



Editorial: Small Non-coding RNAs in *Streptococci*

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The Editorial on the Research Topic

Small Non-coding RNAs in *Streptococci*

Bacterial small RNAs (sRNAs) are post-transcriptional regulators of gene expression and the mechanisms by which this can occur have begun to be understood (Gottesman and Storz, 2011). In pathogenic bacteria, the importance of sRNAs-mediated regulation depends on a fine-tuning of the expression of various virulence genes (Papenfert and Vogel, 2010). For instance, RNAIII, a regulatory RNA from the pathogenic bacterium *Staphylococcus aureus*, controls the expression of different virulence factors such as *rot* (repressor of toxins), *spa* (surface protein A), *sa1000* (fibrinogen binding protein), or *coa* (coagulase) (Boisset et al., 2007). Considering the functional roles of sRNAs, they are categorized in two major classes: (i) cis-acting elements located/acting on untranslated regions (UTRs) of a translated mRNA which controls the expression of their enclosed gene(s) through the modulation of their secondary structures or stability; and (ii) the trans-acting sRNAs that comprise the cis-encoded sRNAs, known as antisense RNAs (asRNAs), and the trans-encoded sRNAs which generally present imperfect base-pairing with the mRNA targets (Zorgani et al., 2016).

The goal of this special research topic is to bring together research reviews and original articles on sRNAs, their identification and the characterization of their mode of action in *Streptococci*. Streptococcal species can colonize and invade humans and animals. They mostly exhibit an asymptomatic interaction within their hosts. However, several of them, such as *Streptococcus pyogenes* and *Streptococcus pneumoniae*, are well-known pathogenic species responsible for severe and life-threatening infections in humans (Lamagni et al., 2009). The development of biocomputational and high throughput screening methods, like next generation sequencing, led to the discovery of hundreds of sRNAs encoded by the bacterial genome, which represent 3–5% of total number of annotated genes regardless of the studied species (Siezen et al., 2010). Currently, the main challenges in the study of these molecules in *Streptococci* are their identification, as well as the identification of their potential targets. Three review papers published in this research topic reported that a large number of putative regulatory sRNAs were recently identified in different streptococcal species (Cho and Kim; Patenge et al.; Wilton et al.).

Most of these studies focus on three pathogenic species: *S. pyogenes* and *S. pneumoniae* and the opportunistic pathogen involved in neonatal infections, *Streptococcus agalactiae*. At present, 75 sRNAs have been identified in *S. pyogenes* (Perez et al., 2009; Patenge et al., 2012; Tesorero et al., 2013) and 179 sRNAs in *S. pneumoniae* (Kumar et al., 2010; Tsui et al., 2010; Acebo et al., 2012; Mann et al., 2012). In *S. agalactiae*, 197 sRNAs were predicted *in silico* and 10 of them were validated by northern blot (Pichon et al., 2012). More recently, 125 sRNAs were identified by dRNA-seq (differential RNA-sequencing) in the strain *S. agalactiae* NEM316 (Rosinski-Chupin et al., 2015).

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Although, several sRNAs have been identified to date in *Streptococci*, very few are characterized. For instance, in *S. pyogenes*, only three sRNAs were characterized: (i) FasX, which binds to the 5' end of *ska* mRNA and activates its translation by avoiding degradation with RNase J1 (Ramirez-Peña et al., 2010); (ii) RivX, which affects the expression of *emm*, *scpA*, *mga*, and *speA* mRNAs (Roberts and Scott, 2007); and (iii) Pel, a pleiotropic sRNA which affects the expression of different virulence factors (*emm*, *sic*, and *speB*) and encodes at the same time an hemolysin (Mangold et al., 2004).

In *S. pneumoniae*, a combination of biocomputational approaches, transcriptomic analysis and RNA-seq were used to identify sRNAs encoded by *S. pneumoniae*. While an *in silico* study identified 128 sRNAs located in intergenic regions (Livny et al., 2008), 50 sRNAs were identified by microarray analysis (Kumar et al., 2010), and 88 sRNAs by RNA-seq (Acebo et al., 2012). A recent study published in our special research topic demonstrated that csRNAs (*cia*-dependent small RNAs) are involved in competence control in *S. pneumoniae* R6 (Laux et al.). The csRNAs are non-coding *trans*-acting sRNAs transcribed from the promoter associated to CiaRH two-component system. They generally present a complementarity to the Shine-Dalgarno sequence and the translation initiation codon (AUG). The authors showed, by using reporter gene fusions, that deletion of a single or two csRNAs was not sufficient to impair competence gene expression. In contrast, combination of three csRNAs, or mutations in the competence gene *comC* and its complemented csRNAs, completely blocked *S. pneumoniae* competence. This study demonstrated that csRNAs are important players in competence regulation for *S. pneumoniae*.

Two original research articles focused on sRNAs in *S. agalactiae* were included in the present topic. The first study is about RNAII, an asRNA involved in replication control

and encoded by pMV158 plasmid (López-Aguilar et al.). One of the main findings is that RNAII do not requires the formation of a kissing complex for efficient binding to its mRNA target or for inhibition of *repB* expression. The *repB* gene encodes the replication initiator protein of the pMV158 plasmid. However, site-directed mutagenesis on the 5' and 3' regions on the 5'-tail of RNAII, revealed that the entire 5'-tail on the asRNA is mandatory for efficient *repB* translation *in vitro* and pMV158 replication *in vivo*.

The second study described the distribution of the type II-A CRISPR-Cas system among the different *S. agalactiae* genetic lineages (Lier et al.). CRISPR RNAs (Clustered Regularly Interspaced Short Palindromic Repeats RNAs) are sRNAs that target invading cognate nucleic acids (Chylinski et al., 2013). Beside the ubiquitous presence and the high sequence polymorphism of CRISPR1 spacers, one of the main findings of this study is that strains belonging to the "highly virulent" sequence type 17 clone showed a lower number of spacers. This result suggests that sequencing of the CRISPR1 locus can be used as an alternative tool for phylogenetic studies.

The sRNAs widespread in the bacterial kingdom, and particularly in *Streptococci*, highlights their importance in regard to regulatory networks and pathogenesis and open new perspectives for future therapeutic and industrial applications. This special research topic was the opportunity to review the different sRNAs categories in *Streptococci* and to emphasize their role in gene regulation, physiology, and virulence.

AUTHOR CONTRIBUTIONS

MZ: Wrote the manuscript; ML, EC, and RQ: Participated in manuscript correction.

REFERENCES

- Acebo, P., Martín-Galiano, A. J., Navarro, S., Zaballos, A., and Amblar, M. (2012). Identification of 88 regulatory small RNAs in the TIGR4 strain of the human pathogen *Streptococcus pneumoniae*. *RNA* 18, 530–546. doi: 10.1261/rna.027359.111
- Boisset, S., Geissmann, T., Huntzinger, E., Fechter, P., Bendridi, N., Possedko, M., et al. (2007). *Staphylococcus aureus* RNAIII coordinately represses the synthesis of virulence factors and the transcription regulator Rot by an antisense mechanism. *Genes Dev.* 21, 1353–1366. doi: 10.1101/gad.423507
- Chylinski, K., Le Rhun, A., and Charpentier, E. (2013). The tracrRNA and Cas9 families of type II CRISPR-Cas immunity systems. *RNA Biol.* 10, 726–737. doi: 10.4161/rna.24321
- Gottesman, S., and Storz, G. (2011). Bacterial small RNA regulators: versatile roles and rapidly evolving variations. *Cold Spring Harb. Perspect. Biol.* 3:a003798. doi: 10.1101/cshperspect.a003798
- Kumar, R., Shah, P., Swiatlo, E., Burgess, S. C., Lawrence, M. L., and Nanduri, B. (2010). Identification of novel non-coding small RNAs from *Streptococcus pneumoniae* TIGR4 using high-resolution genome tiling arrays. *BMC Genomics* 11:350. doi: 10.1186/1471-2164-11-350
- Lamagni, T. L., Neal, S., Keshishian, C., Powell, D., Potz, N., Pebody, R., et al. (2009). Predictors of death after severe *Streptococcus pyogenes* infection. *Emerg. Infect. Dis.* 15, 1304–1307. doi: 10.3201/eid1508.090264
- Livny, J., Teonadi, H., Livny, M., and Waldor, M. K. (2008). High-throughput, kingdom-wide prediction and annotation of bacterial non-coding RNAs. *PLoS ONE* 3:e3197. doi: 10.1371/journal.pone.0003197
- Mangold, M., Siller, M., Roppenser, B., Vlamincx, B. J., Penfound, T. A., Klein, R., et al. (2004). Synthesis of group A streptococcal virulence factors is controlled by a regulatory RNA molecule. *Mol. Microbiol.* 53, 1515–1527. doi: 10.1111/j.1365-2958.2004.04222.x
- Mann, B., van Opijnen, T., Wang, J., Obert, C., Wang, Y. D., Carter, R., et al. (2012). Control of virulence by small RNAs in *Streptococcus pneumoniae*. *PLoS Pathog.* 8:e1002788. doi: 10.1371/journal.ppat.1002788
- Papenfert, K., and Vogel, J. (2010). Regulatory RNA in bacterial pathogens. *Cell Host Microbe* 8, 116–127. doi: 10.1016/j.chom.2010.06.008
- Patenge, N., Billion, A., Raasch, P., Normann, J., Wisniewska-Kucper, A., Retej, J., et al. (2012). Identification of novel growth phase- and media-dependent small non-coding RNAs in *Streptococcus pyogenes* M49 using intergenic tiling arrays. *BMC Genomics* 13:550. doi: 10.1186/1471-2164-13-550
- Perez, N., Treviño, J., Liu, Z., Ho, S. C., Babbitzke, P., and Sumbly, P. (2009). A genome-wide analysis of small regulatory RNAs in the human pathogen group A *Streptococcus*. *PLoS ONE* 4:e7668. doi: 10.1371/journal.pone.0007668
- Pichon, C., du Merle, L., Caliot, M. E., Trieu-Cuot, P., and Le Bouguéneq, C. (2012). An *in silico* model for identification of small RNAs in whole bacterial genomes: characterization of antisense RNAs in pathogenic *Escherichia coli* and *Streptococcus agalactiae* strains. *Nucleic Acids Res.* 40, 2846–2861. doi: 10.1093/nar/gkr1141
- Ramirez-Peña, E., Treviño, J., Liu, Z., Perez, N., and Sumbly, P. (2010). The group A *Streptococcus* small regulatory RNA FasX enhances streptokinase activity by increasing the stability of the *ska* mRNA transcript. *Mol. Microbiol.* 78, 1332–1347. doi: 10.1111/j.1365-2958.2010.07427.x

- Roberts, S. A., and Scott, J. R. (2007). RivR and the small RNA RivX: the missing links between the CovR regulatory cascade and the Mga regulon. *Mol. Microbiol.* 66, 1506–1522. doi: 10.1111/j.1365-2958.2007.06015.x
- Rosinski-Chupin, I., Sauvage, E., Sismeiro, O., Villain, A., Da Cunha, V., Caliot, M. E., et al. (2015). Single nucleotide resolution RNA-seq uncovers new regulatory mechanisms in the opportunistic pathogen *Streptococcus agalactiae*. *BMC Genomics* 16:419. doi: 10.1186/s12864-015-1583-4
- Siezen, R. J., Wilson, G., and Todt, T. (2010). Prokaryotic whole-transcriptome analysis: deep sequencing and tiling arrays. *Microb. Biotechnol.* 3, 125–130. doi: 10.1111/j.1751-7915.2010.00166.x
- Tesorero, R. A., Yu, N., Wright, J. O., Svencionis, J. P., Cheng, Q., Kim, J. H., et al. (2013). Novel regulatory small RNAs in *Streptococcus pyogenes*. *PLoS ONE* 8:e64021. doi: 10.1371/journal.pone.0064021
- Tsui, H. C., Mukherjee, D., Ray, V. A., Sham, L. T., Feig, A. L., and Winkler, M. E. (2010). Identification and characterization of noncoding small RNAs in *Streptococcus pneumoniae* serotype 2 strain D39. *J. Bacteriol.* 192, 264–279. doi: 10.1128/JB.01204-09
- Zorgani, M. A., Quentin, R., and Lartigue, M. F. (2016). Regulatory RNAs in the less studied streptococcal species: from nomenclature to identification. *Front. Microbiol.* 7:1161. doi: 10.3389/fmicb.2016.01161

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