



Regulatory sRNAs in Cyanobacteria

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As the transcriptional and post-transcriptional regulators of gene expression, small RNAs (sRNAs) play important roles in every domain of life in organisms. It has been discovered gradually that bacteria possess multiple means of gene regulation using RNAs. They have been continuously used as model organisms for photosynthesis, metabolism, biotechnology, evolution, and nitrogen fixation for many decades. Cyanobacteria, one of the most ancient life forms, constitute all kinds of photoautotrophic bacteria and exist in almost any environment on this planet. It is believed that a complex RNA-based regulatory mechanism functions in cyanobacteria to help them adapt to changes and stresses in diverse environments. Although lagging far behind other model microorganisms, such as yeast and *Escherichia coli*, more and more non-coding regulatory sRNAs have been recognized in cyanobacteria during the past decades. In this article, by focusing on cyanobacterial sRNAs, the approaches for detection and targeting of sRNAs will be summarized, four major mechanisms and regulatory functions will be generalized, eight types of *cis*-encoded sRNA and four types of *trans*-encoded sRNAs will be reviewed in detail, and their possible physiological functions will be further discussed.

Keywords: small RNA, cyanobacteria, sRNA identification, regulatory functions, physiological roles

INTRODUCTION

RNAs from cells that do not display messenger RNAs (mRNAs), ribosomal RNAs (rRNAs), or transfer RNAs (tRNAs) functions include a wide class of molecules, usually denominated as small RNAs (sRNAs) or non-coding RNAs (ncRNAs) (Storz et al., 2004; Waters and Storz, 2009). As the transcriptional and post-transcriptional regulators of gene expression, sRNAs play important roles in every domain of life in organisms (Raghavan et al., 2011). Different from protein-coding regions specified by genetic codes, sRNAs have no clear-cut signatures that represent their boundaries as regulatory RNAs or even their occurrence in a genome. They play key roles in various basic processes in organisms, including structural maintenance of chromosome (Storz, 2002; Volpe et al., 2002), regulation of stability and translation of mRNAs (Storz et al., 2004), translocation and stability of proteins (Huttenhofer et al., 2005; Hüttenhofer and Vogel, 2006), metabolic reactions (Park et al., 2010), stress responses (Romby et al., 2006), and pathogenesis (Lee and Groisman, 2010).

Abbreviations: nt, nucleotides; sRNA, small RNA.

In prokaryotes, the length of sRNAs usually ranges from 50 to 500 nucleotides (nt). The sRNAs were known in prokaryotes as regulators for years before the first microRNA (miRNA) and short interfering RNA (siRNA) were found in eukaryotes. It was reported that a ~108 nt RNA I blocked ColE1 plasmid replication by base pairing with the RNA, which was cleaved to produce the replication primer (Stougaard et al., 1981; Tomizawa et al., 1981). Shortly after that, a ~70 nt RNA transcribed from the pOUT promoter of Tn10 transposon was discovered preventing translation of the transposase mRNA and repressing transposition (Simons and Kleckner, 1983). Subsequently, the 174 nt MicF RNA was identified as the first chromosomally encoded sRNA in *Escherichia coli* (*E. coli*) and plays a role in inhibiting translation of the mRNA encoding OmpF, the major outer membrane porin (Mizuno et al., 1984). These sRNAs were identified by multicopy phenotypes, by gel analysis due to their abundance, or by serendipity (Wassarman et al., 1999; Waters and Storz, 2009). During the past decade, the importance of this amazing group of RNAs has been widely recognized in various organisms (Mattick, 2001; Huttenhofer et al., 2002; Storz, 2002; Huttenhofer et al., 2005; Raghavan et al., 2011). Due to recent technical advancements, e.g., deep sequencing, computational searches, and tiling microarrays with full-genome coverage (Weinberg et al., 2007; Landt et al., 2008; Livny et al., 2008; Sittka et al., 2008), many new candidate sRNAs have now been predicted in *E. coli* and other bacteria. In *E. coli*, systematic computational analyses have predicted the existence of hundreds of sRNAs in the past few decades (Livny and Waldor, 2007); however, only about 80 sRNAs have been experimentally validated (Waters and Storz, 2009). Above all, the function of many sRNAs remains unknown.

Cyanobacteria constitute a wide variety of photoautotrophic bacteria that undergo oxygenic photosynthesis and grow under extremely diverse environmental conditions on Earth, such as oceans, freshwater, rocks surface, desert soils, or even polar regions. The existence of cyanobacteria can be traced back possibly up to 3–5 billion years (Schopf, 1993), and they have long been recognized as important model organisms for research in aspects such as photosynthesis (Pisciotta et al., 2010; Chen et al., 2015), metabolism (Tamagnini et al., 2002), stress responses (Wang et al., 2008; Ma et al., 2017), biotechnology (Abed et al., 2009; Zhan and Wang, 2018), evolution (Flores and Herrero, 2008), and nitrogen fixation (Goebel et al., 2010). Particularly, cyanobacteria are a group of unicellular aquatic prokaryotes that possess certain properties such as fast growth, short life cycle, and spontaneously transformability, which have entitled them to be one of simple experimental systems and the most promising feedstock for bioenergy generation (Quintana et al., 2011; Giordano and Wang, 2018).

The majority of data on bacterial sRNAs originated from enterobacteria analysis. To date, in cyanobacteria, identified or functionally characterized sRNAs have only been reported in a few studies. In our previous works, by deep RNA-sequencing (RNA-seq), a total number of 5261 putative sRNAs in *Synechocystis* sp. PCC 6803 were revealed from the genome and its four megaplasmids, providing a comprehensive overview of sRNAs in the model organism (Xu et al., 2014; Hu et al., 2017). It is very likely that cyanobacteria, present in

almost any environment with a long evolutionary history, have evolved a complex RNA-based regulatory network to respond to environmental changes and stress, analogously to enterobacteria. Here, we will review the research works on cyanobacterial sRNAs, including approaches for sRNAs detecting and targeting, their mechanisms of action and regulatory functions, and their possible physiological functions.

IDENTIFICATION OF sRNAs AND THEIR TARGETS IN CYANOBACTERIA

Due to divergence in sequences, structures, and functions, cyanobacterial sRNAs have no common identifiers. Nevertheless, biocomputational prediction, tiling microarrays, and pyrosequencing revealed that many candidate sRNAs have been existing in some cyanobacterial model organisms for the past several decades. The combination of computational and experimental methods used to identify sRNAs can help us understand sRNAs better and is likely to provide the complete sRNAs definition soon.

Biocomputational Prediction

The most important aspect of sRNA research includes designing the methods used for the prediction of sRNA and their targets. These investigations have been carried out in-depth in the last few years. The standard procedures used to predict bacterial sRNAs and their targets include these three steps: 1. identifying conserved sequences and/or structure features in intergenic regions, 2. clustering and comparing of pairwise or multiple alignments, and 3. scoring based on structural features of predicted RNAs, which were reviewed in detail by Backofen and Hess (2010).

A mass of sRNAs has been predicted based on comparative genomics analysis and non-comparatives screenings (Backofen and Hess, 2010; Kopf et al., 2014). Meanwhile, as a key bottleneck, the targets identification of these sRNAs is an immediate requirement for further progress in this field. Many developed target-prediction tools and related software for bacteria may be worth learning (Table 1). Some examples include TargetRNA (Tjaden et al., 2006; Tjaden, 2008), sequence-based scoring combined with stacking (Mandin et al., 2007), RNAplex (Tafer and Hofacker, 2008), sRNATarget (Zhao et al., 2008; Cao et al., 2009), RNAup (Muckstein et al., 2006), RNAhybrid (Rehmsmeier et al., 2004), GLASSgo (Lott et al., 2018), especially CopraRNA (Wright et al., 2014), and IntaRNA (Busch et al., 2008). Among those, CopraRNA is the latest asset of the Freiburg RNA tools Web server, which incorporates and extends the functions of IntaRNA to better predict targets and interaction domains of bacterial sRNA. The GLASSgo allows discovery of the homologous sRNA sequences, which is often the first step in the functional characterization and targets prediction of a sRNA. All of the earlier mentioned tools can be used to predict targets of cyanobacterial sRNA, if used reasonably. There is a representative report that possible sRNAs target genes were predicted using the IntaRNA tool, and then functional categorization analysis using gene ontology (GO) assignment programs and functional

TABLE 1 | Summary of sRNA targets prediction tools mentioned in the text.

Tools	Algorithm	Advantage	Limitation	Source
TargetRNA	Target prediction based on sequence	1. Simplicity 2. Easy calculating of the significance of the found matches	Neglecting some factors that may contribute to RNA-RNA interaction, such as RNA secondary structure or the role of Hfq	Tjaden et al., 2006; Tjaden, 2008
Sequence-based scoring combined with stacking	Target prediction based on sequence	1. Simplicity 2. Easy calculating of significance of the found matches	Neglecting some factors that may contribute to RNA-RNA interaction, such as RNA secondary structure or the role of Hfq	Mandin et al., 2007
RNAplex	Thermodynamic scoring of mRNA-sRNA mixed duplexes	1. Providing a much more realistic model of RNA-RNA interaction, as compared to approaches based on sequence complementarity 2. Very fast 3. Easy calculating of significance of the found hits	Neglecting intra-molecular base pairs	Tafer and Hofacker, 2008
sRNATarget	Thermodynamic scoring of mRNA-sRNA mixed duplexes	1. Providing a much more realistic model of RNA-RNA interaction, as compared to approaches based on sequence complementarity 2. Very fast 3. Easily calculating the significance of the found hits	Neglecting intra-molecular base pairs	Zhao et al., 2008; Cao et al., 2009
RNAhybrid	Thermodynamic scoring of mRNA-sRNA mixed duplexes	1. Providing a much more realistic model of RNA-RNA interaction, as compared to approaches based on sequence complementarity 2. Very fast 3. Easy calculating of significance of the found hits	Neglecting intra-molecular base pairs	Rehmsmeier et al., 2004
RNAup	Handling accessibility in mRNA-sRNA interactions	Easily combined with faster methods for assessing RNA-RNA interactions, such as RNAhybrid and RNAplex	By itself not fast enough for genome-wide predictions of microRNA or siRNA targets	Muckstein et al., 2006
IntaRNA	Handling accessibility in mRNA-sRNA interactions	Including extensive postprocessing methods such as functional enrichment analysis and visualization of interacting regions	Predicting interactions in single organisms	Busch et al., 2008
CopraRNA	Handling accessibility in mRNA-sRNA interactions	Incorporating and extending the functionality of IntaRNA to better predict targets and interaction domains of sRNA		Wright et al., 2014
GLASSgo	Combines an iterative BLAST strategy with pairwise identity filtering and a graph-based clustering method	1. Very fast and high specificity 2. Easy-to-use		Lott et al., 2018

annotation tool DAVID (Dennis et al., 2003; Huang et al., 2008) can be used, evidencing that sRNAs are involved in various metabolic pathways (Xu et al., 2014).

However, bioinformatic analysis alone is still full of high false positive rates. There are three main reasons for high false positive rates in cyanobacteria: (1) most target genes are at different genomic loci relative to their sRNAs; (2) although some sRNAs have single targets, others could regulate a multitude of gene expression; and (3) targets predictions of protein-modifying sRNAs also can be a challenge depending on the nature and understanding of the target characteristics. It is significantly

necessary to identify more sRNAs targets of cyanobacteria by combining computational and experimental approaches in future. Georg et al. (2014) proved the interaction between the ribosome-binding regions of the *psaL*, *psaJ*, *chlN*, and *cpcA* mRNA and sRNA PsrR1 by the combination of the CopraRNA tool and experimental analysis.

Microarray Analysis

Microarray analysis is the approach that uses microchips conjugated with probes (short DNA elements) for the screening of large-scale gene expression. Labeled nucleic acid samples

are applied to the microchips and then hybridized to specific probes; the interaction is identified by imaging and subsequent data processing. Microarray analysis is a strong tool for the detection of sRNA, especially antisense sRNA (asRNA), whose function is mainly determined by mere sequence complementarity rather than structural characteristics or specific sequence. Since computational screens rarely find asRNAs, the superiority and preferences are important to novel sRNA by a correlation between the prediction and the actual presence of a terminator.

High-coverage genome-wide microarrays had been used to screen for the presence of sRNAs in *Prochlorococcus* MED4 and *Synechocystis* sp. PCC 6803 (Steglich et al., 2008; Georg et al., 2009). Twelve new ncRNAs and 24 asRNAs were identified through microarray analyses in *Prochlorococcus* MED4 and then 12 ncRNAs were independently verified by experiment (Steglich et al., 2008). In *Synechocystis* sp. PCC 6803, a novel transcriptome microarray was designed as an efficient tool for the verification of the possible regulation of the newly found asRNAs and ncRNAs. This array includes probe sets for all protein-coding genes as well as for all other transcripts. Totally, 11 out of 73 asRNAs and 27 out of 60 intergenic ncRNAs had been predicted with high microarray expression levels, based on the existence of a Rho-independent terminator using this approach (Georg et al., 2009). Five ncRNAs of these have been earlier reported in a comparative genomics study (Voss et al., 2009).

RNA-Sequence Approach

Within the last few years, RNA-sequencing technology has completely changed the global identification of sRNAs in cyanobacteria and evoked a research wave in this field. As a powerful analytical tool, RNA-seq, especially differential RNA-seq (dRNA-seq), not only provides an in-depth understanding of changes in gene expression, but also the details of all promoters active at a given time, thus providing effectively an insight into the state of transcription. A dRNA-seq has established a genome-wide map of *Synechocystis* sp. PCC 6803 with 3,527 transcriptional start sites (TSSs) (Mitschke et al., 2011a). One-thirds (1,112) were found on the reverse complementary strand of 866 genes (aTSS, antisense RNA TSS), suggesting a large number of antisense transcripts. The prediction of ncRNAs came from 429 seemingly orphan TSSs (nTSS, non-coding RNA TSS) located in intergenic regions. Genome-wide maps of active TSSs have been established and identified 4,091 transcriptional units under 10 different conditions related to photosynthetic growth (Kopf et al., 2014). Besides, several sRNAs with an intriguing expression pattern have been reported. In addition, a deep RNA-seq analysis focusing on low molecular weight RNAs (≤ 200 nt) predicted 5,261 and 3,380 novel transcribed sRNA regions from the genome and the four megaplasmids in *Synechocystis* sp. PCC 6803 under normal and high light conditions. Totally, 14 of these molecules, including three from different megaplasmids, were confirmed by RNA blot hybridization (Xu et al., 2014; Hu et al., 2017).

However, there are a lot of data noise and false-positives in the biocomputational prediction, tiling microarrays, or RNA-sequence approaches. So, the subsequent verification by experimental approaches is essential for the identification of

sRNAs and their functional verification, e.g., RACE, Northern blot analysis, and mutant strains of overexpression/knockdown.

REGULATORY FUNCTIONS OF CYANOBACTERIAL sRNAs

The sRNAs are important transcriptional and post-transcriptional regulators of gene expression in every domain of life, modulating mRNA stability, DNA maintenance, DNA silencing, transcription, and translation (Waters and Storz, 2009). They achieve these diverse outcomes by multiple mechanisms, such as changes in protein binding, base pairing with other RNAs, and interactions with DNA.

sRNAs That Modulate Protein Activity

A small class of sRNA would interact with special target protein in order to function. Three classes of the well-studied sRNAs of those have the typical characteristics of protein-binding sRNA. The RNase P RNA has intrinsic activity, e.g., the RNase P RNA is required for survival in *Synechocystis* sp. PCC 6803 (Tous et al., 2001), while 4.5S RNA and tmRNA provide basic functions for a ribonucleoprotein particle. In contrast, another protein-binding sRNA (6S RNA) acts in a regulation to antagonize cognate proteins activities by mimicking other nucleic acids structures (Banta et al., 1992; Watanabe et al., 1997, 1998; Sugita et al., 2007). In *Synechocystis* sp. PCC 6803, nTSS (sRNA TSS) for 4.5S RNA (*ffs*), 6S RNA (*ssaA*), RNase P RNA (*rnpB*), and tmRNA (*ssrA*) were consistent with the published experimental data or genome annotation (Mitschke et al., 2011a).

The >100 new 6S RNA homologs were computationally identified in diverse eubacterial lineages and their conserved features suggested that they bind to RNA polymerase by mimicking the DNA template structure in an open promoter complex (Barrick et al., 2005). In 1997, 6Sa RNA was first isolated as a novel sRNA using an elaborate biochemical protocol from the unicellular cyanobacterium *Synechococcus* PCC 6301 (Watanabe et al., 1997). The accumulation of 6Sa RNA was observed in all cell stages and its level was significantly decreased in the stationary phase, which suggests that 6Sa RNA plays a role in cell division. Later, 6Sa RNA was recognized as 6S RNA homologs by GenBank (Barrick et al., 2005). Axmann et al. (2007) reported that two distinct types of 6S RNAs were accumulated and regulated during growth and the diel cycle in *Prochlorococcus* MED4. In order to control the timing of cell division and rhythmic regulation of gene expression, these 6S RNAs may switch between two different conformations after binding to *trans*-acting proteins or other unknown factors (Nair et al., 2002; Ditty et al., 2003; Axmann et al., 2007).

Ribonuclease P (RNase P) is a universal enzyme necessary for tRNA maturation. In cyanobacteria, the enzyme is composed of a protein subunit by *rnpA* and an RNA subunit by *rnpB*. The RNA subunit was the catalytic component of the enzyme and can precisely perform endonucleolytic cleavage of tRNA precursors to produce a mature 5' end in the absence of the protein subunit (Frank and Pace, 1998; Tous et al., 2001; Schon et al., 2002). In *Synechocystis*, for maturation of the dimeric precursors, it needed

to be cleaved at two positions by RNase P, corresponding to the mature 5' ends of tRNA^{Tyr} and tRNA^{Thr}, respectively (Kaneko and Tabata, 1997; Tous et al., 2001). Besides, RNase P was also involved in the 5' processing of some stable RNAs, e.g., 4.5S RNA and tmRNA (Tous et al., 2001).

The tmRNA, which has been named to reflect its tRNA-like and mRNA-like properties, was first found in *E. coli* (Ray and Apirion, 1979) and then reported in many other bacteria (Brown et al., 1990; Tyagi and Kinger, 1992; Ushida et al., 1994; Fleischmann et al., 1995; Williams and Bartel, 1996). Watanabe et al. (1998) reported the first evidence of tmRNA existence in cyanobacteria, and its coding gene was temporarily named as *ssrA*. This study suggested that tmRNA functioned not only when cells divided actively, but also when cells growth stopped by reason of a fairly high level of tmRNA throughout the cell cycle. Although it has not experimentally analyzed the *Prochlorococcus* tmRNA so far, it would consist of two separate molecules from a common precursor *in silico* analyses (Keiler et al., 2000; Gaudin et al., 2002; Axmann et al., 2005).

Cis-Encoded Base Pairing sRNAs

Different from the protein-binding sRNAs mentioned earlier, most characterized sRNAs have complete complementarity or limited complementarity with their target mRNA by base pairing to regulate gene expression (Waters and Storz, 2009). *Cis*-encoded asRNAs are transcribed from the opposite strand of the target gene and share complete complementarity with target mRNA. Most asRNAs reported are heavy regulatory elements and commonly encoded in accessory elements such as transposons and plasmids in bacteria (Wagner et al., 2002; Brantl, 2007). In cyanobacteria, all asRNAs found to date are chromosomally encoded RNAs (Table 2). *Cis*-encoded asRNAs has a great advantage in a number of cyanobacteria, for example, asRNAs amounting to 26% of all genes were reported in *Synechocystis* sp. PCC 6803 (Georg et al., 2009; Mitschke et al., 2011a) and 39% in *Anabaena* sp. PCC 7120 (Mitschke et al., 2011b). Thus, *cis*-encoded chromosomal asRNAs may have an important role in cyanobacterial regulatory networks. In cyanobacteria, *cis*-encoded asRNA represent the most investigated class of sRNA by far. However, the list of asRNAs is far from complete, especially for detailed mechanisms.

Most of *cis*-encoded chromosomal asRNAs reported so far regulate essential genes expression in response to environmental stresses, optimizing gene function and cell economy. A *cis*-encoded asRNA *IsrR* (iron stress-repressed RNA), which is the first well studied, was reported to play a role in protecting PSI complex from photo-induced damage in *Synechocystis* sp. PCC 6803 (Dühning et al., 2006). The *IsrR* RNA regulates the expression of target gene *isiA*, which forms a supercomplex around PSI under three different stress conditions: oxidative stress, high light, and iron limitation. Analysis clearly showed that *IsrR* overexpression strongly diminishes the *IsiA*-PSI supercomplexes number under iron stress, whereas *isrR* depletion leads to premature *isiA* expression. The *IsrR* appears to be expressed constitutively under moderate iron starvation to suppress *isiA* expression. In this case, *IsrR* filters out transient environmental stress signals and results in a delayed *isiA*

expression in the early phase, but it is depleted more quickly during the stress recovery (Legewie et al., 2008).

An asRNA (α -*furA* RNA) has a complete *furA* mRNA coding sequence in the nitrogen-fixing *Anabaena* sp. PCC 7120, which transcribed in the same transcriptional level as *alr1690* (Hernández et al., 2006). Disruption of the α -*furA*-*alr1690* mRNA increases the level of FurA protein and the resulting mutant shows an iron-deficient phenotype, indicating the biological correlation of this asRNA (Hernandez et al., 2010). Meanwhile, in *Microcystis aeruginosa* PCC 7806 and *Synechocystis* sp. PCC 6803, it has identified other three anti-*fur* RNAs (Martin-Luna et al., 2011; Sevilla et al., 2011), indicating that anti-*fur* RNAs are highly homologous in cyanobacteria.

The *PsbA2R* and *PsbA3R*, two asRNAs located in the 5' untranslated region (5'UTR) of target genes *psbA2* and *psbA3*, encode D1 protein of photosystem II in the thylakoid membrane of *Synechocystis* sp. PCC 6803 (Sakurai et al., 2012). The expression of *PsbA2R* and *PsbA3R* is shown to be upregulated by light and downregulated by darkness, similar to the expression of their target mRNA, and acts as a positive regulator of cell growth. These results of the *PsbA2R*(-) mutant strain showed that *PsbA2R* was a positive factor and could achieve a maximum level of D1 synthesis under some environmental conditions.

The RuBisCO catalyzes carbon fixation and is the most abundant protein in leaves, summing up to 50% of the soluble leaf protein in C3 plants and to 30% in C4 plants (Feller et al., 2008). The large chain of RuBisCO is encoded by the *rbcL* gene in cyanobacteria and land plant (Spreitzer and Salvucci, 2002). In our previous study, the 113 nt asRNA *RblR* that is completely complementary to its target gene *rbcL* was identified in *Synechocystis* sp. PCC 6803 (Hu et al., 2017). Analysis clearly showed that *RblR* has a positive regulation on *rbcL* gene expression under several stress conditions and suppressing *RblR* adversely affects carbon assimilation.

The sRNA-mRNA interactions described earlier have been described one by one. Some asRNAs have been discovered to modulate the expression of genes in operons by coordinate regulation or discoordinate regulation in cyanobacteria (Figure 1). In some cases, the activity of sRNA activates or suppresses all genes in the operon at the same time (coordinate regulation). The first reported asRNA is a *cis*-encoded chromosomal asRNA, which is complementary to the *gvpABC* operon and involved in gas vesicles formation in *Calothrix* sp. PCC 7601 (Csiszár et al., 1987). Therefore, this asRNA can form a homologous duplex with three transcripts of the *gvpABC* operon, which can damage translation and/or modify mRNA stability. Another *cis*-asRNA *As1_flv4*, derived from the *flv4-2* operon, could be transiently expressed in *Synechocystis* sp. PCC 6803 under the limitation of inorganic carbon (Ci) (Eisenhut et al., 2012). During the early phase of low carbon acclimation, *As1_flv4* plays roles in Ci-dependent regulation of *Flv4-2* proteins by establishing safety threshold and transferring expression in time. The respective asRNA of the *flv4-2* operon acts as a buffer mechanism to avoid premature operon expression under stress (Zhang et al., 2009, 2012). The *CoaR* is a negative regulator of *slr0847* (*coaD*) and *slr0848* operon, which is responsible for the synthesis of coenzyme A (CoA) and

TABLE 2 | *Cis*-encoded antisense sRNA identified in cyanobacteria.

sRNA	TSS	Length	Target gene	Function	Species	Source
IsrR	1518034	177 nt	<i>isiA</i>	The protection of the PSI complex against photo-induced damage	<i>Synechocystis</i> sp. PCC 6803	Dühring et al., 2006
α - <i>fur</i> RNA	2018583	2200 nt	α - <i>fur</i> - <i>alr1690</i>	Regulating photosynthetic machinery	Anabaena sp. PCC 7120, conserved in cyanobacteria	Hernández et al., 2006
PsbA2R, PsbA3R	7210, 1820016	130 and 220 nt, 160 and 180 nt	<i>psbA2</i> , <i>psbA3</i>	Achieving a maximum level of D1 synthesis	<i>Synechocystis</i> sp. PCC 6803	Sakurai et al., 2012
RbIR	c2478718	113 nt	<i>rbcL</i>	Regulating photosynthetic machinery	<i>Synechocystis</i> sp. PCC 6803	Hu et al., 2017
<i>gvp</i> asRNA	56 bp upstream from the start codon of the <i>gvpA</i> gene	About 420 nt	<i>gvpABC</i> operon	Involved in the formation of gas vesicles	<i>Calothrix</i> sp. PCC 7601	Csiszár et al., 1987
As1_ <i>flv4</i>	166849	500 and 280 nt	<i>flv4-2</i> operon	Photoprotection of photosystem II under low carbon conditions	<i>Synechocystis</i> sp. PCC 6803	Eisenhut et al., 2012
CoaR	2867076	124 nt	<i>slr0847</i> - <i>slr0848</i> operon	Regulating CoA synthesis and 1-butanol tolerance	<i>Synechocystis</i> sp. PCC 6803	Sun et al., 2017
PilR	758305	210 nt	<i>pilA9</i> - <i>pilA10</i> - <i>pilA11</i> - <i>slr2018</i> operon	Controlling <i>pilA11</i> gene expression and cell motility	<i>Synechocystis</i> sp. PCC 6803	Hu et al., 2018

TSS, transcription start sites; nt, nucleotides.

may regulate the tolerance to 1-butanol by downregulating the synthesis of CoA, thus reducing fatty acid metabolism and energy metabolism (Sun et al., 2017). While in other cases, sRNAs are not coupled with cistrons expression, resulting in uncoordinated regulation of the operon. Cell motility relies on the expression of putative *pilA9-pilA10-pilA11-slr2018* operon in *Synechocystis* sp. PCC 6803 (Panichkin et al., 2006). Recently, an asRNA PilR was identified in *Synechocystis* sp. PCC 6803, and it encoded in the non-coding strand of the prepilin-encoding gene *pilA11* (Hu et al., 2018). The PilR reduced the expression levels of *pilA11*, but no other gene in the *pilA9-pilA10-pilA11-slr2018* operon, while reducing both the number and thickness of pili, limiting the motility of cells.

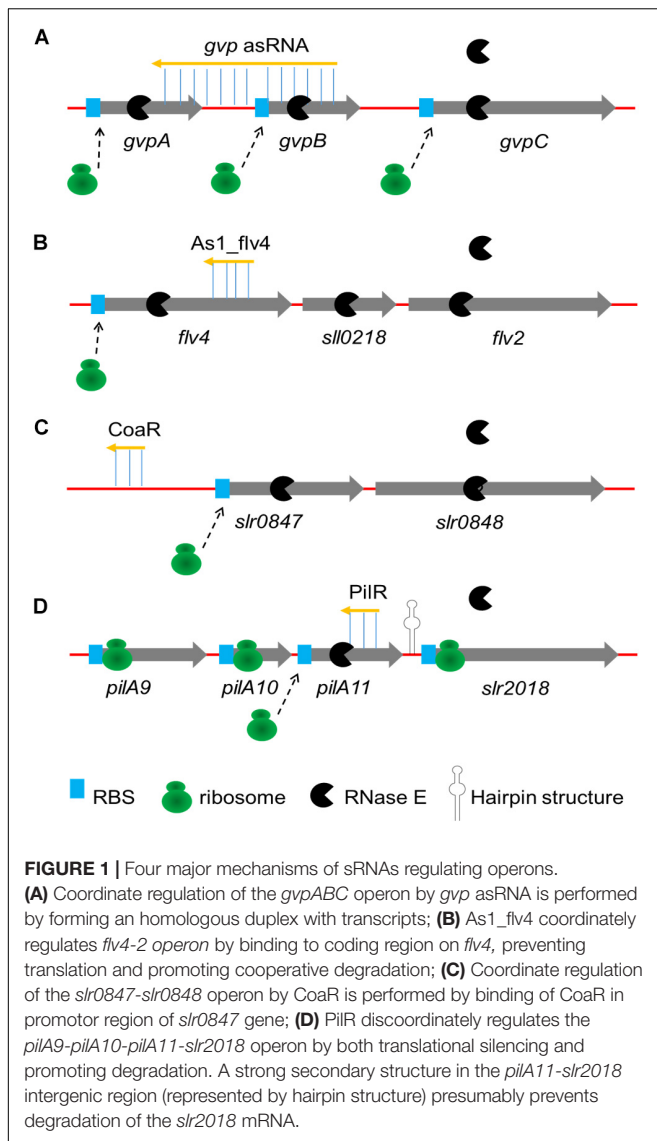
Trans-Encoded Base Pairing sRNAs

In cyanobacteria, in contrast to *cis*-encoded asRNAs, several of which have an in-depth study, another class of incomplete base-pairing sRNAs is the *trans*-encoded sRNAs (Table 3). Sequence comparison of intergenic regions has revealed several *trans*-encoded sRNAs Yfr in several different cyanobacteria of the *Prochlorococcus-Synechococcus* lineage (Axmann et al., 2005). Of these, the Yfr2 sRNAs family (Yfr2-5) is a group of abundant RNA molecules and widely present in nearly all cyanobacteria sequenced to date (Gierga et al., 2009).

For *trans*-encoded sRNAs, the chromosomal location of the sRNA gene has little correlation with the target mRNA gene. The *yfr1* gene, a high conserved gene found in various

cyanobacterial strains, is 65 nucleotides long and positioned between *guaB* and *trxA* in *Synechococcus elongatus* PCC 6301 (genome position: 2,417,299–2,417,363). However, the target *sbtA* transcript of Yfr1 has a distant position (genome position: 2,623,057–2,624,178) (Nakamura et al., 2007). Furthermore, the *yfr1*-disruption mutant, showed growth defects under various stress conditions.

In contrast to the *cis*-encoded asRNAs, the *trans*-encoded sRNAs modulate more than one target by formation of partial RNA-RNA duplexes. These sRNAs control the stability and translation of target mRNAs and have similar functions to eukaryotic miRNAs in many respects (Gottesman, 2005; Aiba, 2007). By computational and experimental data, sRNA PsrR1 has interaction with the ribosome-binding regions of the *psaL*, *psaJ*, *chlN*, and *cpcA* mRNA in *Synechocystis* sp. PCC 6803 (Georg et al., 2014). As a regulatory factor for photosynthetic functions, sRNA PsrR1 and the transcriptional regulator RpaB constituted a feed-forward loop to regulate PSI gene expression to achieve a rapid adaptive response to the high light stress condition (Kadowaki et al., 2016). Oxygenic photosynthesis depends to a large extent on proteins that contain Fe²⁺ or Fe/S complexes as cofactors or prosthetic groups (Keren et al., 2004). Georg et al. showed that sRNA IsaR1 was induced by iron starvation and then acted on photosynthetic apparatus in three specific ways. The IsaR1 had more than 15 direct targets and controlled a complex network to acclimate to low iron condition (Georg et al., 2017). A *trans*-encoded sRNA Nc117 was discovered involved in the tolerance



of *Synechocystis* sp. PCC 6803 to ethanol and 1-butanol (Pei et al., 2017). A total of 119 upregulated and 116 downregulated genes were identified by comparative transcriptomic analysis, among which a gene *slr0007* encoding D-glycero- α -D-mannoheptose 1-phosphate guanylyltransferase was determined to be a potential target of Nc117. These results suggested that the synthesis of lipopolysaccharide or S-layer glycoprotein may be related to the increased tolerance (Bi et al., 2018).

Hfq Protein of RNA Chaperone

In *E. coli* and closely related bacteria, the RNA chaperone Hfq usually participates in *trans*-encoded sRNA-mediated regulation, presumably to facilitate sRNA-mRNA duplex formation (Valentin-Hansen et al., 2004; Aiba, 2007; Brennan and Link, 2007). Several studies have found that hexameric Hfq ring was homologous to Sm and Sm-like proteins involved in mRNA decay and splicing in eukaryotes and archaea (Valentin-Hansen et al., 2004; Nielsen et al., 2007). In cyanobacteria,

preliminary attempts to explore microbial genomes of Hfq homologs failed (Sun et al., 2002). However, combined with motif and pattern sequence searches, Hfq orthologs from various cyanobacteria were identified (Valentin-Hansen et al., 2004). The cyanobacterial Hfq homolog is proved to be essential for motility of *Synechocystis* sp. PCC 6803 through the knockout of *hfq* (Dienst et al., 2008). The crystal structures of Hfq-homolog proteins retained the classic Sm folding despite low-sequence conservation in *Synechocystis* sp. PCC 6803 and *Anabaena* sp. PCC 7120 (Bøggild et al., 2009), suggesting the higher conservation of Hfq proteins in bacteria than initially anticipated (Table 4).

On the contrary, Hfq and homologue proteins are not ubiquitous in all of cyanobacteria. The *hfq* has been lost in several sequenced *Prochlorococcus* strains, e.g., *Prochlorococcus* MED4 (Axmann et al., 2005; Steglich et al., 2008). The naturally *hfq*-deficient *Prochlorococcus* MED4 expresses 24 different sRNAs (Axmann et al., 2005; Steglich et al., 2008). The phenomenon suggests that a class of sRNAs functions that do not require partner support of a chaperone or have a novel chaperone are yet to be identified.

PHYSIOLOGICAL ROLES AND FUNCTIONS OF sRNA

Although there has not been much research done, many sRNAs studied possessed important physiological roles and are related to environmental stresses, photosynthesis, CO₂ fixation, and metabolism in the cyanobacteria (Table 5). Especially, *trans*-coded sRNA and some *cis*-encoded asRNA respond generally to adverse environmental conditions by regulating metabolic pathways or alarm reaction. The asRNAs largely function against various environmental insults by the synthesis of target proteins, e.g., the target protein levels of IsiA (Dürring et al., 2006), Fur (Martin-Luna et al., 2011), PsbA2 and PsbA3 (Sakurai et al., 2012), RbcL (Hu et al., 2017), CoaD (Sun et al., 2017), and Flv4-2 (Eisenhut et al., 2012) are influenced by corresponding asRNA in iron-, light-, oxidative-, 1-butanol-stress, and carbon-limited conditions. Moreover, some sRNAs are required for growth under multiple stress conditions, e.g., the *Synechococcus elongatus* Yfr1, which has homologs in other cyanobacteria (Nakamura et al., 2007; Voss et al., 2007). The 14 new sRNA and 24 asRNA had been identified from intergenic regions of *Prochlorococcus* MED4 under various different stress conditions (Steglich et al., 2008). Some of these sRNAs expression profiles suggested involvement in bacteriophage infection response and/or light stress adaptation.

Cyanobacteria are usually obedient organisms for genetic manipulation, but the lack of powerful metabolic engineering tools has seriously restricted the attempts to implement more complex genetic regulation (Huang et al., 2010). In recent years, much attention has been paid to genetic tools based on artificial sRNAs as a result of their obvious superiority (Zess et al., 2016; Sun et al., 2018). Firstly, sRNA regulatory tools mostly do not impose any metabolic burden on host cells (Gaida et al., 2013). Secondly, the tunable base-pair

TABLE 3 | *Trans*-encoded sRNAs identified in cyanobacteria.

sRNA	TSS	Length	target gene	Function	Species	Source
Yfr1	2417299	65 nt	<i>sbtA</i>	Maintenance of normal growth under various stress conditions	<i>Synechococcus elongatus</i> PCC 6301	Nakamura et al., 2007
SyR1/PsrR1	1671919	131 nt	<i>psaL</i> , <i>psaJ</i> , <i>chlN</i> , and <i>cpcA</i>	Controlling photosynthetic functions	<i>Synechocystis</i> sp. PCC 6803, conserved in cyanobacteria	Georg et al., 2014
IsaR1	3164320	68 nt	More than 15 direct targets, including Fe ²⁺ -containing proteins involved in photosynthetic electron transfer, detoxification of anion radicals, citrate cycle, and tetrapyrrole biogenesis	Acclimating photosynthetic apparatus to iron starvation	<i>Synechocystis</i> sp. PCC 6803, conserved in cyanobacteria	Georg et al., 2017
Nc117	3250530	102 nt	<i>slr0007</i>	Positively regulating biofuels tolerance	<i>Synechocystis</i> sp. PCC 6803	Pei et al., 2017; Bi et al., 2018

TSS, transcription start sites; nt, nucleotides.

TABLE 4 | Hfq protein of RNA chaperone in cyanobacteria.

RNA chaperone	Characteristic	Species	Source
Hfq	Essential for cell motility	<i>Synechocystis</i> sp. PCC 6803	Dienst et al., 2008
Hfq	Retaining the classic Sm fold	<i>Synechocystis</i> sp. PCC 6803, <i>Anabaena</i> sp. PCC 7120	Bøggild et al., 2009

TABLE 5 | Physiological roles and functions of sRNAs in cyanobacteria.

sRNA	Target gene	Physiological roles	Species	Source
IsrR	<i>isiA</i>	Response to iron, light, and oxidative stress	<i>Synechocystis</i> sp. PCC 6803	Dühring et al., 2006
α - <i>fur</i> RNA	<i>α-fur-<i>alr1690</i></i>	Response to light and oxidative stress	<i>Microcystis aeruginosa</i> PCC 7806	Martin-Luna et al., 2011
PsbA2R and PsbA3R	<i>psbA2</i> and <i>psbA3</i>	Response to light stress	<i>Synechocystis</i> sp. PCC 6803	Sakurai et al., 2012
RblR	<i>rbcL</i>	Response to light and carbon-limited stress	<i>Synechocystis</i> sp. PCC 6803	Hu et al., 2017
CoaR	<i>coaD-slr0848</i> operon	Response to 1-butanol stress	<i>Synechocystis</i> sp. PCC 6803	Sun et al., 2017
As1_ <i>flv4</i>	<i>flv4-2</i> operon	Response to carbon-limited stress	<i>Synechocystis</i> sp. PCC 6803	Eisenhut et al., 2012
Yfr1	<i>sbtA</i>	Response to various stress, e.g., oxidative, high salt, Fe ²⁺ limitation, and calcium limitation stress	<i>Prochlorococcus/Synechococcus</i> , conserved in cyanobacteria	Nakamura et al., 2007; Voss et al., 2007
14 New ncRNAs		Response to light qualities and quantities, phage, and phosphorus starvation stress	<i>Prochlorococcus</i> MED4	Steglich et al., 2008

complementation sRNAs permit whole genome regulation of target genes for fine flux control (Rodrigo et al., 2012; Na et al., 2013). The last but not the least, sRNA regulatory tools could effectively knock the essential genes from the host cell, in contrast to the traditional lethal knockout method of these

genes (Nakashima and Tamura, 2009). On the basis of interaction between sRNAs and their target mRNAs, some physiological and metabolic pathways of cyanobacteria can be modulated and altered by artificial sRNA tools. A *trans*-encoded sRNA system was constructed and adapted in *Synechococcus* sp. PCC 7002

(Zess et al., 2016). This system exhibited 70% depression of target gene expression, demonstrating sRNA regulatory tools for differential gene expression in cyanobacteria. Moreover, two sRNA regulatory tools were reported in *Synechocystis* sp. PCC 6803, which manipulated basic metabolic pathways and redirected carbon flux, based on a exogenous Hfq chaperone and a well-studied MicC scaffold previously developed in *E. coli* (Sun et al., 2018). For regulating multiple genes simultaneously and modifying into an inducible system, the Hfq-MicC tool was developed based on the theophylline-induced riboswitch. The sRNA regulatory tools introduce efficient and valuable metabolic and physiological regulatory strategies for cyanobacteria and, therefore, have showed promising applications.

PROSPECT

In recent years, approaches for sRNAs detecting and targeting have become a standard method used to search for several types of sRNA genes within bacterial genomes. Meanwhile, many target prediction tools and related software of bacteria have been developed. The sRNA regulatory systems are identified as efficient and valuable physiological regulatory strategies in cyanobacteria. Therefore, cyanobacterial sRNAs are well worth exploring as a strong and facile regulatory factor in future.

At present, RNA molecules hold many types of regulatory functions in bacteria and control almost all aspects of cell metabolism. A large number of sRNAs are intriguing as they may represent a mode of adaption under various environmental conditions. Regulation by sRNAs may require fewer resources than protein regulators synthesis (Shimoni et al., 2007; Mehta et al., 2008). Furthermore, there might be positive selection

pressure to maintain small regulators rather than large protein regulators during genome reduction. Outstanding progress has been made in the identification and characterization of cyanobacterial sRNAs, indicating clearly that RNA regulators are ubiquitous and often conservative, possibly exceeding the number and diversity of protein regulators. However, many exciting questions about mechanism and function of some sRNAs still remain. The current focus is on developing tools to correctly predict sRNA targets. Several target prediction tools have been developed, but there are still considerable developments to be made. The identification of sRNA targets would allow the unraveling of pathways that involve sRNA-induced regulation. It would also be a further important step for the functional characterization of the many found sRNAs.

AUTHOR CONTRIBUTIONS

JH and QW gathered the information and wrote and edited the manuscript. QW revised and approved the work.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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