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RNA techniques for bacteria

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Bacterial non-protein-coding RNAs (sRNAs)

Bacteria were the first source of the major discoveries that changed our perception of RNA. In 1982 in the laboratory of Sidney Altman, it was found that the catalytic component of RNaseP is an RNA, and in 1967 the first sRNA, the 6S RNA from *Escherichia coli*, was isolated; it took more than 30 years to unravel its function. Today, we know that the 6S RNA binds directly to RNA polymerase and interferes with transcription. The first examples of antisense RNAs were also discovered in bacteria whilst studying plasmid replication. This shows how important bacteria have been as model organisms for the discovery of novel fundamental principles.

The recent hype in bacterial RNA research is due to the significant discovery of sRNAs as central regulatory molecules: especially important is the impact of this on the field of microbial pathogenesis. This field has long focused on the functional role of proteins as regulators in pathogenic events; now it has become evident that sRNAs are key regulatory players for the physiological and virulent functions of bacteria. Their modes of action include transcription and translation control, RNA stability, maturation and processing. This strengthens the fascinating diversity of mechanisms that bacteria have developed in order to fight for survival.

The aim of the articles in this section is to review techniques for the analysis of RNAs in bacteria. A number of these techniques have been especially developed for bacterial sRNAs, others have been adapted for more 'general' RNA research. The idea behind the choice of topics for the section is 'pick your favorite RNA in your favorite bug' and choose the best and most efficient tools to study it. The papers provide a basis for techniques that are available to study their prediction, expression, stability, function and structure.

The papers describe the techniques available to identify novel regulatory sRNAs, search for, validate and characterize sRNA targets (mRNAs and proteins), study RNA expression, stability and possible maturation and processing events, analyze RNA sequences *in silico* and predict their secondary structures, experimentally determine RNA three-dimensional structures (free RNA or in complex with proteins or small molecules) and, finally, analyze gene transcription profiling in biological systems relevant for pathogenic bacteria. All

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together, answering those questions will help to decipher the role and mode of action of the studied RNAs. How are they expressed? How is their expression regulated? In which physiological and pathological events are they involved? What are their primary and secondary targets? How do they control their targets? How do they interconnect in the gene regulatory global network? In each review, the advantages and limitations of the methods are presented and discussed.

Discovery of sRNAs

In the first review, Altuvia provides an overview of the computational and experimental strategies that recently led to the identification of a number of sRNAs in bacteria. Every single method relies on specific parameters that set limits to the type of sRNAs that can be identified. Bioinformatic tools are used to search for sRNAs on the basis of sequence and/or structure conservation features, the parameters of which will differ among algorithms, thus allowing the discovery of distinct sets of sRNAs only. Genetic and biochemical approaches offer several advantages over computational methods, but have their limitations as well. Depending on the strategy used, the physiological conditions (e.g. growth phase, stress) of sRNA expression are taken into account and species-specific, unique, non-conserved, *cis*-encoded and *trans*-encoded or processed RNAs can be detected. All together, Altuvia emphasizes the necessity to use a combination of computational and experimental methods to search for sRNAs in bacteria. Once sRNAs are identified, an independent validation of their expression and biological significance is also essential.

Finding sRNA targets

Vogel and Wagner summarize the methodologies available to search for, validate and characterize cellular sRNA targets. In this paper, primary targets (defined as 'true targets', e.g. mRNAs or proteins that physically interact with an sRNA, leading to modulation of the target function) are distinguished from secondary targets ('downstream targets', the functions of which are regulated by an sRNA true target or another sRNA downstream target). As for the identification of sRNAs, computational, genetic and biochemical methods to find targets are based on specific and distinct parameters and thus have all their advantages and limitations. The combined use of diverse technologies will increase the number of target predictions. These predictions have then to be functionally validated. Vogel and Wagner briefly review strategies that have been employed thus far to study sRNA–target interactions *in vitro* and *in vivo*. This constitutes the first step towards the characterization of the mechanism by which an sRNA can affect the target function and the assignment of a potentially more global role of the sRNA in the physiology or virulence of the bacteria.

Characterization of sRNAs

In his paper, Condon reviews recent advances in the field of RNA decay and maturation in the two model organisms *E. coli* and *Bacillus subtilis*. The methods that enable the analysis of RNA degradation and maturation through endonucleolytic and exonucleolytic cleavage by RNases and associated proteins remain largely 'classical'. Recent studies in *B. subtilis* have uncovered enzymes and pathways strictly different from those already described in *E. coli*. Interestingly, knowledge of RNA decay and maturation in pathogenic bacteria is very limited and recent studies in this field have implicated these processes in the expression of important virulence factors. Considering the role of regulatory sRNAs in mRNA target degradation, future studies in this research area in pathogens will be essential to understand the mechanistic pathways.

Jossinet, Ludwig and Westhof present a selection of the most recently developed computational tools for the analysis of RNA sequences and their putative structures. The novel algorithms have been adapted to the multifaceted RNA functions and are tightly linked to structural information. These tools include algorithms for the construction of multiple alignments for 'structural RNAs', secondary structure prediction (which constitutes an essential step in the determination of the RNA architecture because it produces strong constraints for the tertiary structure), finding common structural motifs among several related RNA sequences and searching for structural signature-based homologies in genomic databases. A number of those bioinformatics resources are publicly accessible through a web interface. Jossinet and colleagues refer to a recently created consortium whose aim is to develop abstraction and mathematical models, which would allow the integration and visualization of all data generated from various algorithms in a reasonably fast and comprehensive manner.

In the next manuscript, Felden compares biochemical and biophysical techniques that have been employed to study the structure and folding of an RNA, either free or in complex with specific ligands. RNAs form intricate 3D structures and can interconvert between multiple functional states, both processes being essential for the proper functioning of the RNA. Felden presents X-ray crystallography as the most powerful technology, which enables the capture of structural atomic details of large, complex and naturally occurring functional RNAs. When X-ray crystallography cannot be used, the best approach is to combine diverse complementary methods for an optimal 3D structure prediction. The application of these methods for the determination of regulatory sRNA structures will definitely help the understanding of their mode of action.

Expression profiling

The last two reviews focus on transcriptional profiling methodologies and recent developments in their application to relevant biological models: biofilm communities and host–pathogen interactions. An and Parsek discuss the suitability of current experimental designs to study the bacterial transcriptome in the context of biofilm formation and development, including motility, production of extracellular matrix or adherence to it, metabolism and stress. Biofilms are complex structures that are temporally and spatially subjected to distinct physiological conditions and whose growth is associated with significant changes in metabolism. An and Parsek comment on the technical limitations in addressing the heterogeneity inherent to biofilms. Future challenges will be to develop novel transcriptional profiling strategies that consider unique patterns of gene expression related to specific regions of biofilms and distinct functional subpopulations in the community. Waddell, Butcher and Stoker review microarray experimental approaches to analyze global expression profiling of both bacterial pathogens and hosts in *ex vivo* tissue culture, animal models and human disease contexts. Strategies to overcome technical problems, such as the RNA extraction process, the requirement to separate eukaryotic from bacterial gene expression patterns and the proper controls to use, are presented. Difficulties in integrating gene expression data obtained from *in vitro*, *in vivo* and *ex vivo* models of infection into a comprehensive network of expression interactions that occur during human disease are finally discussed.

Conclusions

In summary, diverse research areas have recently converged on RNA as the unifying principle in regulating many physiologically relevant processes. This timely section provides an overview of RNA techniques that have been recently developed in a very interdisciplinary effort. We want to stress that, in order to study your choice of RNA in your

favorite bug, you will have to use a panel of different technologies, and the best way to do so is to cooperate with RNA technique experts.

It is evident that most of the techniques we discuss are still in need for further improvement; especially for finding targets and for the discovery of low and short expressed RNAs, the existing technologies are not yet satisfactory. It is our hope that these technical guidelines will help to stimulate further advances in the RNA field in microbiology.