Current Microbiology (2021) 78:2231–2241
https://doi.org/10.1007/s00284-021-02484-y

REVIEW ARTICLE

Recent Research Advances in Small Regulatory RNAs in *Streptococcus*

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Received: 9 September 2020 / Accepted: 29 March 2021 / Published online: 7 May 2021

Abstract
Small non-coding RNAs (sRNAs) are a class of regulatory RNAs 20–500 nucleotides in length, which have recently been discovered in prokaryotic organisms. sRNAs are key regulators in many biological processes, such as sensing various environmental changes and regulating intracellular gene expression through binding target mRNAs or proteins. Bacterial sRNAs have recently been rapidly mined, thus providing new insights into the regulatory network of biological functions in prokaryotes. Although most bacterial sRNAs have been discovered and studied in *Escherichia coli* and other Gram-negative bacteria, sRNAs have increasingly been predicted and verified in Gram-positive bacteria in the past decade. The genus *Streptococcus* includes many commensal and pathogenic Gram-positive bacteria. However, current understanding of sRNA-mediated regulation in *Streptococcus* is limited. Most known sRNAs in *Streptococcus* are associated with the regulation of virulence. In this review, we summarize recent advances in understanding of the functions and mechanisms of sRNAs in *Streptococcus*, and we discuss the RNA chaperone protein and synthetic sRNA-mediated gene regulation, with the aim of providing a reference for the study of microbial sRNAs.

Introduction

The genus *Streptococcus*, comprising a group of commensal and pathogenic low GC Gram-positive bacteria, typically has spherical or ovoid cells with homofermentative, anaerobic/aerotolerant, non-motile, catalase negative, and non-spore forming characteristics [1]. More than 55 species of *Streptococcus* are found in a wide variety of habitats including the mouth, respiratory tract, and skin surfaces of animals and humans, as well as in various environments such as soil and plants [2]. According to its hemolytic properties, *Streptococcus* is divided into three categories: (I) α-hemolytic *Streptococcus* e.g., *S. mutans*; (II) β-hemolytic *Streptococcus*, mainly including *S. pyogenes* (also called group A *Streptococcus* or GAS) [3] and *S. agalactiae*; and (III) γ-hemolytic *Streptococcus*, which are also called non-hemolytic *Streptococcus* and are generally not pathogenic [1]. Some species of *Streptococcus* significantly affect both humans and animals. For example, *S. pyogenes*, *S. pneumoniae*, and *S. agalactiae* are human pathogens causing serious acute infections [1–3], whereas *S. thermophilus* is an important probiotic lactic acid bacterium and the only "generally recognized as safe" species of *Streptococcus* [4].

Small non-coding RNAs (sRNAs) are a novel class of regulatory RNAs that fine-tune biological function networks, e.g., responses to environmental changes [5, 6]. Many sRNAs have been discovered [7, 8], and their detailed regulatory functions have been studied in bacteria [7]. However, compared with model bacteria such as *Escherichia coli* and *Bacillus subtilis*, current knowledge of sRNAs and sRNA-mediated regulation in *Streptococcus* is limited. In this review, we provide a comprehensive overview of the functions and regulatory mechanisms of known sRNAs in *Streptococcus*.

Small Non-coding RNAs

As an important class of regulators, sRNAs 20–500 nucleotides (nt) in length exist in bacteria but are generally not translated into proteins [7, 9]. sRNAs are transcribed from the intergenic regions (IGRs) located between open reading frames, or in the 5′ or 3′ non-coding regions (UTRs)

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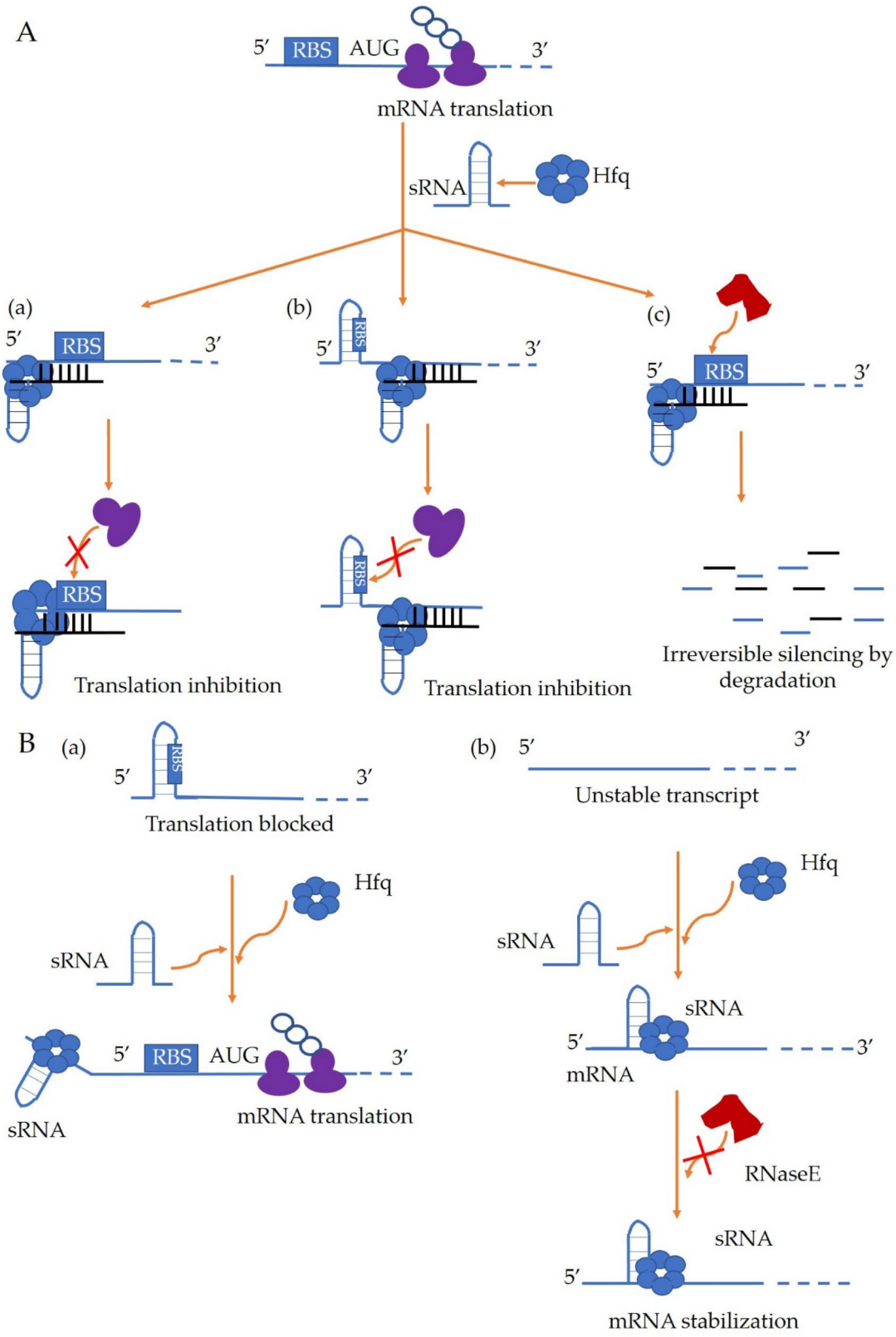


of coding genes [10]. sRNAs play regulatory roles at the post-transcriptional level in the bacterial response to environmental stress, through complementary base-pairing with mRNA molecules or interaction with the corresponding protein molecules [11, 12]. sRNAs are important nodes in many signaling pathways and physiological metabolic pathways

◂**Fig. 1** Regulatory mechanisms of sRNAs. **A** Mechanisms of inhibition of translation. **a** sRNAs prevent the binding of 30S ribosomes and the initiation of translation, through incomplete complementary base-pairing with the ribosome binding site (RBS) located at the 5′-UTR of the target mRNA. **b** sRNAs bind the mRNA being translated with the aid of Hfq, thus resulting in structural recombination of mRNA. The RBS region undergoes complementary base-pairing to form a secondary structure that prevents ribosome binding, thus leading to translational inhibition. **c** After the sRNA base-pairs with the mRNA, the mRNA is degraded by RNase E. In target mRNA degradation, RNase E specifically acts on AU-rich single-stranded RNA, and can simultaneously degrade target mRNA and sRNA. **B** Regulatory mechanisms of activation of translation. **a** Some sRNAs activate translation by binding to the 5′-untranslated region (5′-UTR) of the target mRNA, making the RBS becomes available, allowing initiation of translation. **b** Some sRNAs directly and stringently base-pair with target mRNA with the help of Hfq, thus forming a double-stranded structure, thereby increasing the stability of mRNA and protecting it from degradation by RNase E

[5, 13]. Most known functional sRNAs, in the form of antisense RNAs, specifically bind target mRNAs and regulate target gene expression. Most known bacterial sRNAs act as regulatory elements that respond to extracellular environment stresses and play key roles in intracellular physiological processes [14].

The process of sRNA recognition is not easy to identify through experimental methods. First, sRNAs themselves do not encode proteins, their lengths are short, and they are not easily inhibited by single nucleotide mutations. Second, sRNAs are not translated, and therefore their sequences cannot be obtained by simply recognizing open reading frames. Hence, many sRNAs are discovered and identified through a combination of bioinformatics and experimental methods [15, 16]. For example, Rath et al. [15] have created an RNase III null mutant and used RNA sequencing (RNA-Seq) to analyze the differential expression between this mutant and the wild-type of *S. pyogenes*. Twelve significantly differential transcripts have been obtained using the analysis of Cufflinks and Stringtie softwares, and six putative sRNAs have been visually verified by Artemis and Bamview genome viewers.

The targets of bacterial sRNAs is commonly predicted by bioinformatics methods, mainly on the basis of sequence length, base matching, RNA secondary structure, and sequence conservation and location [17]. Currently used software programs for predicting sRNA target mRNAs include RNAPredator [18], sRNATarBase [19], CopraRNA [20], RNAup [21], RNAhybrid [22], TarPicker [23], IntaRNA [24], and Target RNA2 [25]. The main difference of these in silico sRNA target discovery programs is due to the use of different prediction models, including models for general RNA–RNA interaction and models specifically designed for sRNA–target mRNA interactions in bacteria [17]. Many experimental methods have been used to verify sRNA target genes, among which genetic methods, affinity technology, immune co-precipitation methods, and microarray technology are the most mature and widely used [16, 26]. Ribosome profiling (Ribo-seq) technology is a recently developed method that simultaneously determines transcriptional and translational levels in vivo [27]. Wang et al. [28] have used Ribo-seq to fully validate the known targets of sRNA RyhB from *E. coli* and have identified many novel sRNA targets. Guo et al. [29] have also employed Ribo-seq to discover the target of a new σ^E-dependent sRNA MicL. The experimental data have indicated that Ribo-seq is an effective method for identifying sRNA targets and detecting the extent of regulation by sRNA.

With the progress of bacterial sRNAs, the regulatory mechanisms of sRNAs are gradually being elucidated (Fig. 1A and B). Bacterial sRNAs regulate the virulence of bacteria in host cells and initiate internal mechanisms in bacterial cells, thus allowing bacteria to respond to changes in growth conditions, adapt to the environmental changes in infected cells [30], and control bacterial density by regulating the expression of quorum sensing system-regulated proteins [31]. In the absence of nutrients in the growth environment, bacterial sRNAs such as CsrB and CsrC [32] play important regulatory roles in anti-nutritional stress. Trace elements are indispensable in cell metabolism, and sRNAs regulate the relative balance of trace elements in bacteria. For example, sRNA RyhB regulates the gene expression of Fur (an iron-uptake regulator) in *E. coli* and YdeP (which is responsible for regulation of acidity) in *Shigella flexneri* [33]. In addition to regulating acid tolerance, sRNAs regulate other environmental stress responses, such as MicA, RybB, and MicL to Membrance stress [34], SgrS to phosphosugar stress [34], TisB to SOS stress [34], DsrA, ArcZ, RprA, and OxyS to oxygen stress [35]. sRNAs may have different regulatory mechanisms in different bacteria, thus leading to different functional effects [36].

The Biological Functions of sRNAs in *Streptococcus*

sRNAs from *S. pyogenes*

Streptococcus pyogenes is a bacterial pathogen that causes diseases ranging from superficial infections (e.g., impetigo and pharyngitis) to systemic diseases (e.g., necrotizing fasciitis and toxic shock) [3]. Several sRNAs have been identified in *S. pyogenes* (Table 1), most of which are involved in virulence regulation [37].

sRNA FasX

FasX is an sRNA (~ 200 nt) that affects the expression of proteins (e.g., streptokinase) involved in the conversion

Table 1 Overview of sRNAs in *Streptococcus*

Species	Strains	Predicted sRNAs	Validated sRNAs	Known sRNAs (target mRNA)	References
S. pyogenes	MGAS315	45	14 (NB)	FasX (*ska*, *mrp*, *sagS and fbp54*)	[2, 8, 38, 42, 73]
	SF370	197	30 (NB)	Pel (*speB*, *emm*, *sic* and *nga*)	
	MGAS2221	40	16 (NB)	RivX (*mga*) MarS (*mga*)	
S. pneumoniae	TIGR4	88	13 (qRT-PCR),	csRNA1–5 (*comC*, *spr0081*, *spr0159*, *spr0551*, *spr1097*)	[8, 43, 45, 46, 50, 74]
		50	17 (qRT-PCR or NB)		
	D39	–		F20	
	D39W	109	10 (NB)	F32	
	D39V	34	60 (NB)		
			34 (NB)		
S. mutans	UA159	736	7 (qRT-PCR),	L10-leader (*SMU.488*, *SMU.488* and *SMU.488*)	[51, 52, 56, 57, 75]
		1879	10 (qRT-PCR),	sRNA133474 (*liaR*, *ciaR and covR*)	
		2149	43 (qRT-PCR)	srn884837 (*glnQ*, *glnM*, *brpA*, and *relA*)	
	UA159 and clinical strains	–	9 (qRT-PCR)	srn133480 (*ffh*, *brpA*, and *relA*)	
S. suis	P1/7	29	1 (NB)	srn225147 (*comD*), rss04 (*ccpA* and *luxS*)	[59, 60, 63]
	05ZYH33, 98HAH33	56	–	sRNA34 (*HPA2*, *05SSU0003*, *05SSU1265* and *05SSU0013*)	
S. agalactiae	NEM316	197	26 (qRT-PCR)	SQ18 (*gbs0031*)	[2]
			10 (NB)	SQ485 (*gbs1588*) SQ893 (*gbs1263*)	
S. sanguinis	SK36	219	5 (qRT-PCR or NB)	S.S-1964 (*SSA_0513*)	[9]
	ATCC 10,556	8	2 (qRT-PCR)	csRNA1-1 (*pilT*), csRNA1-2 (*pilT*)	[64]

NB northern blots, *qRT-PCR* quantitative reverse transcription PCR

of plasminogen to plasmin [37]. The mechanism of FasX regulation has been well described elsewhere [38–41]. FasX is part of an operon including genes encoding two histidine kinases (FasBC) and one response regulator (FasA) [37]. FasX influences the expression of virulence factors through three steps [38–41]: (I) FasX enhances the expression of streptokinase (a virulence factor) by stabilizing the mRNA of *ska*, encoding streptokinase; (II) FasX regulates pilus biosynthesis and adherence through destabilizing the mRNA of the pilus biosynthesis operon through base-pairing; and (III) FasX inhibits the translation of *cpa*, which encodes a pilin protein (Fig. 2a). FasX regulation is serotype specific and depends on the presence of target genes [38–41]. For example, FasX can posttranscriptionally regulate the expression of the adhesion- and internalization-promoting, fibronectin-binding proteins *PrtF1* and *PrtF2*. The mechanism is through base-pairing to the *prtF1* and *prtF2* mRNAs within their 5′ untranslated regions, where blocks ribosome access and leads to an inhibition of mRNA translation [41]. As a molecular switch, FasX governs the transition of *S. pyogenes* between the colonization and dissemination stages of infection [41].

sRNAs Pel/*sagA* and RivX

Pleiotropic effect locus/streptolysin-associated gene A (Pel/*sagA*) regulates M and M-related proteins (virulence factor streptolysin S, SLS) in *S. pyogenes* [8]. Pel activates virulence gene expression with strain specificity. RofA-like protein IV regulator X (RivX) is an sRNA that is derived from a longer mRNA encoding the transcription regulator RivR (RALP4, a RofA-like protein family of transcriptional regulators) and corresponds to a processed form of the *rivRX* transcript [8]. Both RivR and RivX positively regulate the *mga* regulon (encoding multiple gene activator), which activates the expression of virulence-associated regulators and factors (Fig. 2b and c).

mga-Activating Regulatory sRNA (MarS)

Recently, Pappesch et al. [42] have reported the novel sRNA MarS in *S. pyogenes* M49591, which modulates the expression of Mga-dependent virulence factor and affects capsule biosynthesis and the fate of *S. pyogenes*. Deletion of MarS results in downregulation of the expression of *mga* and Mga-activated genes. The mode of action of MarS is shown in Fig. 2d.

sRNAs from *S. pneumoniae*

Streptococcus pneumoniae is an opportunistic pathogen associated with a wide range of human diseases [43]. It can cause serious infections such as sepsis, endocarditis, and meningitis. Furthermore, it is a leading cause of pneumonia, which kills more young children than any other infectious diseases [44].

sRNAs F20 and F32

The sRNAs F20 (also called as srn157) and F32 (previously identified as a tmRNA) are associated primarily with the virulence regulation of *S. pneumoniae*. F20 and F32 deletion mutants show decreased adhesion and invasion of nasopharyngeal or endothelial cells, thus resulting in a lack of fitness and competitive index in the environment of the nasopharynx and lungs [44]. Through proteomic analysis, Acebo et al. [45] have found that purine metabolism is significantly downregulated, whereas the pathways of DNA synthesis and repair are strongly upregulated, in an F20 deletion mutant. Kumar et al. [46] have reported that metabolic pathways encompassing the lactose transport system and multiple phosphoglucose transferase systems are depressed in an F32 mutant. F20 deletion thus has pleiotropic effects on virulence attenuation. The F32 mutant also shows a strong effect on bacterial pathogenesis. F32 is associated with deficiencies in the stress response and pathogenicity and has a core role in the *trans*-translation mechanism, which resolves stalled ribosomes on non-stop mRNAs [47]. However, the specific regulatory mechanisms of these two sRNAs and their target genes remains unclear.

cia-Dependent Small sRNAs (csRNAs)

csRNAs are a family of Cia, competence induction and altered cefotaxime susceptibility (CiaR)-regulated sRNAs in *Streptococcus*. CiaR-targeted promoters have been identified through mapping the genome of *S. pneumoniae* [48]. CiaR is a response regulator in the CiaRH two-component system (TCS), which is responsible for β-lactam susceptibility, competence, bacteriocin biosynthesis, autolysis and virulence in bacteria. Sixty-one csRNAs with lengths of 51–202 nt have been identified and classified into 40 different types through a search for CiaR-activated promoters in the genomes of 14 *Streptococcus* species [49]. Among these csRNAs, five pneumococcal csRNAs (csRNA1 to 5) are transcribed by *cia*-controlled non-coding RNA (*ccnA-E*) in the CiaR regulon. Halfmann et al. [48] have reported that csRNA4 and csRNA5 are involved in stationary-phase autolysis in *S. pneumoniae* R6. Microarray analysis of csRNA1 inactivation has uncovered a *cis*-acting effect on the mRNAs of adjacent genes, thus suggesting that csRNA1 is co-transcribed in strain D39 [50]. However, csRNA1 inactivation or activation does not affect cell growth, stress responses, global transcription or virulence.

sRNAs from *S. mutans*

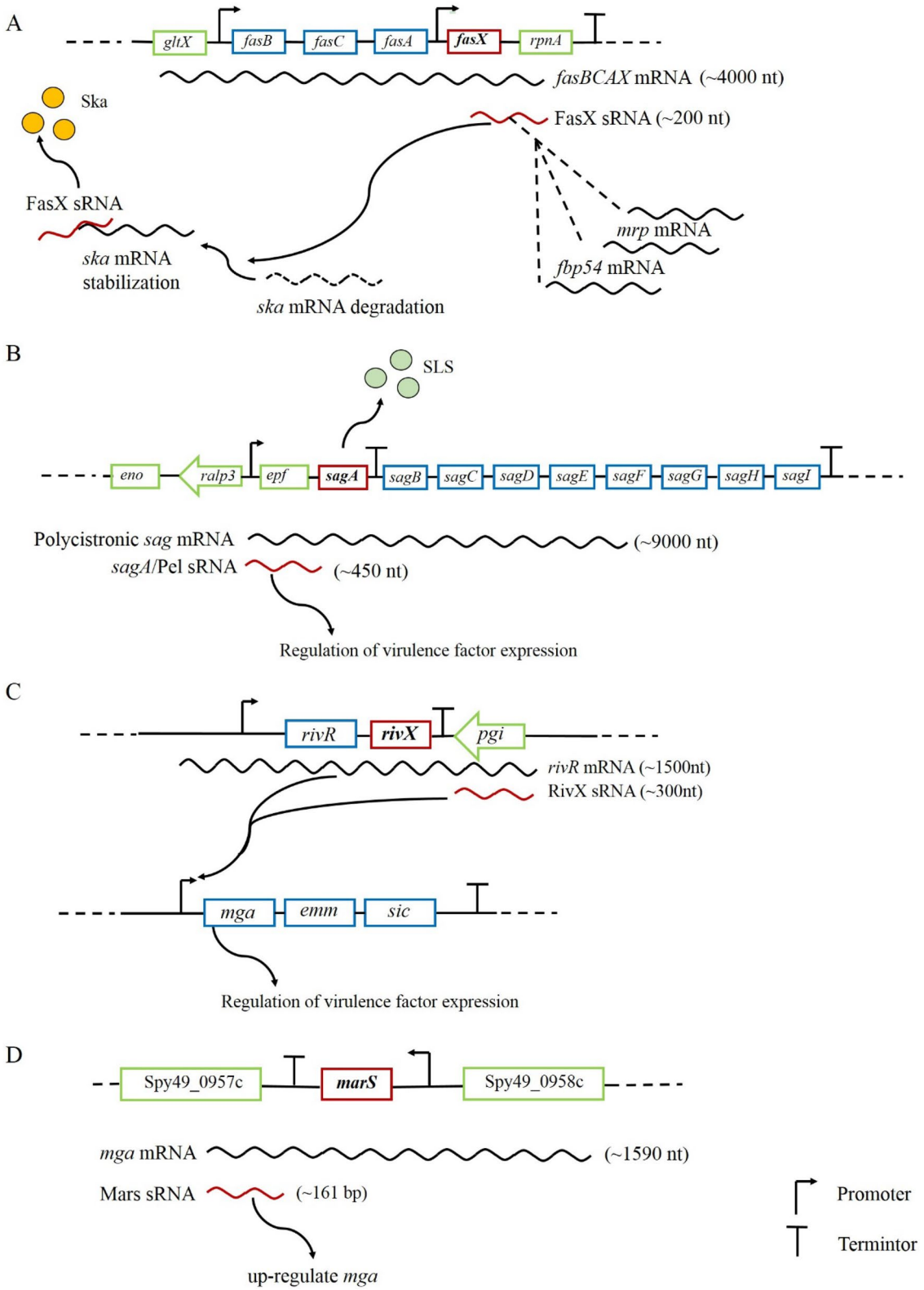
Streptococcus mutans is a major clinical pathogen in dental caries that possesses various virulence factors and consequently can accumulate and colonize the surfaces of teeth [51]. It can utilize diverse carbohydrate sources, and produce and tolerate various acids; therefore, it can survive at low pH. It adheres to the surfaces of teeth, thereby resulting in demineralization of tooth surfaces [52]. Currently, sRNAs are known to regulate adherence and polysaccharide biosynthesis in *S. mutans*. Moreover, sRNAs in *S. mutans* are induced by different concentrations (1–5%) of sucrose during planktonic growth [53]. An array of sRNAs has been found to be induced under acid stress, in comparison with the sRNAs reported by Lee et al.[54]. The study of sRNAs that regulate bacterial virulence in *S. mutans* may be applied to solving the problem of dental caries (Table 1).

sRNA L10-Leader

Xia et al. [55] have predicted 334 sRNAs and verified the existence of L10-Leader in *S. mutans* UA159. The upregulation of L10-Leader in an acidic environment may indicate that L10-Leader binding to target mRNA improves the stability and translation of target mRNA. Consequently, cells can quickly adapt to acidic stress, and correct DNA matching is ensured under various environment stresses. However, the mechanism of L10-Leader in acidic environments remains unclear in *S. mutans*.

sRNA133474

Streptococcus mutans harbors diverse stress response pathways including TCSs to mitigate acid stress in the oral cavity [56]. Nine acid tolerance-associated sRNAs have been validated in *S. mutans* UA159 and other clinical strains cultured at pH 5.5 and 7.5. Compared with *S. mutans* culture at pH 7.5, in culture at pH 5.5, sRNA133474 is the most significantly downregulated among these sRNAs [56]. The


A
gltX
fasB
fasC
fasA
fasX
rpnA
fasBCAX mRNA (~4000 nt)
Ska
FasX sRNA (~200 nt)
FasX sRNA
mrp mRNA
ska mRNA
stabilization
fbp54 mRNA
ska mRNA degradation
B
SLS
eno
ralp3
epf
sagA
sagB
sagC
sagD
sagE
sagF
sagG
sagH
sagI
Polycistronic sag mRNA
(~9000 nt)
sagA/Pel sRNA
(~450 nt)
Regulation of virulence factor expression
C
rivR
rivX
pgi
rivR mRNA (~1500nt)
RivX sRNA (~300nt)
mga
emm
sic
Regulation of virulence factor expression
D
Spy49_0957c
marS
Spy49_0958c
mga mRNA
(~1590 nt)
Mars sRNA
(~161 bp)
up-regulate mga
Promoter
Termintor

◂**Fig. 2** Regulation of sRNAs in *Streptococcus*. **a** FasX regulates the expression of virulence in *S. pyogenes*. FasX is a main effector of the FasBCA three-component system. FasB and FasC are histidine kinases that phosphorylate the response regulator FasA after signal sensing and subsequently upregulate expression of the *fas* operon. FasX inhibits the expression of *sag* (encoding streptolysin S, SLS), *fbp54* (encoding a fibronectin-binding protein), and *mrp* (encoding M-related protein, a fibrinogen-binding protein). FasX increases the stability of *ska* (encoding streptokinase) mRNA through direct targeting, thereby increasing streptokinase synthesis. **b** Pel controls the expression of virulence factors in S. pyogenes. The direction of the arrow of ralp3 represents its direction of transcription. **c** RivX and rivR activate the expression of an operon encoding Mga, M protein (encoded by *emm*), and Sic (streptococcal inhibitor of complement) in *S. pyogenes*. **d** MarS activates the expression of *mga* (encoding multiple gene activator) mRNA

mechanism of action of sRNA133474 involves regulation of the expression of LiaSR, CiaRH, and CovRS—TCSs responsible for acid tolerance.

sRNA srn225147

The bacteriocin mutacin IV from *S. mutans* antagonizes numerous non-mutans streptococcal species (e.g., *S. gordonii*). A target of sRNA srn225147 has been found to be the mutacin IV formation-associated gene *comD*, on the basis of RNAhybrid and RNAPredator prediction [57]. As compared with a negative control condition, *comD* expression has been found to significantly increase with 1400-fold increased srn225147 expression but to decrease with 400-fold increased srn225147 expression, thus suggesting that srn225147 has a two-way regulatory effect on comD expression. However, srn225147′s regulatory effect on mutacin IV biosynthesis is weak [57].

sRNA0426

The virulence of *S. mutans* is dependent on the formation of biofilms, which provide better conditions for bacteria to adapt to the changing environment in the oral cavity [58]. According to bioinformatics analyses, qRT-PCR, and crystal violet staining assays, Yin et al. [58] have found that sRNA0426 has a positive relationship with dynamic biofilm formation and exopolysaccharide production. In the synthesis of exopolysaccharide, sRNA0426 expression is positively correlated with the expression of three target mRNAs (GtfB, GtfC, and CcpA). Therefore, sRNA0426 has been found to play an important positive role in biofilm formation, thus providing novel insights into the biofilm regulatory network in *S. mutans*.

sRNAs from *S. suis*

Although *S. suis serotype 2* (*S. suis 2*) is the main cause of porcine diseases, it can directly infect humans and cause hearing loss, meningitis, and septic shock [59, 60]. Zhang et al. [59] have performed RNA-seq in three strains of *S. suis* 2: P1/7 (reference strain) as well as 05ZYH33 and 98HAH33 (highly virulent isolates). Fifty-six sRNAs were predicted in three isolates, of which 12, particularly rliD and RatA, in all three isolates as well as *cspA* and *rli38* in 05ZYH33 and 98HAH33 were associated with bacterial virulence. For the remaining sRNAs, over 27% (12/44) were found to be involved in riboswitches. Recently, sRNA rss04 has been confirmed to repress capsular polysaccharide biosynthesis in P1/7 [60], thus increasing adhesion and invasion of mouse brain microvascular endothelial cells. Moreover, rss04 regulates catabolite control protein A (CcpA) and the virulence factor LuxS, thereby affecting biofilm formation, capsular polysaccharide biosynthesis, and meningitis progression [61, 62]. In addition, Gong et al. [63] have verified that sRNA34 regulates the virulence of *S. suis* 05ZYH33. Transcriptomic analysis has indicated that deletion of sRNA34 represses the expression of *HPA2* (encoding histone acetyltransferase) and *05SSU0013* (a cell cycle control gene) as well as *05SSU0003* and *05SSU1265*, which are involved in the synthesis of lipoteichoic acids and contribute to cellular chain formation and elongation. Thus, the inactivation of sRNA34 results in longer cellular chains and attenuated virulence [63]. However, the mechanism of action of sRNAs in *S. suis* remains to be elucidated.

sRNAs from *S. sanguinis*

Streptococcus sanguinis, such as *S. mutans*, has been recognized as a pathogen involved in dental caries and periodontal diseases. Choi et al. [9] have predicted 219 sRNAs and characterized five putative sRNAs by RNA-seq, northern blotting, and qRT-PCR in *S. sanguinis* SK36. In the genome of SK36, among these five characterized sRNAs, the sRNA S.S-1964 is located to the right of the gene *SSA_0513*, encoding a putative ATP:cob (I) alamin denosyltransferase, and it may be involved in the conversion of vitamin B_{12} to coenzyme B_{12} through regulation of *SSA_0513* expression [9]. Additionally, Ota et al. [64] have used computational target prediction and luciferase reporter assays to identify csRNA1-1 (a csRNA) as a negative regulator of the expression of pilT (a constituent of the type IV pilus operon) in *S. anguinis*. csRNA1-1 directly binds pilT mRNA, on the basis of RNA–RNA electrophoretic mobility shift assays. Moreover, csRNA1-1 and csRNA1-2 have been found to negatively regulate biofilm formation, thus suggesting that csRNAs are involved in the colonization process in *S. sanguinis* [64].

Perspectives

Although several sRNAs have been predicted and identified in *Streptococcus*, most current studies on sRNAs have focused on those involved in the regulation of virulence, such as FasX, Pel, RivX, and MarS in *S. pyogenes*, as well as F20 *and* F32 in *S. pneumoniae*. Understanding the roles of sRNAs in other cellular functions, particularly in the control of toxin–antitoxin systems and the responses to environmental changes, such as those in pH, oxygen, and nutrients, is very limited in *Streptococcus* but abundant in other bacteria [65]. Moreover, the current understanding of sRNA-mediated regulation, including modes of action and target identification, is largely insufficient in *Streptococcus*. sRNAs and their regulatory mechanisms reported in other bacteria have not yet been studied in *Streptococcus*. Thus, several questions regarding sRNAs in *Streptococcus* remain to be answered.

RNA chaperone protein Hfq (also known as HF-1) is required for the interaction between sRNA and target mRNAs in most sRNAs in Gram-negative bacteria [66]. For example, at least one-third of the experimentally verified *E. coli* sRNAs are Hfq-binding sRNAs [26]. Hfq is also at the core of sRNA-mediated post-transcriptional regulation, although the need for Hfq in the regulation of sRNA depends on multiple factors. The presence of Hfq promotes the pairing of most bacterial sRNAs with target mRNAs [26]. Hfq is involved in regulating the stability, polyadenylation, and translation of many RNAs, as well as in RNA processing. Deletion of the Hfq gene results in growth defects, decreased resistance to environmental stress, and changes in toxicity [67]. Hfq homologues also exist in some Gram-positive bacteria. However, the only known Hfq-dependent sRNA in Gram-positive bacteria is LhrA from *L. monocytogenes* [68]. On the basis of our comparative genome analysis, Hfq and its homologues are not found in *Streptococcus*. Currently known *Streptococcus* sRNAs do not require Hfq to function [66]. It suggests that Hfq and its homologues are not necessary for sRNA regulation in *Streptococcus*. How are sRNAs stabilized and annealed to the targets in *Streptococcus*? Generally, sRNAs are more likely to be associated with RNA-binding proteins than naked in the cellular niche [16]. Thus, whether other RNA chaperone proteins are necessary or helpful for sRNA regulation in *Streptococcus* needs further study.

sRNA-mediated gene silencing with an sRNA-assisted framework can provide a basis to construct artificial synthetic sRNA. The rational design of synthetic sRNAs based on natural sRNAs, can achieve high-throughput knockdown without genetic alterations; this method has been widely applied in metabolic engineering of model bacteria such as *E. coli* and *B. subtilis* [69]. Na et al. [70] have used synthetic sRNAs for combinatorial repression of four genes in 14 strains of *E. coli*. Repression of the expression of *tyrR* and *csrA* caused the mutant strain to produce 2 g/L of tyrosine. Moreover, repression of *murE* led to a 55% increase in the cadaverine titer. Meng et al. [71] have used synthetic sRNA to increase 6-deoxyerythronolide B production in *E. coli*, thus achieving the highest reported titer for heterologous polyketide biosynthesis. Liu et al. [72] have used an sRNA scaffold from *E. coli* to design synthetic sRNA to increase the yield of *N*-acetylglucosamine in *B. subtilis*. Thus, synthetic sRNA might potentially be used for gene regulation in *Streptococcus*. With deeper understanding of sRNAs and their regulation in *Streptococcus*, we believe that sRNAs could be designed and engineered as a genetic toolkit for metabolic engineering and synthetic biology in *Streptococcus* and other prokaryotes.

Acknowledgements This work was supported by the National Natural Science Foundation of China (Grant Nos. 31972101 and 31871776), National Science Fund for Distinguished Young Scholars (Grant No. 32025029), Natural Science Foundation of Shanghai (Grant No. 18ZR1426800), Shanghai Agriculture Applied Technology Development Program (Grant No. 2019-02-08-00-07-F01152), and Shanghai Engineering Research Center of Food Microbiology (Grant No. 19DZ2281100).

Author Contributions ZQX and ZXL contributed equally to this work. ZQX, ZXL, and LZA conceived and wrote the draft manuscript. XS, XXL, and YJX revised the manuscript. All authors read and approved the final manuscript.

Declarations

Conflict of interest There is no conflict of interest among the authors.

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