Cell PRESS
Cell Host & Microbe
Previews

A Bacterial Pathogen Flips the Riboswitch

Bobbi Xayarath[1] and Nancy E. Freitag[1,*]
[1]Department of Microbiology and Immunology, University of Illinois at Chicago, Chicago, IL 60612-7344, USA
*Correspondence: nfreitag@uic.edu
DOI 10.1016/j.chom.2009.10.009

Riboswitches are RNA structures traditionally viewed as acting in *cis* to regulate downstream gene expression in bacteria. In a recent issue of *Cell*, Loh and colleagues report on the ability of a riboswitch to act in *trans* to modulate the expression of a critical bacterial virulence regulator.

Bacteria are masters of adaptability, responding to environmental changes with a flexibility that can put eukaryotic organisms to shame. Bacterial survival in disparate environments is dependent upon changes in gene expression that are responsive to fluctuations in available nutrients or environmental stresses. While attention has traditionally focused on the ability of proteins to act as signaling molecules responsive to environmental stimuli, there is growing recognition of the importance of RNA molecules as mediators of signal transduction and gene regulation (Winkler and Breaker, 2005). RNA can be produced rapidly, and molecules do not require the extra energetic step of translation for functionality (Henkin, 2008). A number of RNA-based regulatory mechanisms have been described, including the existence of mRNA leader sequences or riboswitches that regulate the expression of genes immediately downstream, as well as small RNAs (sRNA) that bind proteins or base pair with target RNA molecules and can act either in *cis* or in *trans* (Waters and Storz, 2009). Most of these regulatory RNA molecules appear to regulate gene expression by influencing mRNA transcription and/or transcript stability, or by altering the efficiency of translational initiation.

Riboswitches have been viewed as one of the simplest forms of RNA regulatory elements (Waters and Storz, 2009). Studies have shown them to be *cis*-encoded and *cis*-acting, being located within the 5′ untranslated region (5′UTR) of the mRNA molecules they regulate. The limitation of acting in *cis* has been used as a means of differentiating riboswitches from sRNAs, which often act in *trans* by base pairing with specific target RNAs or through protein binding. The long leader sequences that constitute the mRNA riboswitches are capable of assuming structures that respond directly to temperature changes or physiological cues. These structures often directly bind metabolites as a means of sensing available cell nutrients (Winkler and Breaker, 2005). Once a physiological signal has been detected, a conformational shift occurs in the RNA secondary structure that serves to influence to expression of the downstream genes.

The environmental bacterium *Listeria monocytogenes* has often served as a model system for the study of fundamental cellular processes, and it is now serving as a vehicle for understanding the regulation of gene expression by RNA. *L. monocytogenes* is a ubiquitous organism that survives primarily as a saprophyte in soils but is capable of transitioning into a pathogen upon ingestion by a susceptible human host (Freitag et al., 2009). The ability of *L. monocytogenes* to cause disease is directly related to the transcriptional induction of a number of gene products required for host cell invasion, cytosolic bacterial replication, and spread to adjacent cells (Scortti et al., 2007). The majority of *L. monocytogenes* gene products thus far associated with bacterial virulence are regulated by a transcriptional activator known as PrfA (or *p*ositive *r*egulatory *f*actor *A*) (Freitag et al., 2009; Scortti et al., 2007). The expression of PrfA-dependent virulence factors is temperature regulated, such that increased levels of gene expression occur at 37°C (host temperature) versus temperatures of 30°C or below. The first identification of a thermosensor riboswitch in *L. monocytogenes* emerged from the work of Johansson et al. (2002), who demonstrated that transcripts directed by the *prfA* P1 promoter form hairpin loops that mask the ribosome binding site at temperatures equal to or less than 30°C but not at 37°C, resulting in the temperature-dependent translation of *prfA* mRNA.

Increasing evidence supports the premise of critical roles played by regulatory RNAs in the modulation of *L. monocytogenes* gene expression. Long UTRs with extensive secondary structure are present in the mRNAs of multiple virulence genes (Shen and Higgins, 2005; Toledo-Arana et al., 2009; Wong et al., 2004). The recent publication of a complete *L. monocytogenes* bacterial transcriptome by Toledo-Arana et al. (2009) identified 29 previously unknown sRNAs and 40 putative riboswitches that appeared to be differentially regulated as *L. monocytogenes* transitioned into different environments. In a recent issue of *Cell*, Loh et al. (2009) reveal a new role for a *cis*-acting riboswitch as a *trans*-acting regulatory RNA that interacts with the 5′UTR of *L. monocytogenes prfA*, the master regulator of virulence gene expression.

Loh et al. (2009) identified seven putative S-adenosylmethionine (SAM)-responsive riboswitches (SAM riboswitches), which they termed Sre for *S*AM-*r*iboswitch *e*lement. The typical small molecule-binding riboswitch consists of two separate elements: a ligand-binding (or aptamer) domain and a gene expression (or platform) domain, both of which are located immediately upstream of the translational start of the gene subject to riboswitch control (Henkin, 2008). The aptamer domain of SAM riboswitches binds SAM in a concentration-dependent manner, whereas the expression platform forms a secondary structure that functions as a transcription antiterminator in the absence of SAM and folds to terminate transcription in the presence of SAM. SAM riboswitches have been described in numerous bacteria, and although bacteria can contain more than one SAM riboswitch, the affinity of the different

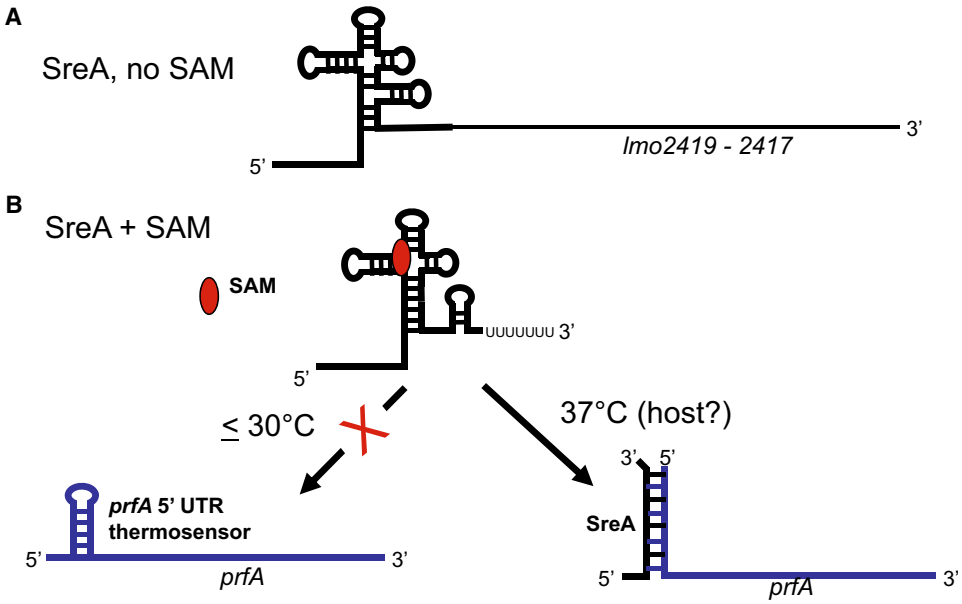

Figure 1. Model of SreA *cis* and *trans* Regulation
(A) The SreA riboswitch is located immediately upstream of three genes, *lmo2491-2418-2417*. In the absence of the metabolite SAM, SreA forms an antitermination structure that allows transcription of the downstream genes.
(B) Binding of SAM to SreA alters its formation and a terminator structure is formed, resulting in the production of a short transcript lacking the downstream genes. This small RNA is capable of binding the *prfA* mRNA at high temperature (37°C) leading to a reduction in PrfA protein synthesis. At low temperatures (≤30°C), the thermosensor element located in the 5′UTR of *prfA* mRNA prevents both SreA binding and *prfA* mRNA translation.

riboswitches for SAM varies (Henkin, 2008). Each of the seven Sres (SreA–SreG) identified by Loh et al. (2009) were found to be located upstream of genes encoding proteins predicted to function in methionine or cysteine metabolism and/or transport. Although riboswitches have been defined as acting strictly in *cis*, the authors wished to explore the possibility that the small RNA produced as a result of riboswitch-induced transcript termination (Figure 1) might itself have the potential to function in *trans* on distally encoded mRNAs.

The authors began by constructing a deletion mutation in the largest and most highly expressed SAM riboswitch, SreA. Tiling array-based transcriptome analysis was used to compare the Δ*sreA* mutant to wild-type *L. monocytogenes* grown in rich media. In the absence of *sreA,* three genes showed a significant increase in expression representing negative regulation by SreA, while six genes had significant reductions in expression levels representing positive regulation by SreA. A representative gene subject to negative SreA regulation (*lmo2230*, encoding an arsenate reductase) and one gene positively regulated by SreA (*lmo0049*, encoding AgrD, a homolog of a *Staphylococcus aureus* autoinducing peptide) were selected for further analysis.

The potential for a direct role of SreA in regulating the expression of *lmo2230* and *lmo0049* was investigated. It was determined that plasmid-based expression of the small SreA riboswitch transcript in *trans* in the Δ*sreA* mutant could restore the transcript levels of *lmo2230* and *lmo0049* to those observed for wild-type bacteria, indicating that SreA could influence the expression of genes other than those located immediately downstream. Interestingly, the regulation of *lmo2230* and *lmo0049* expression by SreA appeared to be independent of SAM binding.

An intriguing connection was then established between SreA and the *L. monocytogenes* master virulence regulator PrfA. Transcriptome analyses previously indicated that the expression of *lmo2230* was PrfA-regulated and a comparison of PrfA protein levels in wild-type and Δ*sreA* strains indicated a 2-fold increase in the amount of PrfA in strains lacking SreA, and a 3-fold increase in strains lacking both SreA and SreB. The increase in PrfA protein synthesis was reflected at the transcript level as measured by northern blot analysis and quantitative RT-PCR (although this change was not evident in the microarray data). The modest increase in PrfA levels correlated with a corresponding increase in the expression of listeriolysin O (LLO), a PrfA-dependent gene product required for bacterial virulence. Loh and colleagues found yet another link between *prfA* and *sreA*/*sreB* when they identified a putative PrfA binding site upstream of *sreA* as well as *sreB;* the expression of both genes was found to be reduced in a Δ*prfA* mutant (Loh et al., 2009). A clue to the mechanism of SreA regulation of PrfA protein production came when the authors noticed a sizeable stretch of complementary nucleic acid sequence existing between a defined region of *sreA* and the distal end of the *prfA* 5′UTR, suggesting the possibility of a direct interaction between *prfA* transcripts and SreA. Subsequent experiments demonstrated that *prfA* expression was reduced by the presence of *sreA* both in an ectopic *Escherichia coli* expression system and in in vitro transcription/translation assays. Direct binding of SreA to the *prfA* 5′UTR was visualized using an electromobility shift assay with SreA binding dependent on *prfA* 5′UTR sequence complementarity. As observed for *lmo2230* and *lmo0049*,

SreA regulation of *prfA* was independent of SAM binding.

The data presented by Loh and colleagues presents the first example of a *trans* acting SAM riboswitch based on the ability of SreA to bind the 5′UTR of *prfA* and influence protein expression (Loh et al., 2009). Interestingly, SreA regulation was only evident at 37°C, suggesting that the *prfA* mRNA thermosensor represents the predominant form of regulation at low temperatures, with SreA capable of functioning at temperatures that are relevant to bacterial infection of mammalian hosts (Figure 1). SreA binding to the 5′UTR of *prfA* was postulated to result in a reduction in *prfA* mRNA translation, although the mechanism behind this reduction remains to be determined. As PrfA is also capable of inducing the expression of *sreA*, there exist additional layers of complexity within the SreA-PrfA regulatory loop. The full impact of the interaction between SreA and the *prfA* 5′UTR on bacterial virulence remains to be determined, but meanwhile SreA regulation would appear to represent yet another mechanism used by *L. monocytogenes* to carefully modulate PrfA activity (in addition to the previously established mechanisms of transcriptional and posttranslation regulation). The potential link forged between nutrient availability and bacterial pathogenesis deserves further investigation, especially given the surprising lack of a requirement for SAM binding by SreA. Clearly, much remains to be explored with regards to the regulation of gene expression by RNA molecules in *L. monocytogenes*. Fortunately, the relative ease of genetic manipulation of this organism combined with the existence of its complete transcriptome has set the stage for new intriguing discoveries.

REFERENCES

Freitag, N.E., Port, G.C., and Miner, M.D. (2009). Nat. Rev. Microbiol. *7*, 623–628.

Henkin, T.M. (2008). Genes Dev. *22*, 3383–3390.

Johansson, J., Mandin, P., Renzoni, A., Chiaruttini, C., Springer, M., and Cossart, P. (2002). Cell *110*, 551–561.

Loh, E., Dussurget, O., Gripenland, J., Vaitkevicius, K., Tiensuu, T., Mandin, P., Repoila, F., Carmen Buchrieser, C., Cossart, P., and Johansson, J. (2009). Cell, in press.

Scortti, M., Monzo, H.J., Lacharme-Lora, L., Lewis, D.A., and Vazquez-Boland, J.A. (2007). Microbes Infect. *9*, 1196–1207.

Shen, A., and Higgins, D.E. (2005). Mol. Microbiol. *57*, 1460–1473.

Toledo-Arana, A., Dussurget, O., Nikitas, G., Sesto, N., Guet-Revillet, H., Balestrino, D., Loh, E., Gripenland, J., Tiensuu, T., Vaitkevicius, K., et al. (2009). Nature *459*, 950–956.

Waters, L.S., and Storz, G. (2009). Cell *136*, 615–628.

Winkler, W.C., and Breaker, R.R. (2005). Annu. Rev. Microbiol. *59*, 487–517.

Wong, K.K., Bouwer, H.G., and Freitag, N.E. (2004). Cell. Microbiol. *6*, 155–166.