Physiological roles of small RNA molecules

Charlotte Michaux, Nicolas Verneuil, Axel Hartke and Jean-Christophe Giard

Introduction

Small non-coding RNAs (sRNAs) occur in all kingdoms of life and have become increasingly recognized as a novel class of gene expression regulators. The eubacterial sRNAs, known in bacteria since the early 1970s (typically, 50–250 nt in length), generally do not contain expressible ORFs, although some of them, such as SgrS or RNAIII of Escherichia coli or Staphylococcus aureus, also contain coding regions. However, only the success of recent systematic genome-wide searches for genes encoding these molecules has led to their full appreciation (Bradley et al., 2011; Ferrara et al., 2012; Mraheil et al., 2010; Pellin et al., 2012; Shioya et al., 2011; Silvaggi et al., 2006). Several screens using various methods have increased the number of known sRNAs (Pichon & Felden, 2008; Vogel & Sharma, 2005). In E. coli, one of the best-studied models, the majority of the expressed sRNAs are conserved in closely related pathogens, such as Salmonella and Yersinia species (Hershberg et al., 2003). It has been estimated that enterobacterial genomes with a mean size of 4–5 Mb might contain 200–300 sRNAs, which correspond to ~5% of the total number of genes (Zhang et al., 2004). Substantial efforts have been made to define sRNA functions in various physiological responses, to identify their targets and the mechanisms by which they affect them, and to analyse their integration into complex regulatory networks in the cell (Richards & Vanderpool, 2011; Vogel & Wagner, 2007; Waters & Storz, 2009). With regard to the regulatory mechanisms, sRNAs can be divided into four main classes: RNAs modulating protein activity, cis-encoded base-pairing RNAs, trans-encoded base-pairing RNAs and CRISPRs (clustered regulatory interspaced short palindromic repeats) (Storz et al., 2011). Cis-acting RNA elements that are encoded on the DNA strand opposite the target RNA share ≥75 nt complementarity with the mRNA targets. Well-studied examples of these sRNAs are encoded by genes located on mobile genetic elements such as those of the toxin–antitoxin system involved in plasmid replication control (Brantl, 2007; Fozo et al., 2008; Weaver, 2012). Trans-encoded sRNAs share limited complementarities with their mRNA targets and the chromosomal locations of the corresponding genes are not correlated. Note that usually the trans-acting sRNAs have more than one mRNA target, contrarily to cis-acting sRNAs. Base-pairing between the sRNA and its target mRNA usually leads to modification
of translation, mRNA degradation by the RNase or both (Aiba, 2007; Gottesman, 2005). sRNAs modulating protein activity are 'non-base-pairing' molecules. They can interact directly with proteins and modify their activities by sequestration, e.g. csrB–csrC sRNA or 6S RNA in E. coli (Babitzke & Romeo, 2007; Steuten et al., 2014; Wassarman, 2007). Other non-base-pairing sRNAs, such as RNase P, 4.5S or transfer-messenger RNA (tmRNA), are involved in the regulation of proteins (Herskovits et al., 2000; Lai et al., 2010; Moore & Sauer, 2007). As an example, the well-known tmRNA is involved in translational surveillance and ribosome rescue. The last discovered regulatory RNA class comprises the CRISPR sequences, highly variable DNA regions of 24–47 bp that consist of a ~550 bp leader sequence followed by a series of 2–249 repeat-spacer units called CRISPR RNA (crRNA) which provide resistance to bacteriophages and prevent plasmid conjugation. crRNAs appear to target foreign DNAs and allow their degradation (Richter et al., 2012; Sorek et al., 2008).

The various biological roles of sRNA elements encompass the regulation of metabolism, growth processes or adaptation to stress. Moreover, recent evidence shows that regulatory RNAs play key roles in microbial pathogenesis (Gong et al., 2011; Koo et al., 2011; Toledo-Araná et al., 2007; Vogel, 2009; Waters & Storz, 2009). During infection, bacterial pathogens have to adapt rapidly to changing environmental conditions in the host, including survival strategies in specific niches, avoidance of exposure to the immune system and systemic toxicity. Among the multiple infectious strategies that bacterial pathogens have developed, regulatory RNAs are considered as signal transducers of environmental cues by participating in the precise coordination of gene expression. Several sRNAs have been shown to regulate the synthesis of virulence factors and other pathogenic traits by adapting the bacterial metabolism in response to the host.

Many reviews deal with the mechanism by which sRNAs interact with their target or experimental procedures leading to the identification of many sRNAs, although only a small proportion of them have been analysed at the functional level (Altuvia, 2007; Gottesman & Storz, 2011; Gottesman, 2005; Mraheil et al., 2010; Sharma & Vogel, 2009; Storz et al., 2011; Vogel & Wagner, 2007; Waters & Storz, 2009). Thus, here, we review examples of sRNAs that are involved in numerous cellular processes, such as energy metabolism, quorum sensing (QS) and biofilm formation, stress response and adaptation to growth conditions, and pathogenesis. We concentrate on the functional aspects of these sRNA molecules corresponding to transcripts that interact with mRNA or protein targets. The regulatory RNAs, such as riboswitches, attenuators, thermosensitive stem–loop structures in the untranslated region and the CRISPR, reviewed extensively elsewhere (Barrangou, 2013; Waters & Storz, 2009; Winkler & Breakey, 2005), will not be presented here. This review aims to illustrate the diversity of physiological characteristics where sRNAs are key regulators and their importance in cell behaviour.

**sRNA and cellular metabolism**

### Carbon metabolism

Many bacteria alternate periods of nutrient abundance and famine. These changes trigger major switches in gene expression and require coordination of global regulatory networks. These fine orchestrations that depend on the nature and the quantity of nutrients usually implicate specific transcriptional regulators and RNA regulators. Recently, some studies showed that sRNA molecules can regulate the expression of other regulators (Table 1). One of them, the Csr (carbon storage regulator) system, ensures connections between carbon metabolism and various other traits in many bacteria. In E. coli, the central component of this system is the repressor CsrA, which is known to be the regulator of carbon starvation and glycogen biosynthesis, mobility, QS, acetate metabolism, and flagellum biosynthesis, and more recently it has been identified to play a prominent role in the virulence of numerous bacteria (Heroven et al., 2012; Romeo et al., 2013). Two sRNAs, CsrB (369 nt) and CsrC (242 nt), antagonize the activity of CsrA during the exponential growth phase and repress metabolic pathways related to the stationary phase (Babitzke & Romeo, 2007; Revelles et al., 2013).

CsrA-like proteins are called RsmA and RsmE in *Pseudomonas* and *Erwinia* species, respectively, where they also play a crucial role in virulence (Lapouge et al., 2008). In *Pseudomonas aeruginosa*, two sRNAs, RsmY and RsmZ, sequester the translational regulator RsmA (González et al., 2008). Note that the overexpression of RsmZ has significant effects on the synthesis of several exoproducts, as observed for the RsmA mutant strain (Heurlier et al., 2004).

Sugar utilization is a key step in bacterial cell life and cellular adaptations are frequently needed according to the energy source available in the environment. Bacterial phosphoenolpyruvate carbohydrate phosphotransferase systems (PTSs) mediate the uptake of sugars and their concomitant phosphorylation (Deutscher et al., 2006). In *E. coli*, the main PTS importing glucose contains the transporter PtsG, generating glucose 6-phosphate in the presence of glucose. This phosphosugar, which is toxic if it accumulates inside cells, causes the phenomenon known as ‘phosphosugar stress’ (Morita et al., 2004). This leads to the activation of SgrR, a transcriptional activator and main coordinator of the response to glucose-phosphate stress, and the synthesis of the SgrS sRNA, responsible for the ptsG mRNA degradation reducing further accumulation of glucose 6-phosphate (Papenfort et al., 2013; Vanderpool & Gottesman, 2004; Zhang et al., 2003). The data were confirmed by the analysis of the sgrS mutant and its growth defect under phosphosugar stress conditions (Morita et al., 2006).

The sRNA Spot42 is another illustration of the role of a small transcript in the control of sugar metabolism. The *E. coli galETK* operon encodes enzymes that convert galactose to the glycolytic intermediate glucose 1-phosphate. The galactokinase activity of GalK depends on the presence...
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<tr>
<td><strong>Cellular metabolism</strong></td>
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<td><strong>Carbon metabolism</strong></td>
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<td></td>
<td>Spot42</td>
<td><em>galK</em></td>
<td>Hfq</td>
<td>Prevents the translation of the <em>galK</em> operon, converting galactose in glucose 1-phosphate and impacts metabolic pathways</td>
<td>Beisel &amp; Storz (2011)</td>
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<td></td>
<td>CsrB–CsrC</td>
<td>CsrA</td>
<td>CsrD, RNase E,</td>
<td>Represses the carbon storage regulator CsrA</td>
<td>Babitzke &amp; Romeo (2007); Modi <em>et al.</em> (2011)</td>
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<td></td>
<td>GlmY–GlmZ</td>
<td><em>glmS</em></td>
<td>Hfq, YhbJ</td>
<td>Involved in cellular response to DNA damage, in addition to allowing <em>glmS</em> activation</td>
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<td><strong>Pseudomonas aeruginosa</strong></td>
<td>RsmY–RsmZ</td>
<td><em>rsmA</em></td>
<td>PNPase</td>
<td>Antagonizes the effect of the CsrA-like protein RsmA on swarming mobility or lipase production</td>
<td>González <em>et al.</em> (2008)</td>
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<td><strong>Amino acid metabolism</strong></td>
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<td><em>E. coli</em></td>
<td>GcvB</td>
<td><em>oppA, dppA, ssT</em></td>
<td>GcvA, GcvR, Hfq</td>
<td>Represses peptide transporters, induced by high glycine levels and enhances bacterial survival under acid pH via RpoS regulation</td>
<td>Jin <em>et al.</em> (2009); Pulvermacher <em>et al.</em> (2009)</td>
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<td></td>
<td>Sr1</td>
<td><em>ahrC</em></td>
<td>CcpA, CcpN</td>
<td>Controls the AhrC transcriptional activator of the two operons encoding catabolic and transport enzymes for arginine catabolism, and stimulated by L-arginine and the stationary phase</td>
<td>Heidrich <em>et al.</em> (2006, 2007)</td>
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<td><strong>Iron regulation</strong></td>
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<td><em>E. coli</em></td>
<td>RhxB</td>
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<td>Leads to the rapid degradation of iron-binding mRNAs; RhxB also activates some mRNAs</td>
<td>Massé &amp; Gottesman (2002); Sorger-Domenigg <em>et al.</em> (2007)</td>
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<td><strong>P. aeruginosa</strong></td>
<td>PrrF1–2</td>
<td><em>bfr, sdh, sodB</em></td>
<td>Fur, Hfq, RNase E/III</td>
<td>Leads to the rapid degradation of iron-binding mRNAs</td>
<td>Wilderman <em>et al.</em> (2004)</td>
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<td><strong>QS and biofilm formation</strong></td>
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<td><em>Staphylococcus aureus</em></td>
<td>RNAIII</td>
<td>mRNAs of QS</td>
<td>RAP/TRAP</td>
<td>Represses expression genes encoding cell adhesion proteins, and induces expression of genes encoding toxins and other virulence factors</td>
<td>Korem <em>et al.</em> (2005)</td>
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<td><em>E. coli</em></td>
<td>CsrB/C</td>
<td>CsrA</td>
<td></td>
<td>Activates <em>flhDC</em>, the regulator of flagella expression, and represses <em>pga</em>, involved in the expression of a biofilm matrix component</td>
<td>Mika &amp; Hengge (2013)</td>
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<td></td>
<td>McaS</td>
<td><em>flhDC</em></td>
<td></td>
<td>Activates the <em>flhDC</em> operon and CsrA, a negative regulator of <em>pgaA</em> translation</td>
<td>Jørgensen <em>et al.</em> (2013)</td>
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<td></td>
<td>MicA</td>
<td><em>ompA</em></td>
<td><em>σE</em></td>
<td>Represses various porins, such as <em>ompA</em>, and its expression is essential for proper biofilm formation</td>
<td>Kint <em>et al.</em> (2010); Mika &amp; Hengge (2013)</td>
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<td>Role of sRNA/species</td>
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<td>Main targets</td>
<td>Partners</td>
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<td><strong>Stress response and adaptation to growth conditions</strong></td>
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<td><strong>Acid stress response</strong></td>
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<td>E. coli</td>
<td>GadY</td>
<td>gadXW</td>
<td>RNase III</td>
<td>Acts as a positive regulator of the gadXW operon where gadX encodes the major acid tolerance regulator</td>
<td>Opdyke et al. (2011)</td>
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<td>6S RNA</td>
<td>pspF</td>
<td></td>
<td>Regulates PspF transcription which leads to altered cell survival at high pH</td>
<td>Trotochaud &amp; Wassarman (2006)</td>
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<td><strong>Oxidative stress response</strong></td>
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<td>E. coli</td>
<td>OxyS</td>
<td>rpoS, fhlA</td>
<td>Hfq</td>
<td>Acts as an antimutator, inhibits rpoS translation, regulates fhlA, activator of the hyp operon and contributes to cell protection against oxidative DNA damage</td>
<td>Altuvia et al. (1997); Johnson et al. (2006)</td>
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<td><strong>Osmotic stress</strong></td>
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<td>E. coli</td>
<td>FnrS</td>
<td>sodB, maeA, gpmA, folEX</td>
<td>Hfq</td>
<td>Expressed under anaerobic conditions; under the control of two O2-dependent FNR and ArcA regulators, downregulates mRNAs encoding enzymes of energy metabolism</td>
<td>Durand &amp; Storz (2010)</td>
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<td>ArcZ</td>
<td>arcB</td>
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<td>Under aerobic conditions, inhibits arcB by downregulation of arcB</td>
<td>Mandin &amp; Gottesman (2010)</td>
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<td><strong>Aerobic or anaerobic growth conditions</strong></td>
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<td>E. coli</td>
<td>MicF</td>
<td>ompF</td>
<td></td>
<td>Regulates the expression of the major outer membrane porin OmpF</td>
<td>Andersen &amp; Delihas (1990)</td>
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<td></td>
<td>OmrA/OmrB</td>
<td>Four porins</td>
<td>Hfq</td>
<td>Represses genes encoding outer membrane proteins cirA, fecA, fepA and ompT</td>
<td>Guillier et al. (2006)</td>
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<td>RybB</td>
<td>omp mRNA</td>
<td>σE</td>
<td>Facilitates omp mRNA decay as part of the envelope stress response</td>
<td>Majdalani et al. (2002)</td>
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<td></td>
<td>RprA</td>
<td>rpoS</td>
<td>Hfq</td>
<td>Stimulates RpoS synthesis in an osmolarity-dependent manner in response to cell envelope stress</td>
<td>Madhugiri et al. (2010)</td>
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<td><strong>Phosphosugar stress</strong></td>
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<td>E. coli</td>
<td>SgrS</td>
<td>ptsG, manXYZ, yigL</td>
<td></td>
<td>Decreases phosphosugar accumulation by repressing translation of sugar transporter mRNA and enhancing translation of sugar phosphatase mRNA</td>
<td>Papenfort et al. (2013); Rice &amp; Vanderpool (2011)</td>
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<td><strong>Pathogenesis</strong></td>
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<td>Staphylococcus aureus</td>
<td>RNAIII</td>
<td>hla, prot A, coagulase, rot</td>
<td></td>
<td>Effector of the agr operon; activates hla translation encoding haemolysin and represses the synthesis of major cell surface virulence factors, such as coagulase, protein A and the transcriptional regulator rot</td>
<td>Boisset et al. (2007)</td>
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<td>Streptococcus pyogenes</td>
<td>FasX</td>
<td>fasBCA</td>
<td></td>
<td>Regulates the fasBCA regulon required for the repression of adhesins FBP54 and MRP, and for the activation of virulence factors such as streptokinase</td>
<td>Kreikemeyer et al. (2001)</td>
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<td>Clostridium perfringens</td>
<td>VR-RNA</td>
<td>colA/plc</td>
<td></td>
<td>Under the dependence of VirRS system; responsible for the regulation of toxin genes, such as κ-toxin (colA) and z-toxin (plc)</td>
<td>Shimizu et al. (2002)</td>
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</table>
of glucose and the 109 nt Spot42 sRNA (Beisel & Storz, 2011; Beisel et al., 2012). Indeed, Spot42 specifically binds to the 5′ part of the galK mRNA and prevents translation of the galK operon.

### Amino acid metabolism

Amino acids may also be used as energy sources by bacteria and some published results showed that the regulation of their catabolism can involve sRNA. In *Bacillus subtilis*, the use of arginine requires the products of the rocABC and rocDEF operons encoding catabolic and transport enzymes. RocR, which negatively regulates its own synthesis, and AhrC, controlled by the sRNA Sr1, are both transcriptional activators of rocABC and rocDEF (Gardan et al., 1997; Heidrich et al., 2006). The interaction between Sr1 and ahrC mRNA prevents the assembly of a translation initiation complex (Heidrich et al., 2006, 2007). Sr1 transcription is stimulated by the presence of L-arginine or stationary-phase entrance and is repressed when sugars are used as the energy source (Heidrich et al., 2007).

Another sRNA involved in the regulation of amino acid transport is the 206 nt sRNA GcvB of *Salmonella enterica* sv. Typhimurium, induced by high concentrations of glycine and activated by GcvA, a regulator known to activate the gcvTHP glycine degradation operon. GcvB regulates oppA and dppA mRNAs, which encode oligopeptide and dipeptide periplasmic binding proteins, respectively (Pulvermacher et al., 2009; Urbanowski et al., 2000). They also repress directly transcripts encoding proteins implicated in peptide transport as well as permeases in *E. coli* (Sharma et al., 2007).

In addition, in *Salmonella*, a *sin* *E. coli*, the mRNAs encoding amino acid transporters are GcvB targets (Kim et al., 2002; Ogawa et al., 1998; Sharma et al., 2007, 2011). Interestingly, experiments using an *E. coli* strain lacking CcvB sRNA and activated by GcvA, a regulator known to activate the gcvTHP operon, showed that this sRNA enhances the ability of bacteria to survive acid pH. This is explained by the upregulation of the alternative sigma factor RpoS (Jin et al., 2009).

### Iron regulation

In *E. coli*, the sRNA RyhB is a key player for adaptation to iron-limiting conditions. It redirects cellular iron use by preventing new synthesis of non-essential iron-containing enzymes (an effect called 'iron sparing') and enhancing the ability of cells to synthesize iron-scavenging siderophores (Richards & Vanderpool, 2011). Indeed, RyhB is known to base-pair with at least 18 transcripts (encoding 56 proteins mainly involved in iron metabolism), some of which encode iron-binding proteins such as *sodB* (encoding iron superoxide dismutase), leading to rapid degradation of the transcripts (Masse et al., 2002; Masse et al., 2003, 2007). The result of destroying the mRNA and therefore stopping the synthesis of these iron-binding proteins is a decrease in the cellular requirement for iron, which therefore limits the ability of cells to synthesize siderophores and thus prevents bacterial iron scavenging. Therefore, RyhB sRNA plays a crucial role in iron regulation by *E. coli*.

### Table 1. cont.

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<tr>
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<tr>
<td>Pel</td>
<td><em>emm</em>, <em>sic</em>, SpeD</td>
<td>Pelemm, Pelsic, SpeD</td>
<td></td>
<td>Regulates virulence factors at transcriptional (emm and sic, respectively, encoding surface-exposed virulence M protein and inhibitor of the complement system) and post-transcriptional levels (SpeD cysteine protease)</td>
<td>Mangold et al. (2004)</td>
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<tr>
<td>RivX</td>
<td><em>mgA</em></td>
<td>rivR</td>
<td>Hfq</td>
<td>Activates a <em>trans</em>-acting RNA, <em>mgA</em> operon, implicated in the expression of virulence genes such as <em>scpA</em>, <em>sic</em>, <em>fba</em> and <em>sclI</em></td>
<td>Roberts &amp; Scott (2007)</td>
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<td><em>P. aeruginosa</em></td>
<td>RsmY–RsmZ</td>
<td>RsmA</td>
<td></td>
<td>Activated by the GacAS system; prevents RsmA (regulator of genes important for virulence and survival) activity by sequestration</td>
<td>Heurlier et al. (2004)</td>
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<td><em>Chlamydia trachomatis</em></td>
<td>IhtA</td>
<td>Hc1</td>
<td></td>
<td>Acts indirectly as an activator by impeding the synthesis of the histone Hc1, required for bacterial differentiation in the infection process</td>
<td>Grieshaber et al. (2006)</td>
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Such results point out the interconnection between energy metabolism and the bacterial stress response in which sRNA molecules can play a role.
makes more iron available for essential proteins. Moreover, physiological studies revealed that a RyhB mutant strain has a significant delay in growth, is defective in both biofilm formation and chemotaxis, and produces significantly less enterobactin than WT cells under conditions of iron starvation (Jacques et al., 2006; Mey et al., 2005; Salvail et al., 2010). sRNAs with similar functions have been identified in different Gram-negative species, such as PrrF1 and PrrF2 of *P. aeruginosa*. In *E. coli* and *P. aeruginosa*, the production of sRNA associated with iron assimilation is regulated by the Fur repressor when iron is bound to it. Under iron limitation, the sRNA is transcribed and consequently reduces synthesis of iron-binding proteins, but can also repress the synthesis of its own regulator Fur (Vecerek et al., 2007).

**Role of sRNA in QS and biofilm formation**

QS is a form of cell communication in bacteria that provides information about the population density, leading to genetic changes allowing in some cases biofilm formation (Waters & Bassler, 2005). It is becoming apparent that integration of information by QS systems is regulated by sRNA molecules (Table 1). sRNAs described in the following examples illustrate how such molecules can be involved in different aspects of cellular physiology, such as the stress response, virulence and energy metabolism.

*Vibrio cholerae* synthesizes and responds to autoinducers with two-component membrane-bound sensor kinases as receptors (Miller et al., 2002). Each sensor transfers information into the phosphorelay protein LuxU, which subsequently transmits the signal to the response regulator protein LuxO (Bassler et al., 1994; Freeman & Bassler, 1999; Lilley & Bassler, 2000). Depending on the cell density, LuxU or LuxO is phosphorylated and *Vibrio* QS genes are expressed. At low cell density, LuxO is phosphorylated and can activate the transcription of five non-coding quorum-regulated sRNAs (*qrr1–5*) that are functionally redundant (Bardill et al., 2011). Qrrs repress their own regulator LuxO (Svenningsen et al., 2009) and inhibit the translation of three other targets, all involved in the global regulation of *V. cholerae* pathogenicity: *hapR* (encoding the transcription factor that represses virulence genes) (Lenz et al., 2004), *aphA* (encoding the transcriptional factor that enhances virulence gene expression) (Rutherford et al., 2011) and *vca0939* (encoding a protein stimulating biofilm formation) (Hammer & Bassler, 2007). Note that, at high cell density, accumulation of autoinducers causes the dephosphorylation of LuxO which cannot further activate transcription of Qrrs.

In *Staphylococcus aureus*, one QS system involves the sRNA RNAIII, which plays an important role in virulence, demonstrating the pivotal role a sRNA can play in diverse parts of bacterial physiology. This micro-organism uses the peptide RNAIII-activating protein (RAP) and its target molecule TRAP as autoinducers, detected by two-component associated membrane sensor kinases that phosphorylate the cognate cytoplasmic response regulators (Lyon & Novick, 2004). At high cell density, binding of the autoinducing peptide triggers expression of the regulatory RNA RNAIII. This RNAIII represses expression of genes encoding cell adhesion proteins important for early colonization stages, and induces expression of genes encoding several toxins, exoproteins and other virulence factors (Johansson & Cossart, 2003; Korem et al., 2005; Toledo-Arana et al., 2007) (see ‘Role of sRNA in pathogenesis’ below).

As mentioned above, QS is used as a signal for the cell to form biofilms, which represent the prevalent microbial mode of existence in nature (Flemming & Wingender, 2010). Such structures confer protection against environmental stresses as well as antibiotics and play critical roles in microbial pathogenesis. In addition, the formation of biofilms (switching from the planktonic to the surface-associated mode of growth) requires complex regulatory cascades. In *E. coli*, the two sRNAs CsrB and CsrC (already known to interact with CsrA, see ‘sRNA and cellular metabolism’ above) activate *FlhDC* (encoding a flagellar master regulator) and down-regulate the expression of the *pga* operon, encoding a poly-N-acetyl-d-glucosamine extracellular matrix, one of the biofilm components (Mika & Hengge, 2013).

Multiple other sRNAs have been identified, modulating the expression or activity of transcriptional regulators and components of regulatory networks required for attachment and biofilm formation, and also linked to the stress response or metabolism (Mika & Hengge, 2013; Romeo et al., 1993). Among them, McaS (multicellular adhesive sRNA) and MicA (regulator of *ompA* mRNA) have important roles in adhesion and motility, respectively. McaS activates directly the *flhDC* mRNA, but also activates synthesis of the exopolysaccharide β-1,6-N-acetyl-d-glucosamine by binding the global RNA-binding protein CsrA, a negative regulator of *pgaA* translation (Jørgensen et al., 2013; Thomason et al., 2012). MicA, whose overexpression increases motility in soft agar plates, down-regulates the expression of several outer membrane porins in *E. coli* and *Salmonella* (De Lay & Gottesman, 2012).

**Role of sRNA in the stress response and in adaptation to growth conditions**

Some sRNA functions in various physiological responses have already been identified, more particularly concerning adaptation of bacteria to chemical stresses and varying culture conditions. Generally, the expression of sRNAs is regulated tightly and induced by specific environmental conditions. The involvement of sRNA in the stress response is closely related to microbial pathogenesis as cells have to cope with stresses during the infection process (i.e. body fluids, phagocytosis and immune response). Here, examples are given to underscore the role of sRNA in acid, oxidative or osmolarity stress, and adaptation to grow under aerobic or anaerobic conditions (Table 1).

**sRNA and the acid stress response**

One of the main defences of *E. coli* against extreme acid pH is the induction of two glutamate decarboxylase enzymes,
GadA and GadB (Castanie-Cornet et al., 1999). Glutamate is transformed to γ-aminobutyric acid by these enzymes, a process that consumes intracellular protons (Homola & Dekker, 1967). Expression of GadA and GadB is under the control of GadX, the major acid tolerance regulator, present in a bicistronic operon structure with gadW (Tramonti et al., 2002). The GadY sRNA overlaps the 3' end of the gadX mRNA, acting as a positive regulator of acid stress response genes by base-pairing to the gadWX mRNA, and contributes to separate and stabilize the bicistronic transcript (Opdyke et al., 2004).

**sRNA and the oxidative stress response**

In *E. coli* and closely related bacteria, OxyR regulates numerous genes whose expression is induced by hydrogen peroxide and defends the bacteria against peroxides. OxyS (a 109 nt sRNA) is part of the OxyR regulon, which acts as a regulator involved in the adaptation to hydrogen peroxide and helps to protect cells against oxidative damage (Altuvia et al., 1997). It is also described as a regulator of at least 40 genes in *E. coli*, including *fhlA* encoding an activator of the formate–hydrogen lyase complex expressed in the presence of formate, and the *hyp* operon encoding accessory proteins essential for the maturation of the [NiFe] hydrogenase enzymes, as well as *rpoS* encoding the 'general stress sigma factor' RpoS (Altuvia et al., 1997, 1998; Zhang et al., 1998). Note that inactivation of *oxyRS* in the WT strain significantly increased its susceptibility to hydrogen peroxide and attenuated its virulence in a mouse model (Johnson et al., 2006).

**sRNA and the osmotic stress response**

Depending on the osmolarity, the OmpR regulator modulates the transcription of the two most abundant outer membrane proteins in *E. coli*, OmpF and OmpC, in different ways. In addition to this regulation, MicF, the first base-pairing RNA discovered in the *E. coli* genome, was shown to regulate the expression of the porin OmpF (Andersen & Delhais, 1990; Coyer et al., 1990). Expression of MicF RNA is regulated by multiple signals, and is essential for osmoregulation at low and medium levels of osmolarity. At higher osmolarity, the role of MicF RNA is masked by the strong transcriptional osmoregulation exerted by OmpR (Raman et al., 1994). Five additional sRNAs, MicG, MicA, RybB, RseX and IpeX, modulators of expression of the most abundant outer membrane porins, OmpA, OmpC and OmpF, have also been identified and reviewed by Guillier et al. (2006). Additional outer membrane proteins are capable of acting as channels and are also subject to post-transcriptional regulation by sRNAs. The OmrA (88 nt) and OmrB (82 nt) sRNA loci are located in the same intergenic region, induced by high osmolarity and regulated by the OmpR response regulator (Guillier et al., 2003; Wassarman et al., 2001; Zhang et al., 2003). Whole-genome expression analysis after overproduction of either OmrA or OmrB indicated that these two sRNAs mainly negatively regulate the expression of several genes encoding multiple outer membrane proteins, including *cirA*, *fepA*, *fepA* and *ompT* (Guillier et al., 2006). Lastly, RybB, an 80 nt sRNA under control of *σ^E^*, targets a large set of porins and facilitates *omp* mRNA decay as part of the envelope stress response (Papenfort et al., 2006). Characterization of a Δ*rybB* mutant showed that its better resistance to cell envelope stress seems to be due to the upregulation of *σ^E^* (Hobbs et al., 2010).

In *E. coli*, the 105 nt regulatory sRNA RprA (*RpoS regulatory RNA A*) stimulates RpoS synthesis by base-pairing in an osmolarity-dependent manner in response to cell envelope stress (Majdalani et al., 2002). Transcription of RprA is regulated by the RcsC/RcsB phosphorelay system, found previously to regulate capsule synthesis and the activity of the promoter of *ftsZ*, the cell division gene. Note that another study has shown that the osmolarity-dependent turnover of RprA contributes to its own abundance (Majdalani et al., 2002).

**sRNA and aerobic or anaerobic growth conditions**

*E. coli* is able to grow under both aerobic and anaerobic environments. Shifts between low and high oxygen concentrations induce profound changes in gene expression (Constantinidou et al., 2006; Kang et al., 2005; Salmon et al., 2003). Two transcriptional regulators, FNR (fumarate and nitrate reduction) and ArcA (aerobic respiratory control), whose activities are modulated by oxygen availability, impact many of the changes in gene expression associated with a transition from aerobic to anaerobic metabolism. FNR, only active under anaerobic conditions, is a direct sensor of oxygen. The sRNA FnrS, whose expression is induced by anaerobic conditions in an FNR- and ArcA-dependent manner, downregulates at least 32 mRNAs, many of which encode enzymes involved directly in energy metabolism, such as *maeA* (malate dehydrogenase), *gpmA* (phosphoglycerate mutase), *folE* (GTP cyclohydrolase), *folX* (dihydroneopterin triphosphate epimerase) or *sodB* (superoxide dismutase) (Durand & Storz, 2010). ArcA is the cytosolic response regulator of a two-component system, in which ArcB is the transmembrane histidine kinase sensor (Georgellis et al., 2001). The sRNA ArcZ, which interacts with *arcB*, is produced under aerobic growth or repressed under anaerobic conditions (Mandin & Gottesman, 2010). ArcZ regulates RdrA morphotype (multicellular behaviour of *Salmonella enterica* and *E. coli*, characterized by the expression of the adhesive extracellular matrix components cellulose and curli fimbriae), and also the transition between sessility and mobility (Monteiro et al., 2012).

**Role of sRNA in pathogenesis**

As sRNAs participate in the genetic regulation of cellular metabolism, biofilm formation and the stress response of Gram-positive and Gram-negative pathogens, they have an impact on the pathogens' ability to persist and consequently...
on their pathogenicity. In addition to this, several studies have identified sRNAs that regulate specifically the expression of virulence factors (Table 1). In the following sections, several examples of such sRNAs are presented from various bacterial species. These works increase our knowledge of the mechanisms of the bacterial infection process, and open a new research path towards innovative therapeutic strategies and new diagnostic tools (Mraheil et al., 2010).

sRNA in the pathogenesis of Staphylococcus aureus
As mentioned in the QS systems section above, in Staphylococcus aureus the sRNA RNAIII acts as a sensor of population density and is required for virulence in animal models (Cheung et al., 1994; Gillaspy et al., 1995; Novick & Geisinger, 2008; Novick & Muir, 1999; Novick, 2003). RNAIII controls the switch between the early expression of surface proteins and the late expression of exotoxins, but it also encodes a 26 aa δ-haemolysin peptide (Novick et al., 1993; Waters & Storz, 2009). The 5’ domain of RNAIII activates translation of hla mRNA encoding α-haemolysin (Morfeldt et al., 1995; Novick et al., 1993), and the 3’ end and the central domain of this sRNA repress the synthesis of major cell surface virulence factors (protein A, coagulase, fibrinogen-binding protein) as well as the transcriptional regulator Rot. By repressing the synthesis of Rot, RNAIII also induces many downstream effects and activates indirectly the synthesis of many exotoxins (Boisset et al., 2005; Morfeldt et al., 1995). Using a ΔRNAIII deletion mutant strain, the role of the sRNA in adherence to host tissues was studied. The study showed that RNAIII downregulated Staphylococcus aureus adherence to fibrinogen and upregulated adherence to fibronectin and endothelial cells (Shenkman et al., 2002).

sRNA in the pathogenesis of Streptococcus pyogenes
In some other Gram-positive pathogens, several sRNAs have been shown to interact with the expression of transcriptional regulators or proteins clearly involved in the virulence of the bacteria. In Streptococcus pyogenes, three sRNAs participate in the control of virulence factor expression. FasX RNA is part of an operon encoding two histidine kinases (FasBC) and one response regulator (FasA) (Kreikemeyer et al., 2001). The fasBCA regulon, via FasX RNA, negatively controls the expression of two matrix protein-binding adhesins (fibrinogen-binding proteins FBP54 and MRP) and has a positive influence on the activities of two secreted virulence factors (streptokinase and streptolysin S) (Kreikemeyer et al., 2001). FasX also controls the interaction of Streptococcus pyogenes with epithelial laryngeal cells (Klenk et al., 2005). The second sRNA, Pel, acts as a regulator of virulence factor expression, e.g. emm (encoding the surface-exposed virulence M protein), sic (encoding a streptococcal inhibitor of the complement system) and speB (encoding a cysteine protease) (Mangold et al., 2004). As for RNAIII of Staphylococcus aureus, Pel sRNA displays bifunctionality, being an effector of virulence factor expression and encoding a haemolysin. Lastly, RivX sRNA is co-expressed with the downstream gene encoding the response regulator RivR and was shown to affect positively the transcription of members of the Mga regulon, e.g. sepA (peptidase), sic (streptococcal inhibitor of complement) and fba (fibrinectin-binding protein) or scll (a collagen-like protein) (Leday et al., 2008; Roberts & Scott, 2007).

sRNA in the pathogenesis of Clostridium perfringens
The sRNA VR-RNA (VirR-regulated RNA) of Clostridium perfringens is positively regulated by the two-component VirR/VirS system, which controls (positively and negatively) the level of many virulence factors sloping letter theta-toxin (perfringolysin O), α-toxin (collagenase), α-toxin (phospholipase C), sialidase, protease and haemagglutinin [Lyristis et al., 1994]. Moreover, study of the VR-RNA mutant revealed the positive effect of this sRNA in the regulation of toxin genes such as plc (α-toxin), ptp (protein tyrosine phosphatase), cpd (nucleotide phosphodiesterase), ycgI, metB, cysK and ygaG (genes coding for other toxins), and colA (κ-toxin) (Awad et al., 2000; Obana et al., 2013; Ohtani et al., 2003; Podkaminski & Vogel, 2010).

sRNA in the pathogenesis of Listeria monocytogenes
The LhrA sRNA of L. monocytogenes acts as a post-transcriptional negative regulator of chiA (encoding chitinase). chiA contributes to the pathogenesis of L. monocytogenes in the mouse model, possibly through the recognition of glycoproteins or other carbohydrate moieties present in the infected host. Their expression depends on at least two regulatory proteins: the central virulence regulator PrfA and the alternative stress sigma factor σB (Mraheil et al., 2010, 2011).

sRNA in the pathogenesis of Pseudomonas aeruginosa
The role of two sRNAs, RsmY and RsmZ, in P. aeruginosa is a nice example of the interconnection between the regulation of metabolism and pathogenesis. In addition to sequestering the transcriptional regulator RsmA (see ‘sRNA and cellular metabolism’ above) they also affect the expression of a variety of genes important for virulence and survival (Heurlier et al., 2004; Kay et al., 2006), such as those implicated in pyocyanin, hydrogen cyanide and PA-IL lectin synthesis. RsmA plays an important role in the control of the switch between acute infection and chronic infection through the regulation of a type 3 secretion system and motility or a type 6 secretion system and biofilm formation (Bordi et al., 2010; Mulcahy et al., 2008). Of note, overexpression of RsmZ coincides with reduced biofilm development that is particularly associated with the pathogenicity of P. aeruginosa (Petrova & Sauer, 2010).

sRNA in the pathogenesis of Chlamydia trachomatis
One last example is the involvement of the sRNA IhtA in the virulence of Chlamydia trachomatis, an obligate
intraocular pathogen, and the leading cause of blindness and sexually transmitted urogenital infections (Moulder, 1991). The chlamydial life cycle is controlled by two histone-like proteins, Hc1 and Hc2, expressed only during the late stages of the cycle. Their expression is concomitant with the differentiation of reticulate bodies into elementary bodies, the infectious but inactive form of the bacteria (Grieshaber et al., 2006; Shaw et al., 2000). When Chlamydia trachomatis undergoes differentiation from the elementary body to the reticulate body, after bacterial entry into host cells, the abundance of the sRNA IhtA increases and the level of Hc1 decreases (Grieshaber et al., 2006). However, when the reticulate body differentiates into the elementary body, IhtA transcription decreases and Hc1 synthesis takes place. Therefore, IhtA acts indirectly as a global transcriptional activator avoiding chromatin condensation during the replicative stage of Chlamydia trachomatis.

Concluding remarks

The transcriptional activity of the bacterial cell is mainly directed through the production of ‘non-translatable’ RNA. The huge amount of data obtained by next-generation sequencing technologies as well as by progress in bioinformatics also reveals that the intergenic fraction of the genome must be taken into consideration as it encodes RNAs important in the fine regulation of cellular processes. Sophisticated mechanisms such as riboswitches, protein-binding sRNAs, trans-encoded base-pairing sRNAs and some cis-encoding base-pairing sRNAs mediate responses to changing environmental conditions by modulating metabolic pathways or stress responses. This highlights the complexity of the regulatory network that exists in prokaryotic cells and we are likely to be only at the beginning of a vast field of research involving RNA molecules. As suggested by Beisel & Storz (2011), this type of regulation seems favourable for the cells by reducing metabolic cost, bringing additional levels of regulation or allowing faster regulation because of the absence of the translation step. In addition, post-transcriptional regulation by sRNA allows bacteria to respond to signals in a rapid and sensitive manner.

In this review, we have pointed out the diversity of physiological processes in which sRNA molecules are key actors: metabolism, QS, stress response and virulence. This shows the central role of sRNAs to help bacteria cope with environmental changes and to survive under stressful conditions present in the host. During the infection process, bacteria have to adapt rapidly to changing environmental conditions and to escape the immune system. The numerous examples presented in this review indicate that sRNAs are required for a coordinated response and thus play a crucial role in these processes. However, in comparison with the high and still increasing number of sRNAs identified in bacterial genomes, only a few of them have been hitherto functionally characterized. The physiological relevance of sRNA studies will be at the crossroads of computational, transcriptomic and proteomic approaches. Nevertheless, an analysis of their physiological role in the cell, technically laborious (i.e. construction of deletion strains or libraries of sRNA-overexpressing plasmids), will constitute one of the main challenges to increase our understanding of bacterial behaviour and metabolism, especially during infection or the stress responses.

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References


Roles of sRNAs


Pseudomonas aeruginosa RsmA plays an important role during murine infection by influencing colonization, virulence, persistence, and pulmonary inflammation. Infect Immun 76, 632–638.


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