REGULATION OF BACTERIAL RIBOFLAVIN GENES BY A CONSERVED RNA STRUCTURAL ELEMENT

Vitreschak A.G. [1]***, Rodionov D.A.*** [2*]***, Mironov A.A.*** [2,3]***, Gelfand M.S.*** [2,3]

[1] Institute for Problems of Information Transmission, Moscow, 101447, Russia
[2] State Scientific Center GosNIIGenetika, Moscow, 113545, Russia, e-mail: rodionov@genetika.ru
[3] Integrated Genomics–Moscow, P.O.Pox 348, Moscow, 117333, Russia
[*]Corresponding author

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Resume

We have identified riboflavin biosynthesis (RB) genes in almost all available bacterial genomes. In many diverse bacteria, the RB genes are regulated by a conserved RNA regulatory element *RFN*. Comparison of the nucleotide sequences around the *RFN* elements has revealed a set of conserved RNA secondary structures. In gram-positive bacteria, it includes the *RFN* element, terminator hairpin, and alternative antiterminator with the main stem overlapping both *RFN* and the terminator. In contrast, gram-negative bacteria have a sequestering hairpin that overlaps the Shine–Dalgarno (SD) sequence or the start codon of the first gene in the operon. Consequently, involvement of transcription and translation attenuation mechanisms in the regulation of the RB genes is proposed. Analysis of the operon structure shows that *RFN* predominantly regulates single RB genes in proteobacteria and the RB operon in most gram-positive bacteria. Moreover, single RB genes seem to be regulated at the level of translation, whereas the RBS operons are predicted to be regulated at the level of transcription. Analysis of the *RFN*-based regulation and operon structure allowed us to predict new riboflavin transporters, namely, *ypaA*, *impX,* and *pnuX* in the gram-positive bacteria and *rfnT* in rhizobia. Analysis of the *RFN* architecture, operon structure, and protein phylogeny identified several cases of likely horizontal transfer in *F. nucleatum* and two proteobacteria.

Introduction and Motivation

Riboflavin (vitamin B2) is an essential component of the basic metabolism because it is a precursor of the coenzymes flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN). Many microorganisms and all plants are able to synthesize riboflavin, but it is not produced by higher animals. The most well-studied system of riboflavin biosynthesis in bacteria is the *ribGBAH* operon of *Bacillus subtilis.* Furthermore, the riboflavin operons were studied in *Bacillus amyloliquefaciens* (Gusarov et al., 1997), *Actinobacillus pleuropneumoniae* (Fuller, Mulks, 1995), and *Photobacterium phosphoreum* (Lee et al., 1994). Recently, *B. subtilis* was shown to contain the riboflavin transport system YpaA (Kreneva et al., 2000). In contrast to *B. subtilis*, the riboflavin biosynthesis genes of *Escherichia coli* do not form a single operon but are scattered over the chromosome. Metabolic studies gave no evidence for any regulation of the riboflavin biosynthesis genes in *E. coli* (*E. coli Book*, ASM, 1994). On the other hand, flavin nucleotides, but not riboflavin, have an effector function for regulation of the riboflavin operon in *B. subtilis* (Lee et al., 2001). The regulatory region *ribO* located between the promoter and the coding region of the *ribGBAH* operon is involved in regulation. Recently, strong conservation of these regions in diverse bacteria was discovered. Moreover, a conserved RNA structure with five hairpins (the *RFN* element) corresponding to the *ribO* region was found to be involved in regulation of the riboflavin operon (Gelfand et al., 1999). However, the regulatory mechanism of riboflavin genes was not known. It is very interesting to find new *RFN* elements in available genomes and to analyze the regulation of riboflavin genes (see Results). Moreover, a possible regulatory mechanism of expression of riboflavin genes is suggested.

Results and Discussion

We have applied the RNA PATTERN program to scan all the available bacterial genomes for candidate *RFN* elements and identified the riboflavin biosynthesis genes in the listed bacterial genomes by similarity search. Totally, 61 *RFN* elements were found in 49 eubacterial genomes. All these elements are located upstream of the RB genes or potential riboflavin transport genes. Only spirochetes, mycoplasmas, and rickettsia have neither riboflavin genes nor *RFN* elements. The traditional RB gene names are different in *E. coli* and *B. subtilis,* and, for consistency, we use the *E. coli* gene names throughout. Thus, the *B. subtilis ribG*, *rib,* and *ribA* genes are renamed here to *ribD*, *ribE* and *ribBA*, respectively.
The riboflavin transporter gene *ypaA* was found in all the studied genomes of the *Bacillus/Clostridium* group except for *Bacillus halodurans*. Moreover, YpaA seems to be the only source of riboflavin in *Enterococcus faecalis* and *Streptococcus pyogenes*, as these genomes lack RB genes. Two more genomes containing *ypaA* are *Atopobium minutum* (actinomycete) and *Thermotoga maritima*. A *RFN* element precedes *ypaA* in the former, but not in the latter genome. Other actinomycetes seem to have a new, different riboflavin transporter. In *Thermomonospora fusca* and *Streptomyces coelicolor*, the RB operon consists of *ribE*, *RTFU01116* (named here *pnuX)*, *ribBA* and *ribH,* and has an upstream *RFN*

element. The *pnuX* gene is homologous to the nicotinamide mononucleotide transporter *pnuC* from enterobacteria and encodes a protein with six predicted transmembrane segments. Orthologs of the *pnuX* gene exist in two other actinomycetes, *Corynebacterium diphtheriae* and *Corynebacterium glutamicum*, and in the latter, *pnuX* is preceded by a *RFN* element. One more candidate riboflavin transporter, *impX*, is found in *Fusobacterium nucleatum* and *Desulfitobacterium halfniense,* with upstream *RFN* elements in both cases.

Most proteobacteria have some redundancy of the RB genes due to paralogs of the *ribH*, *ribBA* and *ribE* genes. Moreover, some genomes contain not only the fused *ribBA* gene, but also additional single *ribB* or *ribA* genes.

Phylogenetic analysis of the RB protein sequences detects possible horizontal transfer of the *ribDE(BA)H* operon from the *Bacillus/Clostridium* group to two Pasteurellaceae genomes, *Haemophilus ducreyi* and *Actinobacillus pleuropneumoniae.* The *RFN* elements upstream of this operon are of the non-gram-negative type.

Recently, it was shown that flavin mononucleotides (FMN) regulate expression of the RB operon in *B. subtilis* (Lee et al., 2001). We propose here a possible mechanism of the FMN-mediated regulation via the *RFN* element (Fig.). In general, two different types of regulation are suggested, the attenuation of transcription via antitermination mechanism and the attenuation of translation by sequestering of the Shine-Dalgarno box.

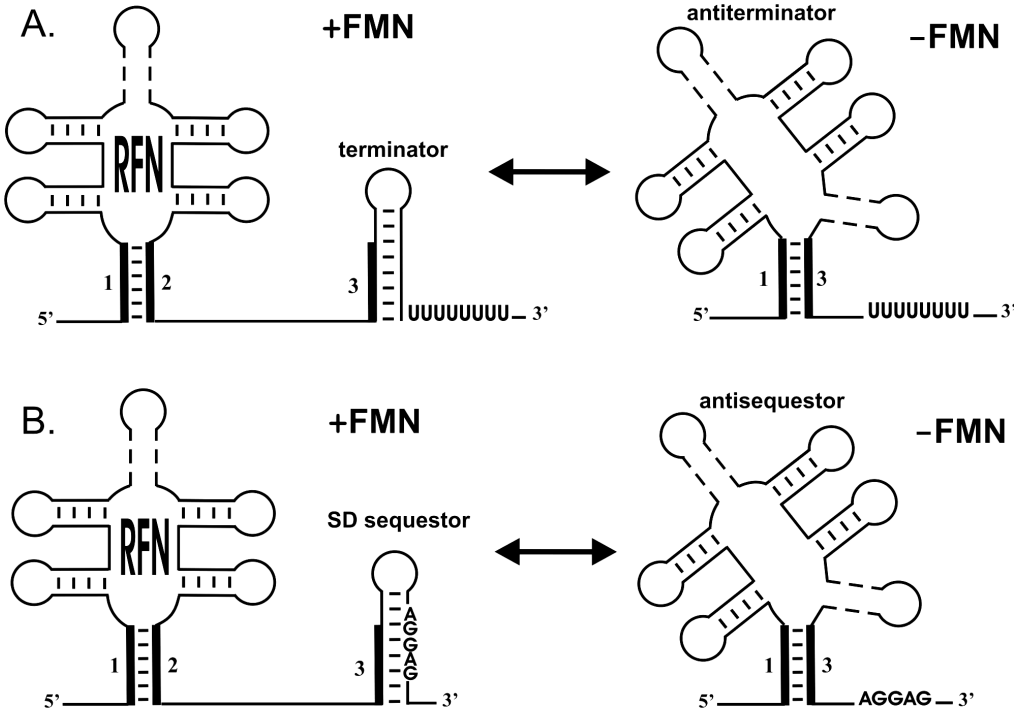

Fig. Predicted mechanism of the *RFN*-mediated regulation of riboflavin genes: (A) transcription attenuation and (B) translation attenuation.

In gram-positive bacteria, *Thermotoga maritima* and *Chloroflexus aurantiacus,* we have found terminator-like RNA structures located between the predicted *RFN* element and translational gene start of RB genes (Fig.). We found complementary fragments of RNA sequences that partially overlap both the first helix of *RFN* and the left stem of the terminator. Furthermore, these complementary fragments always form the first main helix of a more stable new alternative secondary structure with ΔG smaller than ΔG of the *RFN* element. We predict that this structure functions as an antiterminator, which is an alternative to both the *RFN* element and the terminator.

In other cases, mostly in gram-negative bacteria, the RNA hairpins downstream of the *RFN* element sequestor the ribosome-binding site (the Shine–Dalgarno box). In most cases, we have found a highly conserved sequence, GCCCTGA, which overlaps the proposed sequestor hairpin and is complementary to helix 1 of the *RFN* element. These two complementary sequences always form the stem of the RNA secondary structure, called here antisequestor, which is more stable than the *RFN* element. The proposed mechanism of translational regulation of the RB operons is similar to the termination–antitermination mechanism described above, but involves the SD-sequestor instead of the terminator.

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