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A guide to small RNAs in microorganisms

Editorial Overview

Gisela Storz and Dieter Haas

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Gisela Storz

Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, 18 Library Drive, Bethesda, MD 20892-5430, USA
e-mail: storz@helix.nih.gov

Gisela Storz is a Senior Investigator in the Cell Biology and Metabolism Branch of the National Institute of Child Health and Human Development. Since her discovery of the OxyS RNA, the identification and characterization of small RNAs in *Escherichia coli* has become an ever-increasing focus of research by her group.

Dieter Haas

Département de Microbiologie Fondamentale, Université de Lausanne, CH-1015 Lausanne, Switzerland
e-mail: Dieter.Haas@unil.ch

Dieter Haas is a Professor of Microbiology at the University of Lausanne (Switzerland) where he teaches general microbiology and bacterial genetics. His main research interests are the metabolic versatility and regulatory mechanisms of pseudomonads, currently with an emphasis on the role of small RNAs in the expression of secondary metabolites and extracellular enzymes.

Overview

When regulation of the lactose metabolism genes was first discovered in *Escherichia coli*, Jacob and Monod [1] proposed that the regulator was an RNA that would block the expression of *lac* mRNA at the *lac* operator. This proposal was largely forgotten with the discovery of the LacI repressor protein, along with the hundreds of other specific DNA binding proteins that activate or repress transcription. A hint that RNAs could in fact function as regulators came from findings that replication of some plasmids is modulated by antisense RNAs. In general however these RNAs, as well as a few small chromosomally-encoded RNAs that were discovered fortuitously, were considered oddities until the recent realization that microbial genomes encode numerous small, regulatory RNAs. The reviews in this issue of *Current Opinion in Microbiology* summarize what is currently known about bacterial and fungal regulatory RNAs, with an emphasis on their physiological roles and mechanisms of action. The methods used to characterize these RNAs will be the subject of several reviews in the next issue of *Current Opinion in Microbiology*.

Unfortunately, the nomenclature for describing small, regulatory RNAs in bacteria has been neither uniform nor entirely satisfactory. Noncoding RNAs (ncRNAs) has been the predominant term for denoting these RNAs in eukaryotes and also has been used in some papers discussing bacterial RNAs. However, some bacterial RNAs that act as regulators, such as RNA III of *Staphylococcus aureus*, have been shown to encode small proteins and thus are not 'noncoding'. In most other cases, the existence of a peptide product has not been excluded. Riboregulator and regulatory RNAs are also used, but have the disadvantage that an abbreviation of these terms, rRNA, is associated ribosomal RNAs. Another term used to describe the bacterial regulatory RNAs is small RNA (sRNA). A caveat to this name is the fact that some RNAs are in the range of 500 nucleotides and thus are not very 'small'. In addition, the term sRNA historically was applied to tRNAs (which are also small). However, given that sRNA has been predominant in recent bacterial literature, it will be adopted here.

The first sRNAs were discovered on the basis of abundance, fortuitously or because of a phenotype observed for multicopy plasmids expressing the RNAs. In recent years, however, a number of groups carried out systematic screens to identify sRNAs in a variety of microorganisms. Many of these approaches were computational as described in the review by Livny and Waldor. Other approaches to systematically identify sRNAs relied on direct detection. These methods will be covered in *RNA Techniques*, in the next issue of *Current Opinion in Microbiology*.

As mentioned above, plasmid sRNAs were the first to be discovered. Almost all of these sRNAs act by basepairing and are encoded in *cis*, opposite the

RNA that is the basepairing target. As a consequence the potential for basepairing is extensive. Brantl provides a nice overview of this entire class of *cis*-encoded sRNAs and the mechanisms by which they act. In recent years, there has been a renaissance in the characterization of plasmid-encoded sRNAs, and Weaver summarizes what is known about these newly identified antisense RNAs. Many of the *cis*-encoded sRNAs control the expression of toxic proteins, encoded either by plasmids and or by bacterial chromosomes, as is discussed by Gerdes and Wagner. Because plasmid-encoded sRNAs have been characterized most extensively, the steps in sRNA-target RNA basepairing are best understood for these sRNAs. For example, these studies have shown that the initial sRNA–target RNA interaction occurs through limited basepairing in what is referred to as the kissing complex. In addition, the initial basepairing often involves an RNA loop structure termed a 'U-turn'. A U-turn was first described for the anticodon loop of yeast $tRNA^{Phe}$ and corresponds to an RNA structural motif (YUNR), which has a sharp bend in the RNA backbone between the conserved U and the following N base (N is any nucleotide, Y is a U or C, and R is a G or A).

Most of the sRNAs encoded by bacterial chromosomes that have been characterized thus far, have been found to act by basepairing with mRNA targets that are encoded in *trans*, at a chromosomal position different from their target. In contrast to the *cis*-encoded sRNAs, the potential for basepairing between *trans*-encoded sRNAs and their targets is generally more limited. So far most of these *trans*-encoded RNAs have been found to require the Hfq protein, an abundant RNA binding protein that is homologous to the Sm and Lsm protein that form the core of splicing and mRNA degradation complexes in eukaryotes. Brennan and Link discuss how the properties of the Hfq protein enable this hexameric ring to facilitate basepairing and modulate mRNA stability. Basepairing between the *trans*-encoded RNAs can impact both mRNA stability and translation. The review by Aiba focuses on what is known about these regulatory outcomes. Many parallels can be drawn between targeted mRNA degradation in bacteria and gene silencing by microRNAs and small interfering RNAs (siRNAs) in eukaryotic organisms. The physiological roles of several of the *E. coli trans*-encoded sRNA have been elucidated. The crucial roles of RyhB and other sRNA in iron homeostasis are described by Massé, Salvail, Desnoyers and Arguin. The roles of sRNAs, particularly SgrS, in sugar metabolism are the subject of the review by Vanderpool. Finally, Valentin-Hansen, Johansen and Rasmussen summarize the roles of a number of sRNAs in controlling outer membrane protein synthesis. Experimental approaches to identify mRNA targets of these *trans*-encoded RNAs will be covered in *RNA Techniques*, in the next issue of *Current Opinion in Microbiology*.

A few bacterial sRNAs have been found to act in ways other than basepairing with target RNAs. Two that mimic the structures of other nucleic acids are the CsrB and 6S-family RNAs. As discussed by Babitzke and Romeo, the sRNAs of the CsrB family carry multiple repeats of sequences found in the 5′ leaders of mRNAs that are bound by RNA-binding proteins of the CsrA family. CsrB-like RNAs control a variety of global regulatory circuits by antagonizing the activities of the CsrA-type proteins. The 6S RNA impacts transcription by binding RNA polymerase in a way that mimics the DNA corresponding to an open promoter. Wassarman describes what is known about the 6S RNA interaction with RNA polymerase and how this binding contributes to stationary phase survival of *E. coli*. Interestingly, homologs of the CsrB and 6S RNAs can be detected in a much broader range of bacteria than has been the case for the basepairing RNAs.

One RNA found in all bacteria is the tmRNA, which functions as both a tRNA and mRNA to mediate the release of stalled ribosomes. The properties of this fascinating RNA and its roles in cellular physiology are summarized by Keiler. Another class of RNA regulators that are present in organisms ranging from bacteria to plants are the so-called 'riboswitches'. These RNA structures correspond to the 5′-untranslated region of certain mRNAs and are able to bind specific small ligands such as lysine or flavin mononucleotide (FMN). Riboswitches form different structures in the presence and absence of the ligand affecting transcription elongation or translation. When riboswitches regulate the termination of leader mRNA transcription, sRNAs can appear as reaction products. Recent information about riboswitch architecture, the role of magnesium in the structure as well as the mechanism of riboswitch control are the focus of the review by Coppins, Hall and Groisman.

With the identification of more and more sRNAs, the roles of these regulators in global bacterial responses have received increasing attention. In this issue, Toledo-Arana, Repoila and Cossart evaluate the contribution of sRNAs in controlling pathogenesis, and Bejerano-Sagie and Xavier summarize what is known about the roles of sRNAs in quorum sensing. These reviews illustrate the importance of sRNAs in bacterial adaptation. They also provide the opportunity to reflect on the benefits of sRNAs as regulators including the low cost of synthesis and degradation, and the ability to integrate multiple inputs via seemingly redundant sRNAs. The ability of some sRNAs to promote mRNA degradation also can provide an irreversible step in signal transduction pathways. Other possible advantages such as the possibility for secretion and coupling of more than one function remain to be explored.

The topic of the final review in this series is quelling in *Neurospora crassa*. As described by Fulci and Macino,

quelling is a mechanism of post-transcriptional gene silencing that is related to RNA interference in animals and plants. Because many of the proteins required for quelling are homologs of the proteins required for RNA interference, *Neurospora* can serve as a microbial model organism for the study of the ubiquitous gene silencing phenomenon.

Outlook

This is an exciting time for the study of sRNAs; many of these interesting regulators have been identified but much remains to be learned.

Thus far, sRNAs have only been characterized in a relatively limited number of microorganisms. As more sRNAs are identified, will there be significant differences in the number of sRNAs encoded by different microorganisms? In addition, will the distribution of sRNA function vary between microorganisms? For example, will sRNAs that act by limited basepairing predominate in some organisms and sRNAs that act by extensive basepairing predominate in others?

sRNAs have been shown to have crucial roles in the regulation of iron homeostasis, outer membrane protein biogenesis, sugar metabolism, quorum sensing and survival in stationary phase. In what other physiological responses will sRNAs be found to play a role? Will sRNAs be found associated with every major stress response or are RNA regulators more advantageous under certain types of growth condition or stress?

What new functions of sRNA regulators remain to be identified? In addition, how many sRNAs will be found to have more than one function, as is the case for RNA III and might be the case for some riboswitches?

Although it has been generally established how sRNAs modulate mRNA stability or translation initiation by basepairing or mimicking secondary structures, many aspects of the regulation are not yet understood. For example, what constitutes productive basepairing and how does basepairing influence the regulatory outcome? Another unexplored area is the competition among sRNAs for targets and RNA binding proteins. Massé and colleagues describe the modelling of cellular iron metabolism including the role of the RyhB RNA, but efforts to model sRNA regulation are in their infancy.

Can sRNAs or what is learned about these regulators be exploited for biotechnological purposes? Generally applicable RNA silencing methods would be valuable tools for the study of bacteria in which knockouts are notoriously tedious to perform. One could imagine that synthetic oligonucleotides developed on the basis of what is known about sRNAs could potentially serve as antibiotics.

How are the different sRNAs related and how have sRNAs evolved? Are there common ancestors for sRNAs in bacteria? For example, are RsmXYZ of *Pseudomonas fluorescens* and CsrBC of *E. coli* true homologs or 'functional homologs' that have arisen from different ancestral genes? Are some sRNAs recent adaptations to stresses? The evolution of a transcript with limited basepairing with another RNA could occur reasonably frequently. In addition, what are the evolutionary relationships between the proteins that bind and act on the sRNAs? For example, how did the Dicer and RISC proteins of *N. crassa* evolve from bacterial RNase III and Argonaute-like proteins, which occur in *Streptomyces*, *Aquifex*, *Pyrococcus* and *Methanococcus*?

We look forward to following progress in addressing these exciting questions.

Reference

1. Jacob F, Monod J: **Genetic regulatory mechanisms in the synthesis of proteins**. *J Mol Biol* 1961, **3**:318-356.