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Forum

RNA Chaperones Step Out of Hfq's Shadow

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The stability and function of regulatory small RNAs (sRNAs) often require a specialized RNA-binding protein called an RNA chaperone. Recent findings show that proteins containing a ProQ/FinO domain constitute a new class of RNA chaperones that could play key roles in post-transcriptional gene regulation throughout bacterial species.

Deep-sequencing transcriptional analyses have revealed the extent by which bacterial genomes are pervasively and unexpectedly transcribed. This transcription generates thousands of RNA species that may have critical roles in the cell [1,2]. Among these, regulatory noncoding sRNAs function by duplexing with their mRNA targets. This base pairing can have positive or negative effects on the stability and/or the translation of the targeted mRNAs, thereby directly affecting cell physiology. Regulatory RNAs can be *cis*- or *trans*-acting. *Cis*-acting RNAs, also called antisense RNAs, are encoded by the non-protein-coding DNA strand of the target gene, and thus they are perfectly complementary to their partner mRNA (for review, see [3,4]). In contrast, *trans*-acting sRNAs are transcribed from a unique, independent locus and target multiple mRNAs through interactions that involve a much more limited complementarity than is observed between sense–antisense RNA pairs (for review, see [5]). The interactions between regulatory sRNAs and their targets are often assisted by specialized RNA-binding proteins called RNA chaperones (Box 1). For over two decades, the Hfq protein was the only identified bacterial RNA chaperone able to assist *trans*-acting sRNAs in their function [5]. However, in light of many studies obtained in diverse bacterial species, its role outside of Enterobacteriaceae has been debated and controversial [6]. In addition, a number of *trans*-acting sRNAs do not require Hfq for base-pairing, and many species appear to lack Hfq altogether, suggesting that other classes of bacterial RNA chaperones may exist [5,7]. Indeed, the RocC protein of *Legionella pneumophila* was recently characterized as a non-Hfq-like RNA chaperone of a *trans*-acting and multitarget sRNA, RocR [8]. RocC stabilizes RocR and, together, they post-transcriptionally control the expression of genes that are important for natural transformation, a major mechanism of horizontal gene transfer [8]. Another recent study showed that the ProQ protein of *Salmonella enterica* is an RNA-binding protein that interacts with and stabilizes over 50 highly structured antisense and *trans*-acting sRNAs [9]. Interestingly, RocC and ProQ are two of the many proteins distributed throughout the bacterial taxa that possess a conserved domain initially characterized in the plasmid-encoded FinO protein and annotated as the 'ProQ/FinO' domain (PFAM domain 04352) [8] (Figure 1). Although most of the members of this diverse family of proteins are of unknown function, many studies, essentially on the FinO protein, helped shed light on the function of this domain and its potential importance in gene regulatory mechanisms (see [10] and references therein).

The well characterized FinO protein is encoded by genes born by plasmids of the IncF family. FinO inhibits the conjugation of these plasmids by controlling the function of the antisense sRNA FinP [10]. It acts as an RNA chaperone for this sRNA, stabilizing it and facilitating its interaction with the complementary 5' untranslated region (UTR) of the *traJ* mRNA. The sense–antisense pairing FinP/*traJ* mRNA prevents translation of the TraJ transcriptional activator of the plasmid *tra* operon which encodes essential proteins of the conjugation machinery, thereby preventing conjugation [10]. Structural studies and assessment of the biochemical properties of FinO indicate that the ProQ/FinO domain of this protein has a strong affinity for structured RNAs, but that the N-terminal domain (PFAM domain 12602, FinO_N) is essential to stimulate the sense–antisense RNA interaction between FinP and the 5′ UTR of *traJ* mRNA [10]. *In vitro* studies using the FinP/*traJ* system also confirmed the RNA-binding nature of the ProQ/FinO domain in two other proteins: NMB1681 of *Neisseria meningitidis* and ProQ of *Escherichia coli*. Interestingly, these proteins were also capable of enhancing the FinP/*traJ* mRNA base pairing even though they lack a FinO_N domain [10]. It was hypothesized that the domains outside of the ProQ/FinO domain (the N- and C-terminal extensions of NMB1681 and the ~100-amino acid C-terminal domain of ProQ) were responsible for the strand-exchange activity observed in those experiments [10]. But, as *in vivo* studies of these proteins in their natural context were lacking, it was difficult to assess their real function. The

Box 1. RNA Chaperones, RNA-Binding Proteins with a Twist

An RNA-Binding Protein (RBP) is a protein with a specific or unspecific affinity for RNA molecules (e.g., ribosomal proteins, the translation repressor CsrA). An RNA chaperone is a type of RBP that facilitates or enhances the proper folding of an RNA molecule into its functional secondary structure without the need for ATP consumption (e.g., the cold shock proteins CspA and CsdA of *Escherichia coli*) (see [12] and references therein). RNA chaperones can function to prevent RNA misfolding by binding directly to the nascent RNA and can also enhance and stabilize the annealing of internal RNA–RNA pairs. Alternatively, certain RNA chaperones have been hypothesized to bind misfolded RNA, thereby provoking the unfolding of the structure and its subsequent refolding. In addition, certain RNA chaperones possess a strand-exchange activity which allows the duplexing of RNA molecules (e.g., Hfq, FinO, RocC).

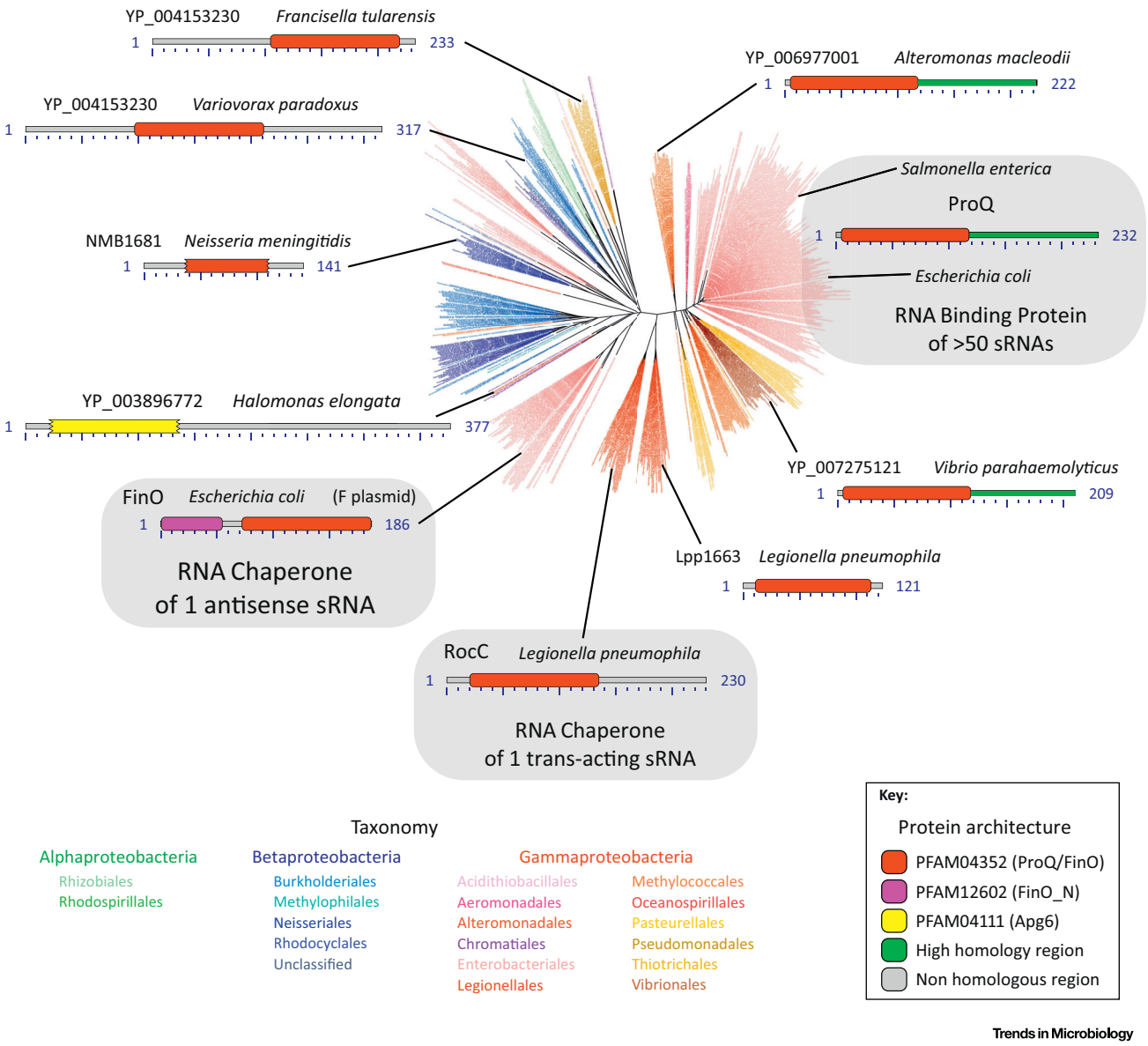

Figure 1. Diversity of the ProQ/FinO Domain-Containing Proteins. The maximum likelihood phylogenetic tree of the 674 protein sequences containing a ProQ/FinO domain found in 2775 complete prokaryotic proteomes is presented with colors corresponding to taxonomic main lineages. The cartoons show the architecture of different ProQ/FinO domain-containing proteins. For each, conserved domains are shown, as well as regions of high similarity. This figure is adapted from reference [8].

recent study on RocC adds weight to the idea that the domains associated with the ProQ/FinO domain can influence the function of the protein. It showed that even though the ProQ/FinO domain is responsible for the interaction with RocR, the C-terminal domain of RocC is essential to observe an effect on the mRNA targets [8]. It is not yet known whether *S. enterica* ProQ (92% identity with *E. coli* ProQ) acts as an RNA chaperone for all its partners, that is, if it facilitates base pairing with their targets [9]. However, if this is the case, it would be interesting to know if the C-terminal domain is particularly important for this function.

Importantly, the homology between the three studied proteins of the ProQ/FinO domain-containing protein family (FinO, RocC, and ProQ) is restricted to the ProQ/FinO domain. In fact, a comparison between all the different members of this family shows a great variability of the C- and N-terminal domains associated with the ProQ/FinO domain (Figure 1) [8]. Since FinO, RocC, and ProQ each appear to carry out different functions (RNA chaperone of an antisense sRNA, RNA chaperone of a *trans*-acting multitarget sRNA, and general RNA-binding protein), it is tempting to speculate that the C- and N-terminal extensions provide specificity

for these proteins. For example, the ProQ/FinO domain-containing proteins found in the Vibrionales, Alteromonadales, Aeromonadales, and Enterobacteriales orders all share the same C-terminal region (high homology region, Figure 1). It might mean that these proteins function similarly in all these species as they do in *Salmonella*, especially since it was hypothesized that this sequence could constitute a functional domain on its own [11]. Future molecular and structural studies will undoubtedly provide more insights into the mode of action of these different proteins and give us a better understanding of the molecular mechanisms underlying their ability to facilitate diverse RNA–RNA interactions. Yet, the diversity of sequences flanking the ProQ/FinO domains remains intriguing (Figure 1). Can all these diverging protein sequences contribute to RNA–RNA interactions, or do they perform other function(s)? For instance, what may be the specificity or function conferred by a coil-coiled domain when associated with a ProQ/FinO domain, as found in *Halomonas* species (PFAM domain 04111, Figure 1)? Furthermore, the taxonomy revealed that no ProQ/FinO domain-containing protein is present in Firmicutes. As Hfq is also not always present in these Gram-positive bacteria, it suggests that other types of proteins can fulfill the role of RNA chaperones.

A diverse family of proteins with RNA chaperone properties has finally emerged from the shadow cast by Hfq but we have yet to unveil the various functions they play in so many species. The evolutionary history of these proteins, how they potentiate the function of their target sRNA, and most importantly, the biological processes they control, remain open questions that need to be answered.

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