Scholars Academic Journal of Biosciences
Abbreviated Key Title: Sch Acad J Biosci
ISSN 2347-9515 (Print) | ISSN 2321-6883 (Online)
Journal homepage: https://saspublishers.com
NLM ID:101629416
OPEN ACCESS
Biological Sciences

Hidden Messengers: The Uncharted Role of Microbial Small RNAs in Reprogramming Host Immunity During Chronic Infections

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DOI: https://doi.org/10.36347/sajb.2025.v13i09.010 | **Received:** 12.07.2025 | **Accepted:** 17.09.2025 | **Published:** 24.09.2025

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Abstract | **Review Article**

Small RNAs (sRNAs) have emerged as versatile regulators of gene expression in both prokaryotes and eukaryotes. Beyond their intracellular roles, an increasing body of evidence suggests that microbial pathogens release sRNAs that can be sensed, internalized, or even hijacked by host cells. This cross-kingdom communication reprograms immune pathways, shaping the delicate balance between pathogen persistence and host defense. In chronic infections—such as tuberculosis, hepatitis, and persistent fungal or parasitic diseases—microbial sRNAs act as hidden messengers, modulating cytokine networks, evading immune surveillance, and rewiring host epigenetic landscapes.

Keywords: Microbial small RNAs, cross-kingdom communication, host immunity, chronic infections, RNA-mediated regulation, immune evasion.

1. INTRODUCTION

Chronic infections remain one of the most pressing challenges in global health, accounting for significant morbidity and mortality worldwide [1]. Pathogens such as *Mycobacterium tuberculosis*, human immunodeficiency virus (HIV), hepatitis B and C viruses, *Helicobacter pylori*, and persistent fungal and parasitic organisms have evolved sophisticated strategies to evade host defenses and establish long-lasting infections [2,3]. These infections are typically characterized by persistent inflammation, tissue remodeling, immune exhaustion, and in some cases, progression to cancer [4]. The mechanisms enabling pathogens to survive within the hostile environment of the host immune system are multifaceted, ranging from antigenic variation and secretion of immunomodulatory proteins to the manipulation of host signaling cascades [5].

Traditionally, research into host–pathogen interactions has emphasized the role of microbial proteins, toxins, and structural components such as lipopolysaccharides and peptidoglycans [6]. However, recent discoveries have revealed a previously underestimated dimension of microbial virulence: small RNAs (sRNAs) [7]. These short, noncoding RNA molecules, ranging between 20–300 nucleotides in length, were initially identified as regulators of microbial gene expression, fine-tuning stress responses, metabolism, and virulence factor production [8,9]. Yet, emerging evidence suggests that microbial sRNAs are not confined to intracellular regulation but can cross species boundaries, directly impacting host cellular processes [10].

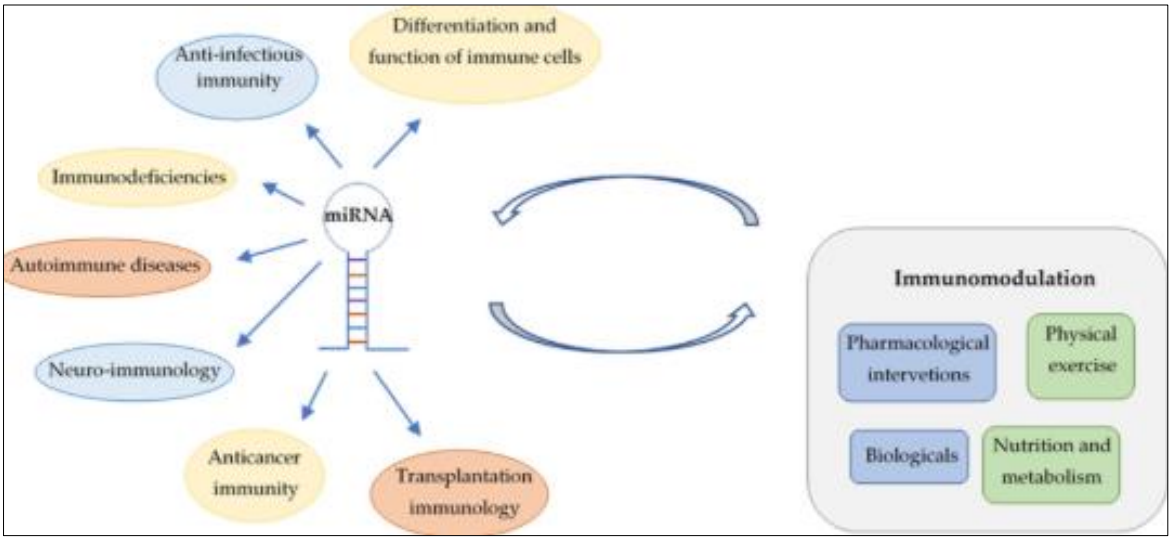
The concept of cross-kingdom RNA communication has revolutionized our understanding of microbial pathogenesis [11]. Pathogens release sRNAs via extracellular vesicles, secretion systems, or lysis,

which can be internalized by host immune and non-immune cells [12]. Once inside, microbial sRNAs mimic host regulatory RNAs or directly interact with host transcripts and signaling molecules, thereby modulating immune pathways [13]. This molecular mimicry enables pathogens to suppress inflammatory signaling, reprogram macrophage or dendritic cell function, interfere with antigen presentation, and skew T-cell responses [14]. Such strategies create a permissive environment for pathogen persistence, driving the transition from acute infection to chronic disease [15].

From an immunological perspective, the ability of microbial sRNAs to reprogram host immunity represents a paradigm shift. Unlike proteins, which are more readily detected by pattern recognition receptors (PRRs), sRNAs can function more subtly, either by engaging RNA-sensing receptors such as Toll-like receptor 7 (TLR7) and RIG-I-like receptors or by hijacking the host RNA interference (RNAi) machinery [16,17]. This allows pathogens to fine-tune immune responses with remarkable precision, often evading detection while ensuring their long-term survival [18].

Importantly, the study of microbial sRNAs in chronic infections is still in its infancy. While a growing number of sRNAs have been identified in bacteria, viruses, fungi, and parasites, the functional characterization of these molecules within the host context remains limited [19,20]. Nevertheless, the potential implications are vast. Understanding the mechanisms of microbial sRNA–host interactions could uncover novel biomarkers for persistent infections, provide insights into host immune dysregulation, and open new avenues for therapeutic interventions, including RNA-based antivirals or antisense strategies targeting pathogen-derived sRNAs [21].

In this review, we explore the hidden messenger role of microbial sRNAs in reprogramming host immunity during chronic infections. We discuss their biogenesis and diversity across microbial kingdoms, their mechanistic interactions with innate and adaptive immunity, the current tools used for their detection, and the challenges and opportunities in translating this knowledge into clinical applications. By shedding light on this emerging field, we aim to underscore the importance of microbial sRNAs as key players in the complex dialogue between pathogens and the host immune system [22].



2. Microbial sRNAs: Biogenesis and Functional Diversity

Microbial small RNAs (sRNAs) represent a highly diverse group of noncoding RNAs, ranging from 20 to 300 nucleotides in length, with critical functions in regulating gene expression, environmental adaptation, and virulence [23]. Unlike messenger RNAs, these molecules do not encode proteins but act through base-pairing interactions or by binding to proteins, thereby influencing transcriptional and post-transcriptional processes [24]. Importantly, their functional diversity extends beyond microbial physiology into host–pathogen interactions, where they serve as molecular messengers capable of reprogramming host immunity.

2.1 Bacterial sRNAs

In bacteria, sRNAs are primarily transcribed from intergenic regions and typically function through imperfect base-pairing with target mRNAs, modulating translation or mRNA stability [25]. Many bacterial sRNAs require the RNA-binding protein Hfq (or ProQ in some species) to facilitate stability and interaction with their targets [26].

Pathogenic bacteria exploit sRNAs to adapt during infection. For example, in *Salmonella enterica*, the sRNAs DsrA, RyhB, and SgrS regulate stress responses and virulence gene expression, enhancing bacterial survival in macrophages [27]. Similarly, *Mycobacterium tuberculosis* expresses sRNAs such as

Mcr11 and MrsI that fine-tune metabolic pathways under hypoxia and iron limitation, conditions commonly encountered within granulomas during chronic infection [28]. Intriguingly, extracellular vesicle-associated sRNAs from *M. tuberculosis* have been detected in infected macrophages, suggesting that bacterial sRNAs can directly influence host immune responses [29].

2.2 Viral miRNA-like RNAs

Viruses, especially large DNA viruses, encode their own microRNA-like RNAs (v-miRNAs) to manipulate host gene expression. These viral RNAs are processed by the host's microRNA machinery (Drosha, Dicer, and Argonaute proteins) and function similarly to host miRNAs [30]. For instance, Epstein–Barr virus (EBV) encodes over 20 miRNAs that suppress host immune genes, including those involved in antigen presentation (e.g., MICB), thereby evading natural killer (NK) cell recognition [31]. Human cytomegalovirus (HCMV) produces miR-UL112, which downregulates major histocompatibility complex (MHC) class I–related molecules, further impairing immune surveillance [32]. In hepatitis B virus (HBV), viral miRNA-like RNAs have been reported to target host apoptotic pathways, promoting persistence [33]. By co-opting host RNA pathways, viral sRNAs act as stealth regulators, blunting antiviral immunity while ensuring viral latency and chronic infection [34].

2.3 Fungal sRNAs

Fungi also generate sRNAs through Dicer-dependent or Dicer-independent pathways, often packaged into extracellular vesicles for delivery to host cells [35]. *Candida albicans* produces sRNAs that modulate its morphological transitions between yeast and hyphae, which are critical for pathogenicity [36]. Importantly, studies have shown that *Cryptococcus neoformans* secretes RNA-containing vesicles capable of modulating macrophage activity, dampening antifungal responses [37].

Emerging evidence indicates that fungal sRNAs can also target host transcripts, functioning similarly to plant–fungus cross-kingdom RNA interference (RNAi) systems, where fungal pathogens deliver sRNAs into host cells to silence immunity-related genes [38].

2.4 Parasitic sRNAs

Protozoan parasites such as *Leishmania* and *Plasmodium* secrete sRNAs via extracellular vesicles, which are internalized by host macrophages and hepatocytes [39]. In *Leishmania donovani*, exosome-associated sRNAs were shown to modulate host cytokine secretion, skewing immune responses toward parasite survival [40]. Similarly, *Plasmodium falciparum*–derived RNAs are transferred into human erythrocytes and immune cells, influencing host signaling pathways and contributing to malaria chronicity [41].

Parasitic helminths also release extracellular vesicles carrying sRNAs that mimic host miRNAs, interfering with host immune regulatory networks [42]. For example, *Schistosoma japonicum* secretes miRNA-like RNAs that suppress Toll-like receptor–mediated responses, allowing the parasite to persist within the host [43].

3. Mechanisms of Host Immune Reprogramming by Microbial sRNAs

Microbial small RNAs (sRNAs) have emerged as potent immunomodulators, reprogramming host immunity through diverse mechanisms. These include direct modulation of innate immune pathways, alteration of adaptive immune responses, and epigenetic reprogramming of host gene expression. By exploiting these strategies, pathogens ensure persistence, immune evasion, and in many cases, chronic disease progression [45].

3.1 Modulation of Innate Immunity

The innate immune system serves as the first line of defense against invading microbes. Microbial sRNAs interfere with this process by modulating pattern recognition receptors (PRRs), cytokine secretion, and phagocyte activation.

Evasion of RNA-sensing receptors:

Several viral miRNAs mimic host microRNAs or evade detection by Toll-like receptor 7 (TLR7) and RIG-I-like receptors, thereby preventing the induction of interferon-stimulated genes (ISGs) [46]. For instance, *Hepatitis C virus* (HCV)–derived sRNAs dampen RIG-I signaling, reducing antiviral interferon responses [47].

Reprogramming macrophage function:

Mycobacterium tuberculosis sRNAs suppress macrophage activation by targeting pathways involved in nitric oxide production and autophagy [48]. This not only allows bacterial survival within phagosomes but also contributes to granuloma persistence [49].

Neutrophil modulation:

Extracellular vesicle–derived fungal sRNAs from *Candida albicans* can alter neutrophil chemotaxis and reactive oxygen species (ROS) production, weakening antifungal immunity [50]. Through these mechanisms, microbial sRNAs fine-tune innate immune responses, creating a permissive environment for persistent infection.

3.2 Rewiring Adaptive Immunity

Chronic infections are often associated with dysfunctional adaptive immunity, including T-cell exhaustion, impaired antigen presentation, and imbalanced cytokine responses. Microbial sRNAs play a central role in orchestrating these dysfunctions.

Antigen presentation suppression:

Viral miRNAs from Epstein–Barr virus (EBV) and cytomegalovirus (CMV) directly downregulate expression of MHC class I–related molecules, impairing antigen presentation to cytotoxic T lymphocytes. This shields infected cells from immune recognition.

T-**cell polarization:**

U-Bacterial sRNAs can indirectly influence T-cell differentiation by modulating dendritic cell cytokine secretion. For example, *Salmonella* sRNAs alter IL-12 and IL-10 production, skewing T-cell responses toward a less protective Th2 phenotype.

T-cell exhaustion:

Persistent viral infections, such as HIV and HBV, exploit viral miRNAs to enhance inhibitory receptor expression (e.g., PD-1, CTLA-4) on T cells, driving exhaustion and impaired effector function.

B-cell regulation:

Viral sRNAs also interfere with B-cell activation and antibody production. EBV miR-BHRF1 has been shown to modulate B-cell receptor signaling, enhancing viral latency in B cells. These findings suggest that microbial sRNAs are critical in reshaping adaptive immunity to favor pathogen persistence.

3.3 Epigenetic Reprogramming of Host Immunity

Perhaps the most intriguing aspect of microbial sRNA function is their ability to reprogram host epigenetics. By interacting with chromatin regulators and RNA interference pathways, microbial sRNAs induce long-term changes in host immune gene expression.

Histone modification and chromatin remodeling:

Some bacterial sRNAs recruit host histone-modifying enzymes to immune gene promoters, leading to transcriptional silencing.

DNA methylation alterations:

Viral miRNAs, such as those from HBV and Kaposi's sarcoma-associated herpesvirus (KSHV), indirectly regulate DNA methyltransferases, leading to hypermethylation of antiviral genes.

Noncoding RNA network interference:

Microbial sRNAs can compete with host microRNAs for Argonaute proteins, disrupting host RNA silencing networks. This reprogramming alters cytokine expression and dampens immune activation.

Such epigenetic alterations provide pathogens with a mechanism to establish long-lasting immune tolerance, even in the absence of active replication.

3.4 Crosstalk Between Mechanisms

These mechanisms rarely act in isolation. Instead, microbial sRNAs often employ a multi-layered strategy—simultaneously dampening innate responses, altering T-cell function, and reprogramming epigenetic landscapes. This synergy ensures that pathogens not only evade immediate clearance but also establish environments conducive to chronic persistence.

4. Detection and Characterization of Microbial sRNAs

The discovery of microbial sRNAs as key regulators of host–pathogen interactions has been largely driven by advances in high-throughput sequencing and molecular biology techniques. Identifying and characterizing these small RNAs presents unique challenges, including their short length, structural variability, and similarity to host noncoding RNAs. Over the past decade, a range of experimental and computational approaches have been developed to address these challenges.

4.1 High-Throughput Sequencing Approaches sRNA sequencing (sRNA-seq):

Next-generation sequencing (NGS) has revolutionized the discovery of microbial sRNAs. By selectively enriching for RNAs shorter than 200 nucleotides, sRNA-seq allows unbiased profiling of microbial sRNA populations. For example, sRNA-seq has identified novel sRNAs in *Mycobacterium tuberculosis* during macrophage infection, revealing infection-specific expression patterns.

Dual RNA-seq:

This technique simultaneously captures both host and pathogen transcriptomes, enabling the identification of sRNAs that are differentially expressed during infection. Dual RNA-seq has been applied to *Salmonella enterica* and macrophages, uncovering sRNAs that coordinate bacterial virulence with host immune responses.

Cross-kingdom RNA sequencing:

Specialized approaches, including RNA-seq combined with extracellular vesicle purification, allow detection of microbial sRNAs trafficked into host cells. This is particularly valuable in distinguishing microbial sRNAs from host-derived RNAs.

4.2 Bioinformatic Prediction and Target Analysis

Identifying sRNA targets requires computational pipelines that can predict RNA–RNA base-pairing and RNA–protein interactions.

sRNATarget and IntaRNA:

Algorithms designed to predict microbial sRNA–mRNA interactions based on sequence complementarity and accessibility.

miRanda and TargetScan adaptations:

Viral and parasitic miRNA-like RNAs are often studied using modified host miRNA prediction tools.

Cross-kingdom prediction tools:

Emerging computational models integrate dual RNA-seq data to predict microbial sRNA targets in host genomes. Despite advances, computational predictions require experimental validation to confirm physiological relevance.

4.3 Extracellular Vesicle (EV) Profiling

Extracellular vehicles (EVs), including outer membrane vesicles (OMVs) from bacteria and exosomes from eukaryotic pathogens, serve as carriers for sRNAs.

Isolation and profiling:

Ultracentrifugation, density gradient separation, and nanoparticle tracking analysis are employed to isolate EVs. RNA sequencing of vesicular contents has revealed microbial sRNAs capable of modulating host responses.

Functional assays:

Transfer of microbial EVs to host immune cells has demonstrated sRNA-mediated suppression of cytokine production, providing functional proof of cross-kingdom RNA transfer.

4.4 Functional Validation Approaches

Establishing the biological role of microbial sRNAs requires loss-of-function and gain-of-function studies.

CRISPR/Cas9 and CRISPRi:

These tools enable targeted deletion or repression of sRNA genes in bacteria and parasites, allowing assessment of their role in infection.

Antisense oligonucleotides (ASOs):

Synthetic ASOs can block microbial sRNA function inside host cells, providing direct evidence of immune modulation.

Reporter assays: Luciferase-based systems are widely used to validate predicted sRNA–host mRNA interactions.

4.5 Emerging Tools

Single-molecule sequencing (PacBio, Oxford Nanopore): Allows detection of sRNAs with complex secondary structures and modifications often missed by short-read sequencing.

RNA immunoprecipitation (RIP-Seq):

Used to identify microbial sRNAs bound to host Argonaute proteins, confirming their functional incorporation into host RNA interference machinery.

Spatial transcriptomics:

Novel methods are being explored to localize microbial sRNAs within infected tissues, providing spatial context to their function.

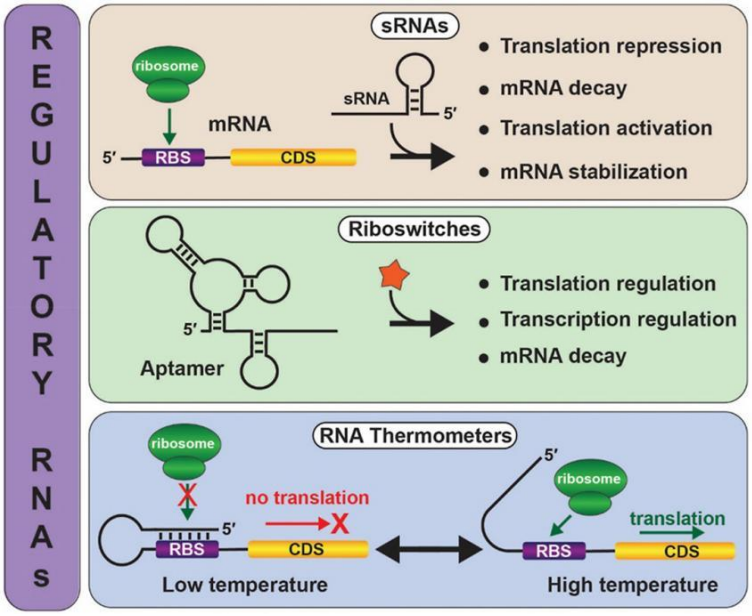


Fig. 1: Key players in regulatory RNA realm

5. Current Challenges and Future Directions

Despite significant progress in identifying microbial sRNAs and their roles in host–pathogen interactions, the field remains in its early stages. Several challenges hinder our full understanding of these molecules, ranging from technical limitations to biological complexity. At the same time, the therapeutic and diagnostic potential of microbial sRNAs presents exciting opportunities for future research.

5.1 Technical Challenges

1. Distinguishing microbial sRNAs from host RNAs:

One of the major obstacles is the difficulty in separating microbial sRNAs from the host's abundant

small RNAs. During infection, microbial sRNAs often represent only a minor fraction of the total RNA pool, making them difficult to detect with confidence. Cross-contamination during extracellular vesicle isolation further complicates accurate attribution.

2. Functional annotation gaps:

Although thousands of microbial sRNAs have been identified by sequencing, only a small percentage have been functionally characterized. This is partly due to the lack of robust high-throughput functional validation methods.

3. Target prediction limitations:

Current computational tools for predicting RNA–RNA interactions often yield high false-positive rates, particularly in cross-kingdom contexts**,** where base-pairing rules and RNA-binding proteins differ significantly between host and pathogen.

4. Lack of standardized pipelines:

Different laboratories use variable approaches for RNA isolation, sequencing, and analysis, making it challenging to compare datasets across studies.

5.2 Biological Challenges

1. Context-dependent expression:

Microbial sRNA expression is highly dynamic and often condition-specific. For example, *Mycobacterium tuberculosis* sRNAs are expressed differently in hypoxic granulomas compared to in vitro cultures. This variability complicates efforts to assign consistent functional roles.

2. Functional redundancy:

Many pathogens encode multiple sRNAs that may act redundantly or synergistically, making knockout studies insufficient to reveal their full biological importance.

3. Host variability:

Host genetic background, immune status, and microbiome composition significantly influence the effect of microbial sRNAs, adding an additional layer of complexity to interpretation.

5.3 Future Directions

1. Development of advanced detection technologies:

Single-cell RNA-seq, spatial transcriptomics, and long-read sequencing (e.g., Nanopore, PacBio) are poised to reveal cell-type–specific roles of microbial sRNAs within infected tissues.

2. Integration of multi-omics:

Combining transcriptomics, proteomics, and epigenomics will provide a holistic view of how microbial sRNAs reprogram host immunity at multiple regulatory levels.

3. Therapeutic applications:

Targeting microbial sRNAs with antisense oligonucleotides (ASOs), locked nucleic acids (LNAs), or CRISPR-based approaches could provide novel anti-infective strategies. For instance, inhibiting viral miRNAs that suppress antigen presentation may restore effective antiviral immunity.

4. Diagnostic potential:

Circulating microbial sRNAs in patient blood, urine, or saliva hold promise as non-invasive biomarkers for chronic infections such as tuberculosis, hepatitis, or leishmaniasis. Standardized detection platforms will be critical for clinical translation.

5. Synthetic biology approaches:

Engineering beneficial microbes to deliver therapeutic sRNAs capable of reprogramming host immunity offers an innovative frontier. Such approaches could be applied in microbiome engineering or live-attenuated vaccine design.

6. CONCLUSION

Microbial small RNAs (sRNAs) have emerged as hidden messengers in host–pathogen interactions, capable of reprogramming immunity in ways that extend far beyond traditional virulence factors. Once considered mere regulators of microbial physiology, these molecules are now recognized as critical modulators of both innate and adaptive immune responses, and in some cases, as drivers of epigenetic reprogramming that ensures pathogen persistence. The discovery that bacteria, viruses, fungi, and parasites all employ sRNAs to influence host biology reflects an evolutionary convergence on RNA-mediated strategies for immune evasion. By blunting interferon responses, suppressing antigen presentation, skewing T-cell polarization, and altering host chromatin landscapes, microbial sRNAs exploit vulnerabilities in the immune system to establish chronic infections. At the same time, advances in sequencing, bioinformatics, and molecular biology are rapidly accelerating our ability to detect and characterize these molecules. Techniques such as dual RNA-seq, extracellular vesicle profiling, and CRISPR-based functional assays are uncovering a previously hidden layer of cross-kingdom communication.

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