roles in the host cells response to bacteria [25–28]. These miRNAs regulate the immune responses in order to clear bacterial infection, while preserving the organism from deleterious effects of inflammation. They will be described below.

![Figure 1. Host microRNA variation upon infection](image)

Upon pathogen exposure, miRNA variability occurs at different levels. (a) The abundance of miRNAs is modified to face infection, and miRNAs are up- or down-regulated early or late in response to pathogens, leading to the expression of a core temporal response common to all pathogens, as well as a specific response; (b) The choice of the arm of pre-miRNA loaded into the RISC complex may be changed by infection, in a process called “arm-switching”; (c) Generation of isomiRs in addition to canonical miRNAs is observed (inspired from [29]).

A recent analysis of the miRNAs expressed in dendritic cells in response to six different bacteria has revealed a core temporal response to infection comprising 49 miRNAs that may play an essential role in response to infection, in addition to subtle variability for each bacterium, that can be considered as a specific signature [29] (Fig. 1a). Moreover, infection can induce a switch in the
relative abundance of mature miRNAs deriving from the 5’ and 3’ arms of the precursor (a process described as “arm-switching”, [30], Fig. 1b), and changes the proportion of miRNA variants (isomiRs, Fig. 1c). Such variability in miRNA identity is thought to affect their regulatory potential, but this aspect deserves more investigations [29].

Key microRNAs involved in immunity

Upon infection, miR-155 and miR-146 are two miRNAs induced by the NF-κB pathway through pattern recognition receptors (PRR) sensing of pathogen motifs, in particular LPS (Fig. 2a). These miRNAs regulate distinct sets of genes [23,31].

Expression of miR-146 is induced by sub-inflamatoty levels of NF-κB activity. It acts as an anti-inflammatory regulator, by targeting TRAF6 (TNF Receptor-associated factor 6) and IRAK1 (IL-1R-associated kinase 1), which are involved in the NF-κB pathway (Fig. 2b), thus promoting tolerance to low doses of LPS [23,31]. This desensitized state is necessary to protect the organism against septic shock. A tolerance state is also crucial during the postnatal establishment of the intestinal microbiota in the newborn gut, where miR-146 prevents inappropriate inflammation (for a review, [32]).

In contrast, miR-155 is induced by higher doses of LPS, at levels which result in pro-inflammatory NF-κB activity, as well as by TNF-α and interferon β, via TAB2 [31,33,34], miR-155 is known to amplify the expression of pro-inflammatory factors, thereby acting in defence against pathogens, and also to exert negative feedback on the immune system, thus protecting the host from potentially damaging overreaction [31]. The involvement of this miRNA in the pro-inflammatory response has been thoroughly investigated; among others, miR-155 targets SHIP1 (Fig. 2c), a negative regulator of the NF-κB pathway [35,36], and SOCS1 (suppressor of cytokine signalling 1), an effector involved in the homeostasis of Treg cells [37]. This in turn, stimulates the expression of the pro-inflammatory cytokines TNF-α, IL-6, IL-1β, IL-8 and IL-12, while it reduces the expression of the anti-inflammatory cytokine IL-10 [38,39]. A complex interplay occurs upon infection, as IL-10 itself can reduce miR-155 expression in response to LPS [40]. miR-155 also increases TNF-α production upon LPS stimulation (Fig. 2d), probably by stabilizing the transcript or by promoting its translation when targeting the expression of proteins that binds the transcript 3’-UTR [34]. Multiple miR-155 targets are also involved in T helper cell development, or promote autophagy by inhibiting the mTOR pathway [41–43]. In line with these data, miR-155 is essential for an efficient immune response to several bacterial pathogens. Indeed, miR-155 null mice show a slower clearance upon Citrobacter rodentium infection [44], as well as an impaired CD8+ T-cell response to Listeria monocytogenes [45]. Moreover, miR-155 is essential in the vaccination process against Salmonella typhimurium [46]. However, this miRNA has also been shown to repress genes such as NIK, IKKe and TAB2, which encode proteins involved in the inflammatory pathway (Fig. 2e); accordingly, it is proposed that miR-155 could act as a limiter of inflammation [31,34,47]. Interestingly, miR-155 expression is also stimulated by the intracellular NOD2 receptor (Fig. 2f). In tolerant macrophages, where miR-146 prevents inflammation, stimulation of NOD2 can restore the NF-κB pathway activation, as well as the feedback control by miR-155 [31].

Another well-described miRNA family is the let-7 family, which targets different genes involved in immunity. This family is repressed upon infection by various pathogens as well as by exposure to LPS [48–52]. Indeed, NF-κB activation induces Lin-28B expression, a protein that blocks let-7 maturation [53]. In addition, various studies have highlighted an active repression of these miRNAs mediated by the bacteria themselves [54–56]. Several targets have been described for let-7 family miRNAs. let-7b targets the TLR4 transcript and as a consequence, bacterial infection-mediated let-7b repression promotes TLR sensing and subsequent NF-κB activation [51]. let-7a and let-7d target
IL-6 and IL-10 transcripts; their down-regulation could contribute to maintaining a balanced immune response upon bacterial exposure [50]. let-7c targets the mTOR pathway, thus modulating T-cell activity [57]. let-7i reduces expression of SNAP23 protein, which is involved in exosome release, a process that is part of the antimicrobial response [52]. The abundant let-7f targets the deubiquinating enzyme A20, an inhibitor of the NF-κB pathway [56]; its downregulation by Mycobacterium tuberculosis favours bacterial survival in macrophages [56].

Figure 2. Mammalian non-coding RNAs at work during host-pathogen crosstalk

A complex interplay of microRNA and IncRNA occurs during bacterial infection. miRNAs miR-155 and -146 are induced by PRRs in a NF-κB-dependent pathway (a), and regulate different sets of transcripts (b,c,d,e) thus promoting or dampening inflammation. miR-155 is also induced by NOD2 cytoplasmic receptors (f). Exosomal transfer of miRNAs allows cell-to-cell communication (g) and extracellular miRNAs might regulate bacterial gene expression (h). To subvert host defences, bacteria can perturb inflammatory response (i,j). Bacteria might also generate miR-like molecules, and thereby interfere with host gene expression (k). IncRNAs are key players in inflammatory response, as they affect chromatin structure (l), thus leading to expression of antimicrobial molecules. They can also hamper inflammation, by sequestering RelA/p65 and thus preventing its DNA binding activity (m).
In murine leucocytes, miR-29 was shown to repress interferon (IFN)-γ expression, and thereby control immune responses to intracellular bacteria [58]. During infection of natural killer cells, CD4⁺ and CD8⁺ T-cells, by *Listeria monocytogenes* or *Mycobacterium bovis* bacillus Calmette-Guérin (BCG), miR-29 expression is down-regulated, thereby facilitating IFN-γ production and subsequent bacterial clearance. Strikingly, miR-29 is one of the microRNAs triggered during infection of macrophages by *L. monocytogenes* [59]. Even though the effect of this up-regulation is unknown, it suggests that infection can differentially control the expression of miRNAs, and as a consequence, that of their targets, depending on the affected cell type.

Altogether, these examples provide evidence that miRNAs act as major components of regulatory networks in immune pathways. Their production is temporally regulated in a dose-dependent manner, resulting in the induction or dampening of immunity, and allowing a rapid and potent response against pathogens.

**Circulating microRNAs during infection**

Aside from their activity inside the cell, miRNAs are powerful molecules for cell-to-cell communication. Indeed, miRNAs are found in many body fluids, including plasma, saliva, tears, urine, amniotic fluid, colostrum, breast milk, stool, etc. [60,61]. Exosomes, which are circulating small lipid vesicles formed from cell plasma membranes, can transport molecules. Exosomal transfer of miRNAs is a powerful mechanism to expand the host response to bacterial infection. Recently, it has been shown that upon LPS exposure, miR-155 and miR-146a are released from bone marrow-derived dendritic cells (BMDCs) within exosomes and are successfully transferred to recipient cells [62] (Fig. 2g). Upon uptake, miRNAs regulate gene expression and reprogram the response of BMDCs to LPS. The authors propose several hypotheses; one of them is that miRNA release in exosomes could be a dynamically regulated process where the ratio of miR-155/miR-146a changes over time, thus enhancing the inflammation upon infection, and then dampening it during recovery.

Exosomal miRNAs could give rise to therapeutic perspectives: miR-146-containing exosomes could be used to treat inflammatory diseases, while miR-155-containing exosomes could be used as an adjuvant to improve vaccine efficacy, or to fight infections as it has been proposed for *Helicobacter pylori* [63]. In addition, these circulating miRNAs are now used or proposed as biomarkers in diagnostic tools for diseases, including sepsis and tuberculosis [64–67]. For instance, the signature pattern of circulating miRNAs in the blood of mice shows a differential response to either LPS [68] or lipoteichoic acids (LTA, a major constituent of the cell wall of Gram positive bacteria) [69]. Nine specific circulating miRNAs were also identified in the serum of mice infected by *Staphylococcus aureus*, but not by *Escherichia coli* [70].

Finally, a recent article relates the *in vitro* ability of miRNAs isolated from faeces to penetrate inside *E. coli* and *Fusobacterium nucleatum* and to regulate the expression of bacterial genes in a sequence-specific manner (Fig. 2h). Whether this phenomenon occurs *in vivo* remains to be determined, nevertheless this discovery opens new perspectives about the possible roles of miRNAs in host-pathogen interaction [60].

**Influence of commensals on the microRNA response to a food-borne pathogen**

The resident intestinal microbiota has been long known to play a major role in intestinal homeostasis by shaping the gut transcriptome. An increasing number of studies indicate that miRNAs are participating in this host/commensal crosstalk, and largely contribute to regulatory networks controlling tissue integrity, immunity and metabolism (reviewed in [71]). For instance, miRNA expression influences the microbiota composition as well as host resistance to dextran sulphate sodium (DSS)-induced colitis [60]. The presence and nature of the intestinal flora can also
tune infection by a bacterial pathogen such as *L. monocytogenes*, as well as the host mRNA and miRNA responses [72,73]. Oral pre-treatment with lactobacilli of otherwise germ-free mice significantly reduces their sensitivity to orally-acquired listeriosis and reshapes host gene expression, notably of genes coding for proteins in the interferon pathway. Furthermore, lactobacilli oral pre-treatment can counteract the down-regulation of miR-192, miR-200b, and miR-215 induced by *L. monocytogenes* infection [73]. A study comparing the intestinal response to *Listeria* infection in conventional versus germ-free mice has additionally highlighted five microRNAs down-regulated by *Listeria* infection only in the presence of the microbiota, and not affected in germ-free mice (miR-143, miR-148a, miR-200b, miR-200c, and miR-378), suggesting that commensals can prime the host microRNA response to infection [72]. This work has also outlined a network of putative regulatory interactions between host microRNAs and their target mRNAs impacted by infection, paving the way to future mechanistic studies aiming at understanding how these microRNAs modulate the intestinal response to infection.

### 2. Subversion of microRNA pathways by bacterial pathogens

**Manipulation of cell physiology**

During infection, bacteria can interfere with many cellular processes using effector molecules [74]. In particular, by modulating the production of miRNAs, they manipulate their host cell physiology and defences, with favourable consequences for pathogen survival (Table 1). For example, *Helicobacter pylori* induces the increase of miR-1289, which represses HKα, a component of the gastric H^+\slash K^+ ATPase. This results in a transient decrease of gastric acidity that promotes *H. pylori* colonization. The mechanism is dependent on CagA, a bacterial effector known to activate the NF-κB pathway [75]. *H. pylori* also interferes with autophagy by inducing the expression of miR-30b, which targets BECN1 and ATG12 transcripts that encode proteins involved in the formation and maturation of autophagosomes [76].

Another example of this subversion is the reduction of host SUMOylation by *Salmonella*, via the stimulation of two miR-30 family miRNAs, miR-30c and miR-30e, and subsequent down-regulation of their target Ubc9, the only cellular E2 SUMO-conjugating enzyme [77]. The mechanism used by *Salmonella* to up-regulate these microRNAs is unknown.

![Figure 3. Modulation of microRNA by pathogens can perturb the cell cycle.](image)

*By inhibiting different sets of miRNAs, *Salmonella* (a) and *Helicobacter pylori* (b) perturb the host cell cycle, thus promoting their survival and proliferation.*
The cell cycle is an important target for pathogens, as it influences their ability to survive and/or proliferate. The perturbation of specific miRNAs is used to this end by *Salmonella*. Indeed, by inhibiting the production of the transcriptional factor E2F1, *Salmonella* reduces the expression of the miR-15 family [78]. This induces the de-repression of cyclin D1 and thus promotes the G1/S cell cycle transition, which is favouring bacterial intracellular replication (Fig. 3a). A similar mechanism has been described for *H. pylori*, which down-regulates miR-372 and miR-373 expression in a CagA-dependent manner. This induces a cell cycle arrest at the G1/S transition and inhibits renewal of the gastric epithelium (Fig. 3b), a major host defence mechanism against bacterial infection [79]. CagA also promotes the expression of miR-584 and miR-1290, which target FOXA1, a negative regulator of the epithelial-mesenchymal transition [80]. In addition to short-term, acute bacterial infections, pathogens also induce long-term effects, as it is exemplified by *H. pylori* in carcinogenesis. The investigation of the roles of miRNAs in this process has revealed that this bacterium induces the expression of miR-21 and miR-222, two miRNAs that target the tumor suppressor RECK [81,82].

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>miRNA</th>
<th>Up/down-regulation</th>
<th>Targeted cellular function</th>
<th>Cell lines</th>
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<tr>
<td><em>Helicobacter pylori</em></td>
<td>miR-1289</td>
<td>up</td>
<td>gastric acidity</td>
<td>AGS</td>
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<td></td>
<td>miR-30b</td>
<td>up</td>
<td>autophagy</td>
<td>AGS and human gastric tissues</td>
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<td></td>
<td>miR-372 and miR-373</td>
<td>down</td>
<td>cell cycle</td>
<td>AGS</td>
<td>[80]</td>
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<td></td>
<td>miR-584 and miR-1290</td>
<td>up</td>
<td>epithelial-mesenchymal transition</td>
<td>AGS, SGC7901 and 293T</td>
<td>[81]</td>
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<td></td>
<td>miR-21 and miR-222</td>
<td>up</td>
<td>carcinogenesis</td>
<td>AGS, SGC7901, MKN28, MKN45, HGC-27, BGC-823, GE5-1</td>
<td>[82,83]</td>
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<td><em>Salmonella spp.</em></td>
<td>miR-30c and miR-30e</td>
<td>up</td>
<td>SUMOylation</td>
<td>HCT-8, HeLa, J774A.2</td>
<td>[78]</td>
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<td>miR-15 family</td>
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<td>cell cycle</td>
<td>HeLa, HT-29, RAW 264.7</td>
<td>[79]</td>
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<tr>
<td></td>
<td>miR-128</td>
<td>up</td>
<td>macrophage recruitment</td>
<td>HT29</td>
<td>[84]</td>
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<tr>
<td><em>Mycobacterium tuberculosis</em></td>
<td>miR-132, miR-26a</td>
<td>up</td>
<td>macrophage response to IFN-γ</td>
<td>hMDM</td>
<td>[85]</td>
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<td>miR-125b, miR-99b, miR-155</td>
<td>up, up, down</td>
<td>production of TNF-α</td>
<td>hMDM, mDC</td>
<td>[86,87]</td>
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<td>let-7f</td>
<td>down</td>
<td>NF-κB pathway</td>
<td>RAW264.7, BMDM, and hMDM</td>
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<td></td>
<td>miR-155</td>
<td>up</td>
<td>NF-κB pathway</td>
<td>RAW264.7 and BMDM</td>
<td>[88]</td>
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**Table 1. Bacteria subvert host defences by modulating microRNA expression.**

Bacteria manipulate the expression of various miRNAs in order to modulate cellular processes, favouring their survival and/or proliferation. AGS, SGC7901, MKN28, MKN45, HGC-27, BGC-823, GE5-1 and 293T: human gastric cell lines; HCT-8 and HT-29: human colonic cell lines; HeLa: human cervix cell line; J774A.2 and RAW 264.7: murine macrophage cell lines; hMDM: human monocyte-derived macrophage cell line; BMDM: murine bone marrow-derived macrophage cell line; mDC: mouse dendritic cell line.

**Effect on immune response**

Bacteria deploy diverse mechanisms to counteract macrophage recruitment and/or activation, as well as induction of pro-inflammatory factors. For instance, secreted effectors from *Salmonella* stimulate, via the p53 signalling pathway, the expression of miR-128, which targets the macrophage...
colony-stimulating factor (M-CSF) and thereby leads to impaired M-CSF–mediated macrophage recruitment [83]. Likewise, *M. tuberculosis* can limit macrophage response to IFN-γ by up-regulating miR-132 and miR-26a [84]. These two miRNAs down-regulate the transcriptional coactivator p300, a component of the IFN-γ signalling cascade. *Mycobacterium* also reduces the production of TNF-α, a potent mediator of macrophage activation and bacterial clearance, by regulating the abundance of miR-125b, miR-155 and miR-99b [85,86] (Fig. 2i). This leads to the destabilization of TNF-α mRNA transcripts and an increase of SHIP1 production, subsequently reducing the production of TNF-α. In addition, the secreted effector from *M. tuberculosis* ESAT-6 down-regulates the expression of let-7f in macrophages, which leads to increased expression of the deubiquitinating enzyme A20, a negative regulator of the NF-κB pathway [56]. *M. tuberculosis* ESAT-6 also stimulates miR-155 expression in macrophages, thus repressing BACH1 and SHIP1. This, in turn, induces the expression of the heme oxygenase 1, and activates the serine/threonine kinase AKT, which respectively promote bacterial dormancy and survival [87] (Fig. 2j). However, the ESAT-6-dependent induction of miR-155 also induces macrophage apoptosis by down-regulating SOCS1 protein [88] and targeting Rheb [43] which is deleterious for the pathogen.

Taken together, these studies indicate that bacteria can control miRNA expression in order to subvert host defences.

### Regulation of host gene expression by bacterial non-coding RNAs

In addition to deregulation of endogenous microRNAs, emerging data suggest that bacteria could also produce regulatory RNAs that would modulate the host gene expression, as previously shown for viruses (reviewed in [89]). A typical example of this system is the *E. coli* OxyS and DsrA ncRNAs which, after ingestion by *Caenorhabditis elegans*, modulate che-2 and F-42G9.6 gene expression, probably by promoting the degradation of the host transcripts [90]. Recently, two studies have investigated bacterial microRNA-like molecules. First, an *in silico* search for bacterial ncRNAs harbouring a secondary structure that might generate miRNA if processed by the host has identified 68 candidate bacterial RNAs from 28 bacterial genomes [91]. These putative bacterial miRNAs are predicted to target mRNAs involved in 47 different human diseases, including cancer and diabetes, thus providing a new perspective for bacterial influence on health and disease. In an independent study, a pre-microRNA was identified in *Mycobacterium marinum*, for which a mature 23 nucleotide-long form was found associated with the host RISC complex upon infection (Fig. 2k) [92]. While the abundance of the endogenous pre-microRNA was too low to induce any detectable effect, their overexpression efficiently decreased mRNA target expression. This type of bacterial-derived miRNA molecules may thus represent a novel class of regulatory factors in the host pathogen crosstalk.

### 3. Long noncoding RNAs at play in response to bacterial infection

The involvement of IncRNAs in defence against viruses has been well described (reviewed in [8]), and recent publications show that several IncRNAs are involved in inflammatory response to LPS and live bacteria. Among them, LINoCR (for LPS Inducible NonCoding RNA) activates the expression of lysozyme in a chicken macrophage cell line challenged with LPS, by remodelling the chromatin (Fig. 2l) [93]. More recently, IncRNA profiles in response to infection or LPS stimulation have been analysed in several mammalian cell lines, and the mechanism of action for some of these IncRNAs has been elucidated.

In human monocytes, 76 enhancer RNAs (eRNA), 40 canonical IncRNAs, 65 antisense IncRNAs and 35 regions of bidirectional transcription are differently expressed upon LPS stimulation, [94]. Knockdowns of IL1β-eRNA and of a region of bidirectional transcription (*IL1β-RBT46*) attenuate
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LPS-induced mRNA transcription and release of proinflammatory mediators, highlighting their implication in the inflammatory response. One lncRNA, lnc-IL7R, has been well characterized: this lncRNA is a negative regulator of proinflammatory mediators such as IL-8, IL-6, E-selectin and VCAM-1, by maintaining a repressive chromatin mark, trimethylation of histone H3 at lysine 27 (H3K27me3) at their gene promoters [95]. In contrast, NeST lncRNA expressed in mice CD4+/CD8+ T cells promotes IFN-γ production by methylating lysine 4 of histone H3 – a mark of active transcription – at the IFN-γ locus [96]. In mouse bone-marrow derived macrophages (BMDMs), 27 lncRNAs are differentially expressed upon LPS stimulation, and their de-regulation is associated with histone trimethylation or acetylation of neighbouring genes, suggesting a possible regulatory role in innate-immune response [97]. In HeLa cells infected by Salmonella, a variation of 44% of total lncRNAs has been monitored, leading the authors to propose that lncRNAs could be used as sensitive markers for pathogen activity in the early infection phase [98]. Last, HOTAIR, a lncRNA first shown to participate in the transcriptional repression of HOX genes [99], is also a positive regulator of inflammation [100]. Indeed, HOTAIR up-regulation in mice cardiomyocytes after LPS-induced sepsis induces TNF-α production by promoting phosphorylation of p65 protein and NF-κB activation.

A subtype of lncRNAs named lincRNAs (long intergenic non-coding RNAs), which are expressed from intergenic regions, have also been studied. In bone marrow dendritic cells, LPS stimulation induces the expression of about 20 lincRNAs, most of them being dependent of NF-κB. Among them, lincRNA-Cox2 is the most expressed [101]. This lincRNA is also up-regulated in mouse bone-marrow derived macrophages upon stimulation by TLR ligands, as well as by Listeria monocytogenes [102], and modulates the expression of many genes involved in inflammation. More precisely, the authors could identify that lincRNA-Cox2 forms a complex with hnRNP-A/B and -A2/B1, two nuclear RNA binding proteins, and thereby repress the transcription of immune genes [102].

LncRNAs can also arise from the expression of pseudogenes. Among them Lethe, which is induced by NF-κB upon TNF-α stimulation, was recently found to contribute to a negative feedback regulation of this pathway, by sequestering the RelA/p65 subunit and thus inactivating its DNA binding activity [103] (Fig. 2m). The active regulation of pseudogene lncRNA expression can thus constitute a means to control inflammatory signalling.

The above-reported studies highlight the involvement of lncRNAs in inflammatory response. Even though some of them were performed using live bacteria, whether bacterial pathogens can subvert lncRNA-mediated regulation remains to be further explored. A first hint of bacterial subversion of lncRNA was obtained in macrophages infected by BCG, showing that 11 lncRNAs were less expressed during a challenge with live bacteria compared to heat inactivated bacteria [104].

4. Conclusions

The major property of ncRNAs is certainly their ability to regulate several targets by specific base-pairing; thereby, they constitute a potent tool allowing cells to tune the expression of a whole regulon with one simple mechanism. As described above, ncRNAs can affect all steps in the gene expression process. When affecting the translation and stability of mature mRNAs, their action takes place at a downstream step in the pathway; as a consequence, their effects are more immediate and flexible than those of transcriptional regulators. Regulation by ncRNAs thus provide a diversity of responses by the host cell during the early stages of bacterial infection, when cells readjust promptly their gene expression programme to face damage and organize immune defences. Given the various microRNAs and lncRNAs that are differentially regulated in response to bacterial
pathogens, in diverse cell types and tissues, they constitute a reservoir of gene expression that can fine-tune an impressive number of putative targets.

In addition to their direct regulatory activity on target gene expression, IncRNA and miRNA may also regulate each other; IncRNAs can act as miRNA sponges, in a process described as “competitive endogenous RNA” (ceRNA, reviewed in [105]). Reciprocally, miRNAs may indirectly influence gene expression by targeting IncRNAs [104]. Thus, a complex regulatory network is at play in the host cell to tightly modulate gene expression. This type of interplay might be involved in host response against bacterial infection, as recently shown for cancer [106]. Given the arising data on the ability of regulatory RNAs produced by pathogens to regulate host gene expression and also the possible regulatory function of cellular RNAs on the pathogen, the role of RNA in the host-pathogen crosstalk might be even more sophisticated than previously anticipated (Fig. 4).

Figure 4. The coding/non-coding RNA interplay in host-pathogen crosstalk

During host-pathogen interaction, RNA is a key regulatory molecule allowing bacterial adaptation, subversion and survival, as well as host response and immunity. Eukaryotic ncRNAs are able to cross-regulate themselves, and could even regulate bacterial genes. In addition, bacterial regulatory RNAs as well as secreted effectors are able to modulate the host transcriptome. Thus, a complex RNA network is at play in the host/pathogen crosstalk.

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