

The Case of Agentive Metaphors Bacterial RNA Biology

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Description

Bacterial RNA biology represents a fascinating and intricate field of study that delves into the diverse roles and regulatory mechanisms governing RNA molecules within bacterial cells. As essential players in gene expression and cellular function, bacterial RNAs contribute significantly to the adaptability, survival and virulence of these microorganisms. This commentary aims to shed light on some key aspects of bacterial RNA biology, highlighting recent advancements and the broader implications for our understanding of bacterial physiology.

Bacteria are an exceedingly diverse group of organisms whose molecular exploration is experiencing a renaissance. Moreover, increasing interest in members of the human microbiota and environmental microbial communities has highlighted the importance of understudied bacterial species with largely unknown transcriptome structures and RNAbased control mechanisms. Collectively, this creates a need for global RNA biology approaches that can rapidly and comprehensively analyze the RNA composition of a bacterium of interest. We review such approaches with a focus on RNA-seq as a versatile tool to investigate the different layers of gene expression in which RNA is made, processed, regulated, modified, translated and turned over.

Given the central importance of proteostasis in co and post-translational folding of Cystic Fibrosis Transmembrane Regulator (CFTR), we performed an siRNA High-Throughput Screen (HTS) using a library composed of 2569 siRNAs targeting individual PN components, including the translational machinery, cytosolic and ER luminal chaperones, the degradation system and post-translational regulatory proteins. The library was composed of four siRNA sequences per target and the screen was performed in triplicate.

Role of Small Regulatory RNAs (sRNAs)

Small regulatory RNAs have gained prominence in recent years as key regulators of gene expression in bacteria. These short transcripts, typically ranging from 50 to 500 nucleotides, function by base-pairing with target mRNAs, affecting their stability and translation. The discovery of numerous sRNAs has expanded our understanding of post-transcriptional regulation,

revealing a complex network of interactions that fine-tune bacterial responses to changing environmental conditions.

We developed a novel algorithm to address how these non-CFTR interacting, siRNA hits are connected to CFTR, which we called Network-Augmented Genomic Analysis (NAGA). We first expanded the CFTR interaction network from its 576 proteins, as characterized in the CFTR interactome. To accomplish this, we utilized publicly available protein interaction databases to assemble an undirected network of human protein interactions. For each interaction, a confidence score was calculated, reflecting the reliability of its combined experimental evidence, providing weight to the CINK, where more reliable interactions are favored during a shortest path analysis. CFTR-interacting proteins that connected two or more siRNA hits to CFTR were classified as hub proteins.

The foundations of bacterial RNA biology were laid by detailed genetic and biochemical studies of individual transcripts in a relatively small number of model bacteria. While bottom-up analyses of individual transcripts such as by northern blot detection remain of value, particularly in the quest to elucidate molecular mechanisms, the field has increasingly embraced global methods to experimentally study entire transcriptomes and discover RNA functions. There are obvious reasons why studying RNA on a global level is advantageous; it facilitates the transition from reductionist to systems-level analyses, placing observations from studying individual molecules or genomic loci into an overall cellular context. This not only helps to reveal their general importance but also uncovers major features of whole RNA classes. To provide a few examples, it was global RNA ligand profiling that defined Hfq-associated sRNAs as a distinct class of post-transcriptional regulators in bacteria. Likewise, global RNA analysis highlighted the abundant tracrRNA in Streptococcus pyogenes, a key component in developing CRISPR-Cas into a revolutionary genome-editing tool. More recently, a transcriptome-wide assessment of biochemical behavior guided the discovery of ProQ as a previously unrecognized global RBP in E. coli and Salmonella, two organisms in which post-transcriptional regulation had been intensively studied for decades already.

Annotation of Transcripts

RNA-seq has revolutionized transcriptomics with its high sensitivity and quantitative digital output. For example,



RNA-seq on total RNA from the gastric pathogen Helicobacter pylori provided the first full singlenucleotide-resolution transcriptome map of a bacterium. RNA-seq rapidly became a popular approach to studying gene expression changes in many other pathogens.

More recently, growing sequencing capacity has enabled the development of dual RNA-seq, in which the full transcriptomes of a bacterial pathogen and its host are sequenced in tandem without prior physical separation. Dual RNA-seq enables the correlation of gene expression changes in the pathogen with those occurring in the host.