ExRNA

The roles of small RNAs: insights from bacterial quorum sensing

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Abstract

Ribonucleic acids (RNAs) mainly played auxiliary roles in regulations of genetic processes while recent explorations into small non-coding RNAs (sRNAs) in bacteria have broadened the scope of RNAs studies in these processes. sRNAs have been demonstrated to be involved in various genetic processes and to regulate a variety of bacterial physiologies. Comparatively, quorum sensing (QS) is a mature bacterial cell signaling system which regulates bacteria physiologies as well. Prokaryotic sRNAs studies in the status quo have revealed an emerging picture of trans-kingdom signaling regulation and increasing investigations have demonstrated the feasibility of inter-kingdom signaling as the consequence of QS. We therefore review such phenomena and their similarities to investigate the potential of prokaryote-sourced interkingdom signaling and regulation.

Keywords: Small RNA, Quorum sensing, Inter-kingdom communication, Prokaryotes, Eukaryotes, Extracellular micro-vesicles

Introduction

The first bacterial sRNA, MicF, was reported and characterised by Takeshi Mizuno and his group in 1984 [1]. It was observed to regulate the translation of trans-membrane protein OmpF in *Escherichia coli*. More sRNAs were gel separated and identified in 1999 [2]. Facilitated by computational analysis, many groups initiated genome-wide searches [3, 4] for sRNAs in the next few years [4] and the field of sRNA studies experienced a boom. As this field attracted increasing attention, the study expanded towards eukaryotic cells. Many other concepts stemmed from sRNAs in other kingdoms, e.g. micro RNAs and siRNAs. Nevertheless, all non-coding regulatory RNAs in bacteria are classified as sRNA [5, 6].

sRNAs in Bacteria

These advances provided a solid ground for further characterisation of sRNA. Bacterial sRNAs are 50–500 nucleotide long non-coding RNAs [7, 8]. sRNAs are highly structured due to intramolecular base pairing, forming secondary structures such hairpins and stem loops [8]. Sandra Cristina Viegas with her group

proposed that these stem-loops stabilise [8] the sRNA. The gene sequences coding for sRNAs are mainly located in intergenic regions (IGRs) [9]. However, there was one case [9, 10] which reported that sRNA genes exist in the 3'UTR region of mRNA genes. sRNAs are transcribed from DNA, in contrast to mRNA, sRNAs are untranslated and usually regulate the translation by interference with mRNA. This concept apparently goes against [11] Francis Crick's Central Dogma of molecular biology [12] because the classical RNA transcripts (mRNA, tRNA and rRNA) [11–13] are translated into proteins whereas sRNAs are not. Gradually, as the field is broadening, this formerly controversial concept is now commonly accepted as an exception of Central Dogma [11, 12].

A variety of approaches have been developed over the past decades to identify and characterise sRNAs. There are either laboratory-based techniques or computational techniques. Northern blotting, as one of the modified gel analysis techniques, identifies sRNAs by sizes. Most of the novel sRNA sequences are predicted and characterised in silico [14, 15] such as comparative genomics-based models [6, 14, 16, 17], RNA sequencing [15, 18], microarrays [14] and even machine-learning based models [16] in these days.

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Small RNAs interact with messenger RNAs (mRNAs) to regulate gene expression [1, 8, 19–22]. These RNA regulations are classified into negative regulation [8] and positive regulation [19] by the type of sRNAs, their binding patterns and the extent to which sRNAs impact post-transcriptional process [5]. Negative regulation impacts the level of translation adversely. Its characterized by either true anti-sense or limited-complementary RNAs [5]. Anti-sense RNAs modulate [23] activities of single type of mRNA due to its full complementarities [5, 23, 24] to the target mRNA sequences. Similarly, limited complementary RNAs bind to the target by complementary base pairing whereas the essential difference between anti-sense and limited complementary is that the latter ones have partial [5, 23] complementary sequences to the target mRNA sequence. This feature enables limited complementary sRNAs to target multiple types of mRNA because they only need to have a part of the sequences that is complementary to mRNA sequences. RNA is degraded [5] and the translation is interrupted when the mRNA is bound by anti-sense sRNA. The level of translation is reduced when mRNA is bound by limited-complementary sRNAs. In contrast to negative regulation, positive regulation upregulates the translation. This involves an RNA chaperone protein [25] named Hfq which was firstly discovered [26] as *E. coli* host factor in 1960s. Until recent years has the role of Hfq protein been redefined [19, 24, 27, 28] to either stabilise or destabilise [24] RNAs by causing structural changes to RNA molecules including sRNAs and mRNAs [5].

Wassarman and his group demonstrated [2, 24] that, in response to stimuli, sRNAs cofunction with Hfq proteins to upregulate translation of rpoS mRNA [21, 22, 24] by forming sRNA-Hfq complexes. Normally, ribosomal binding site (RBS) of rpoS mRNA is buried in the secondary structure, which inhibits the translation. However, the sRNA-Hfq complex modifies [19] the structure by exposing RBS (Fig. 1). Ribosomes can therefore bind to RBS and expression increases dramatically.

Current well-documented model bacteria that harbor sRNAs include *Escherichia coli* [5, 29–31], *Salmonella* [32], *Cyanobacteria, Streptococcus pyogenes* [33–35], *Pseudomonas aeruginosa* [5, 36], *Vibrio harveyi* [37], *Vibrio cholerae* [37], *Vibrio fischeri* [37]. Apart from regulating genetic activities of bacterial species above, sRNA plays a versatile role in regulating bacterial physiologies [23]. These mainly include virulence, outer-membrane regulations and quorum sensing. They are inter-connected and discussed later in this review.

Intra/inter-species and Interkingdom quorum sensing

Bioluminescence was studied in *V. harveyi* and *V. fischeri* for its ecological effect back at the 1980s [38, 39] while another aspect [40] hidden behind bioluminescence was unraveled. It was observed that only when bacterial cell density reached a threshold would the cells emit light. Light was then associated with enzyme termed luciferase [39] and its Lux operon LuxCDABEG [41–43]. In later investigations, same group found evidence that cell density threshold was in accordance to the concentration of an extracellular molecule [38]. Only if when enough of these molecules [39] were secreted at high cell density would bioluminescence be triggered. This type of molecules was defined as auto-inducers (AI) and this was the first time [41] that scholars proved the existence of bacterial cell communication extracellularly and this AI-associated bacterial 'social behaviour' was named Quorum Sensing (QS) [38, 40, 41, 44, 45]. For the past decades, from investigating bioluminescence, the scope of studying QS has been expanded towards other group behaviours and it has been confirmed QS regulate bacterial pathogenicity and virulence [41, 42].
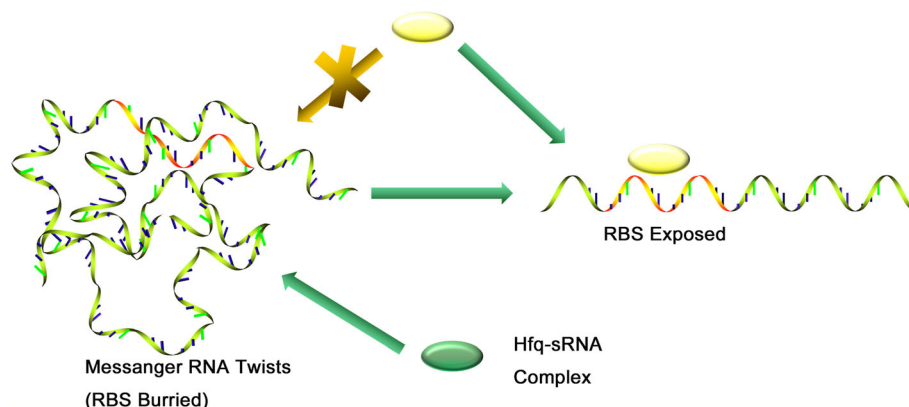

Fig. 1 Action of Hfq-sRNA complex on mRNA. The RBS is buried within the mRNA secondary structure, inhibiting translation. The Hfq-sRNA complex upregulates the translation by interaction with mRNA, exposing the RBS outside by modifying the structure [2, 19, 21, 22, 24]

Quorum sensing allows a single bacterial cell to sense the number of cells around and exchange information about itself with the rest of cells [41, 42, 46]. Both processes are achieved by AI secretion and a reception. There are two stages for completing a group behaviour such as pathogenicity. Firstly, bacterial cells secret AIs to sense the cell density. AIs then bind to the receptors to trigger the collective behaviour gene. Only if when the population reached a threshold to which the expression of gene causes dramatic influence (e.g. concentration of toxin) will the gene of interest be expressed [46].

All bacterial harbor this machinery to regulate their collective behaviours. Gram-negative and gram-positive bacteria both use QS to regulate subsequent group behaviour although there are subtle variations in type of autoinducers and their mechanisms [41, 42]. In gram-negative bacteria, auto-inducer refers to N-Acyl homoserine lactone derivatives (AHL) [39, 43, 47].

Nonetheless, the functional group (usually fatty acid) ensures the specie-specificity [41] of quorum sensing. Majority of the gram-negative bacteria quorum sensing is regulated by highly conserved LuxR/LuxI family [48] although there are bacteria species like *V. harveyi, Staphylococcus aureus* and *Bacillus subtilis* which use two-component systems [42, 49, 50, 51]. Generally speaking, in gram-negative bacteria, LuxI synthases synthesise acyl homoserine lactone (AHL) and they diffuse outside the cells [41, 52–54]. AHL molecules diffuse into other cells freely and bind to LuxR receptors in other cells. LuxR protein molecules consist of two domains for DNA binding and autoinducer binding respectively [54]. DNA binding domain is only activated once the autoinducers bind [53]. AHL-bound-LuxR protein complex binds to the promotor and initiates transcription of gene of interest [52]. LuxR regulates transcription of many genes typically bioluminescence, biofilm formation and virulence (Fig. 2). Other species like *P. aeruginosa* (LasI-LasR) [55] uses LuxI-LuxR-type system [41] as well [52]. As mentioned above, there are difference between autoinducers while parent core is identical. This ensures that only intra-species communication is constructed. Gram-positive quorum sensing mainly involves two-component system [41] and the signaling molecule have difference [41] from the one of gram-negatives. Two-component system consists of a sensor kinase and a response regulator [41, 42, 56].Instead of AHL being secreted, precursor proteins are translated from gene followed by a processing step which cleaves precursors into oligopeptides [41, 57]. These peptides are then secreted out. Two-component system functions when concentration of autoinducer peptides reaches a threshold. Transmembrane sensor kinase receives the signal (AIs) and initiate phosphorylation [41, 56]. Transcription factor enables the gene transcription as the consequence of response regulator involvement. The general signaling procedures are the same even though the mechanisms are different in gram-positive/negative bacteria.

QS does not only exist at intra-species level but also at inter-species level. Bacteria have their universal language when various bacterial communities co-exist (Fig. 3). Bassler et al. discovered a universal signaling molecule and its according mechanism [41]. This varies from existing signaling mechanism of a bacterial species yet it co-functions with the latter. Bacteria apply either inter-species language or generic language when it is needed to interact internally and externally.
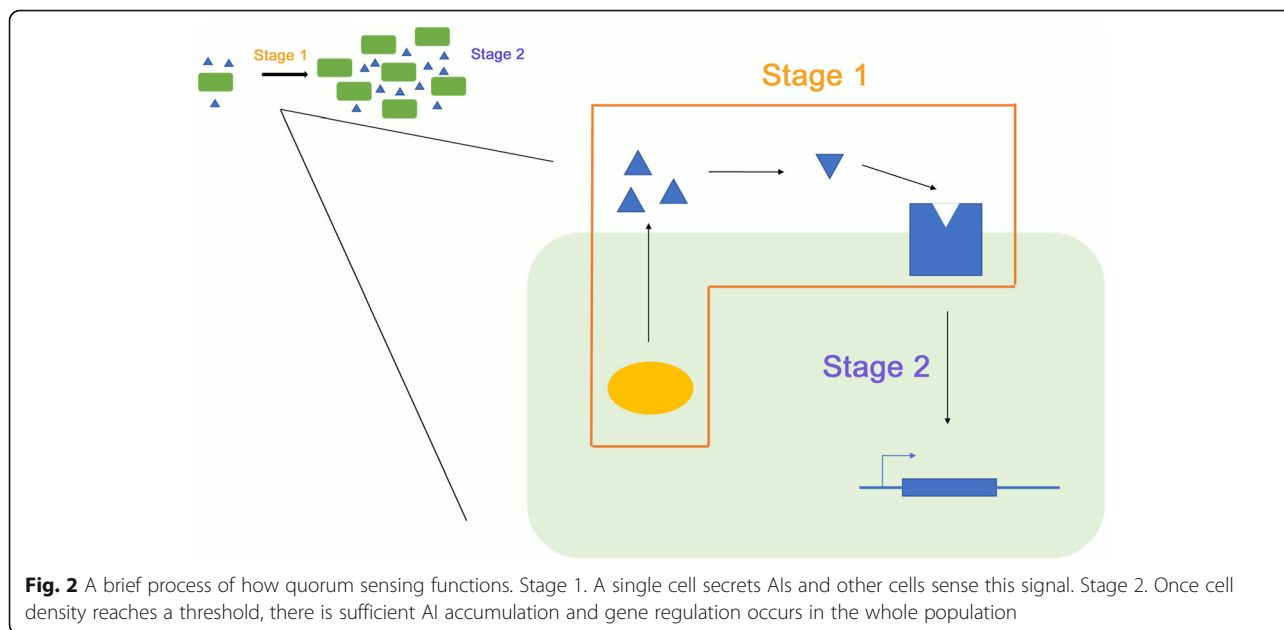

Fig. 2 A brief process of how quorum sensing functions. Stage 1. A single cell secrets AIs and other cells sense this signal. Stage 2. Once cell density reaches a threshold, there is sufficient AI accumulation and gene regulation occurs in the whole population
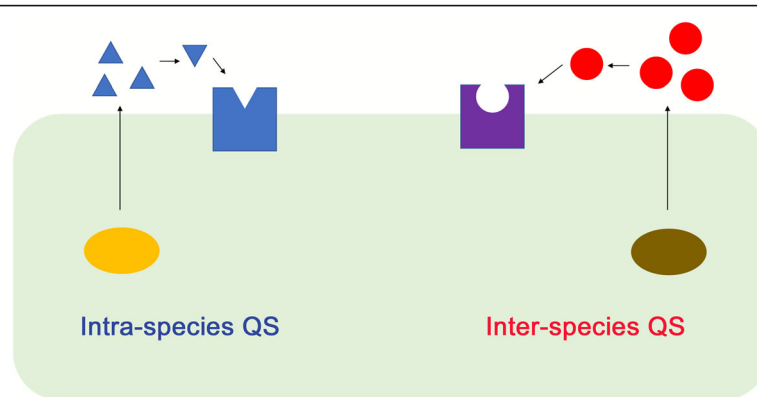
Fig. 3 Two systems for intra/inter-species communication respectively. A single bacterium can have two sets of signaling methods for intraspecies and interspecies communication. The mechanism is identical while the signaling molecule is different between two systems. All discovered bacteria which utilise quorum sensing communicate through the same signaling molecule where inter-species communication is needed

Various cases have demonstrated the existence of interkingdom signaling by QS machineries [58–60]. In, *Legionella pneumophila,* QS is facilitated by AI LAI-1coded by LqsA. This gram-positive bacterium lives at which its AI affects the same species, but also interacts with its eukaryotic host cells, affecting their migration. LqsA encodes for autoinducers LAI-1 and this autoinducer is transported extracellularly. LAI-1 is uptaken by trans-membrane receptor in host cells and its involved in production of cell migration microtubules [58]. The mechanism of host cell uptaking is unknown and neither does the receptor, whereas it suggested the feasibility of interkingdom communication. Other pro-to-eukaryotic communications have been identified [60]. A typical example of co-functioning is seaweed *Enteromorpha* and *Vibrio anguillarum. Enteromorpha* starts the growth based on the biofilm produced by *Vibrio anguillarum.* As proposed by this group, the growth of seaweed is highly dependent to the concentration of autoinducer from *Vibrio anguillarum* [60].
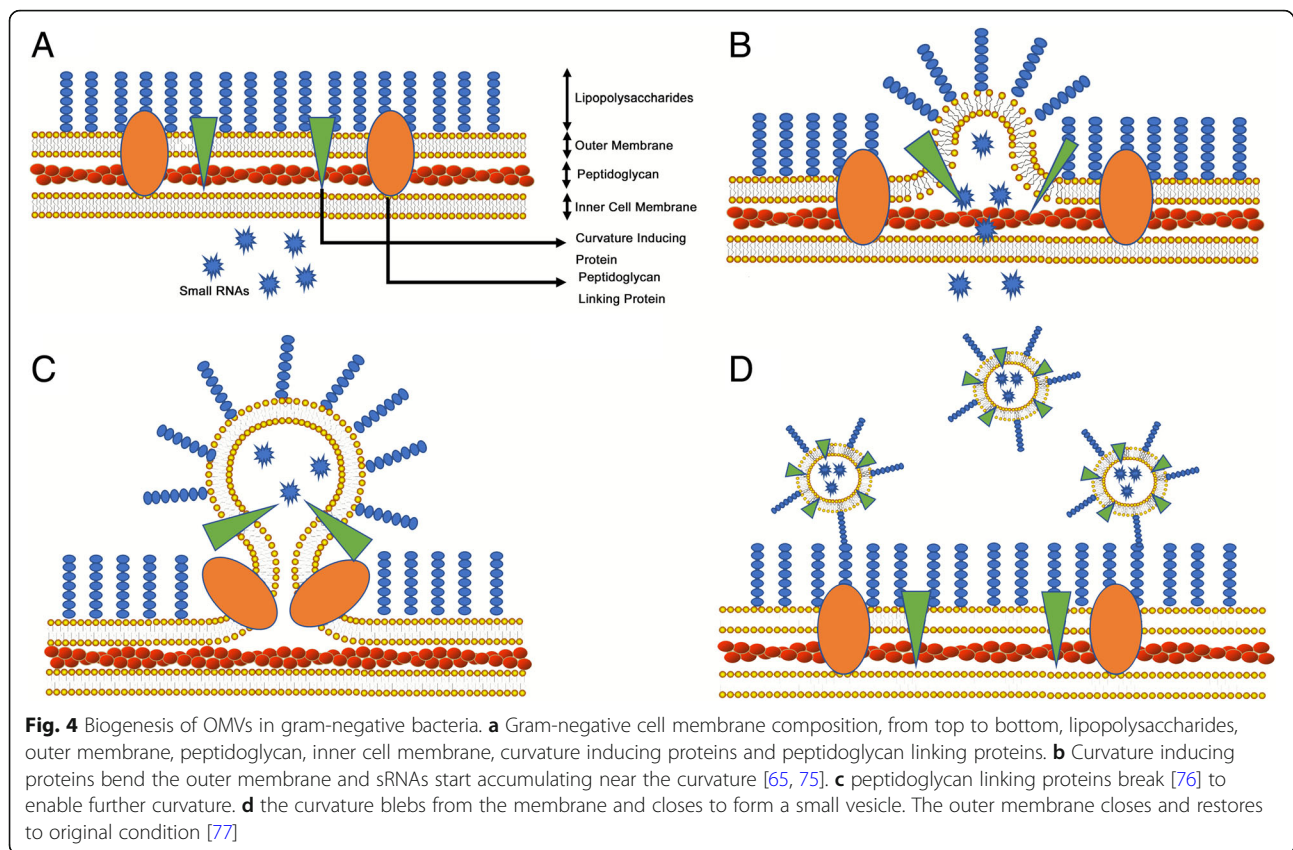
Inter-kingdom signaling through sRNA

QS-mediated interkingdom communication provided an insight of whether or not can sRNA interact with eukaryotic cells for the reason that autoinducer AHL and sRNA both regulate inter-cellular activities at molecular level. The answer is confirmative. There has been a number of evidences showing sRNA interferes with LuxR in quorum sensing [42]. In addition to that, discovery of quorum regulatory RNAs (Qrr) [30, 61], demonstrated sRNA can regulate interkingdom communication. Yet it is not going to be discussed in detail as a directly related communication is arising over the last years [62–65]. Extracellular vesicles (EVs) were initially thought to only exist in eukaryotes [66] and play a critical role in intercellular communication by carrying RNA and proteins [62, 67, 68]. It has also been described

that EVs can carry miRNAs and aid in their horizontal transfer [67–70] and that sRNA may be secreted extracellularly by similar means in prokaryotes [63, 69, 71–74]. EVs in prokaryotes are named outer membrane microvesicles (OMVs) [62] since they are released from the outer membrane [75]. Unlike EVs, the exact mechanism of OMV biosynthesis remains unclear. A proposed model is depicted in Fig. 4. First, breakage of peptidoglycan linking proteins bound to both the inner and outer cell membranes is necessary to produce OMVs [76]. Molecules to be bound to OMVs accumulate in the periplasm. Aided by the curvature inducing protein, the outer membrane is curved out [65, 75]. Eventually the OMVs are formed, blistered out and the outer membrane closes completely [77]. As part of this, sRNA can be packed in OMVs and transported out, yet the mechanism of how sRNAs enter these vesicles is not well understood. OMVs can then enter other cells, including eukaryotes, by membrane fusion [77, 78] thus releasing their content, which may include exogenous sRNA. This proposed mechanism has been described in *P. aeruginosa* host-pathogen interactions by Koeppen et al. [79], despite it is not specified how sRNA can affect host cells in this context.

In another example of trans-kingdom cross-talk due to sRNA, sRNA from *E. coli* may hijack the RNAi of *C. elegans* as illustrated in Fig. 5. A protein similar to Hfq, RDE-2 [78], is endogenous to *C. elegans* but may bind to *E. coli* sRNAs, resulting in post-transcriptional silencing [78].

In addition to these unidirectional interkingdom communications, recent research has related gut microbiota to sRNA. It has been depicted that eukaryotic cells can respond and interact with bacteria in gut through miRNA [80, 81]. Subsequently, the interactions between these two communities may even have impact on brain through the gut-brain axis [81] even though this observation was not systematically assessed. A similar

Fig. 4 Biogenesis of OMVs in gram-negative bacteria. **a** Gram-negative cell membrane composition, from top to bottom, lipopolysaccharides, outer membrane, peptidoglycan, inner cell membrane, curvature inducing proteins and peptidoglycan linking proteins. **b** Curvature inducing proteins bend the outer membrane and sRNAs start accumulating near the curvature [65, 75]. **c** peptidoglycan linking proteins break [76] to enable further curvature. **d** the curvature blebs from the membrane and closes to form a small vesicle. The outer membrane closes and restores to original condition [77]
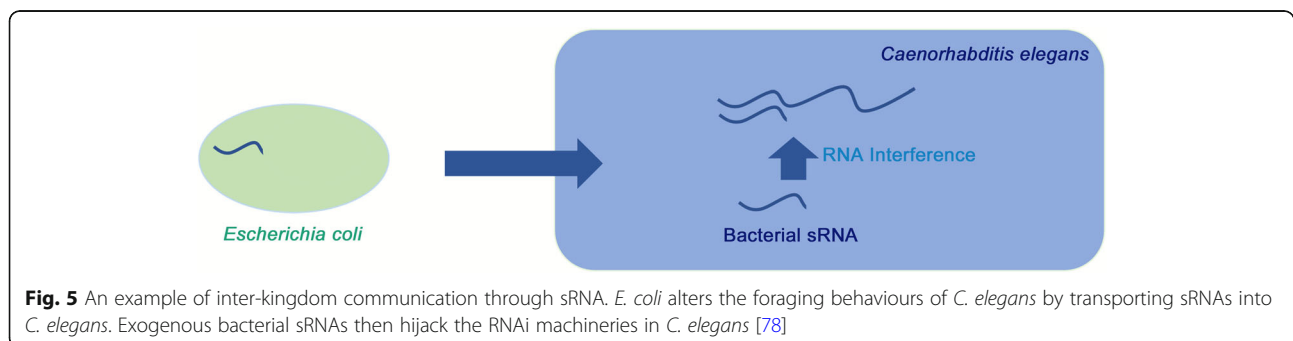
phenomenon [82] has also been reported by other groups on cooperation of intestinal microbiota with eukaryotic miRNA response to counter against the *Listeria* infection in gut [83].

Conclusions and prospective

Even though there has not been solid evidence showing direct accordance between QS and sRNA interkingdom regulations, it is thought that the QS does have impact on extracellular journey of sRNAs. sRNAs concentration decreases when sRNAS enter target cells although they are described to be resistant to harsh environments. sRNAs may be degraded by target cells defenses like by target cell defences such as RNAses. Bacterial QS may assist against reduction on concentration of sRNAs by raising the threshold of sRNA concentration and secrete more of them before entering target cells so that enough of them can interfere with target cell mRNAs.

The impact on sRNA on bacterial intercellular physiology and the mechanism of QS at both an inter-species and inter-kingdom level are well understood. With recent advances in OMVs studies in prokaryotes, we see the tip of the iceberg of interkingdom communication directly through sRNAs. Yet the mechanisms of how sRNAs are accumulated for EVs enrichment, how these vesicles selectively travel from prokaryotic to eukaryotic cells and how sRNAs hijack the target cell machineries without being degraded are left unclear and to be unveiled.

Fig. 5 An example of inter-kingdom communication through sRNA. *E. coli* alters the foraging behaviours of *C. elegans* by transporting sRNAs into *C. elegans*. Exogenous bacterial sRNAs then hijack the RNAi machineries in *C. elegans* [78]

This may hint at how sRNAs can be exploited for several applications. With the aid of computational analysis like comparative genomics [84], sRNA-based gene therapy in cancer treatment is feasible. Given the mRNA sequence of the carcinogenic proteins, a target-based therapeutic sRNA can be found and synthesised [85]. Small RNA can then be delivered into target cells using OMVs as vector, silence these cancer-related gene expressions and disrupt the cancer cell physiologies [82, 84, 85]. Additionally, it is noteworthy pointing out another potential therapy based on gut-brain axis due to the observation described above. The consequence of microbiota-gut interaction [82] subsequently affects the brain development, which indicate the possibility of sRNA-based therapy [85] to potentially delivery drugs [81, 82] or cure the neural diseases by gut-brain axis as it passes the blood-brain barrier.

Abbreviations
AHL: Acyl homoserine lactone; AI: Autoinducer; DNA: Deoxyribonucleic acid; EV: Extracellular vesicle; IGR: Intergenic regions; miRNA: Micro ribonucleic acid; mRNA: Messenger ribonucleic acid; OmpF: Outer membrane protein F; OMV: Outer membrane vesicle; QS: Quorum sensing; RBS: Ribosomal binding site; RNA: Ribonucleic acid; RNAi: Ribonucleic acid interfering; rRNA: Ribosomal ribonucleic acid; siRNA: Small interfering ribonucleic acid; sRNA: Small ribonucleic acid; tRNA: Transfer ribonucleic acid; UTR: Untranslated region

Availability of data and materials
Not applicable.

Authors' contributions
HF drafted the manuscript. RC contributed to review and made a number of valuable comments to the manuscript. PH commented the review and corrected the grammatical error. All authors read and approved the final manuscript.

Ethics approval and consent to participate
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Consent for publication
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Competing interests
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