The Influence of Polyamines on the Expression of *Escherichia Coli* Ribosome Hibernation Factor Raia

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Abstract. RaiA is one of the main ribosome hibernation factors in *Escherichia coli.* Like other ribosome hibernation factors, this protein reversibly inhibits translation under stress conditions. According to published data, being induced by indole, RaiA is involved in bacterial persistence, which is considered to play important role in the recalcitrance of chronic infections to antibiotics. Previously, we showed that the *raiA* expression on the transcriptional level is stimulated by polyamines, in addition to indole. In this work, we investigated the influence of polyamines on the *raiA* expression on the translational level. We obtained the predicted secondary structures of *raiA* mRNA, the analysis of which showed the presence of the bulged-out region in the initiation site with a high probability. This may be a sign of gene involvement in the polyamine modulon. We constructed translational *raiA::lacZ* reporter fusion. Using this genetic construct, we studied the effects of polyamines on the *raiA* expression through an addition of putrescine, cadaverine or spermidine at concentrations of 1 mM and 2 mM. According to the results, the *raiA* expression is primarily stimulated by cadaverine at the stationary phase.

1 Introduction

RaiA is one of the main ribosome hibernation factors in *Escherichia coli.* RaiA has been shown to bind and inactivate 70S ribosomes. This factor blocks A- and P-sites of ribosomes. Moreover, the binding site of this protein overlaps with the binding sites of the factors IF1, IF3, EF-G that disrupts the normal conduct of the initiation and elongation processes. Like other ribosome hibernation factors, the function of RaiA is the reverse inhibition of costly protein synthesis under the nutrient limitation and other stress conditions. This is important for the preventing the waste of resources, as well as the preservation of functional ribosomes to the rapid restoration of translation, when the favorable conditions return [1]. Due to their ability to inhibit protein synthesis, the ribosome hibernation factors are able to be involved in the formation of the dormant state and persistence [1, 2]. The dormancy is the metabolically inactive, nondividing state. In

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turn, persisters are rare dormant variants of regular cells that are highly tolerant to antibiotics. After removing the antibiotics, persister cells are able to resume the growth. Due to this, the persistence is able to be the cause of relapses of infectious diseases [3]. Being induced by indole, RaiA is involved in a persister cell formation [4]. Previously, we showed that, in addition to indole, polyamines are able to stimulate the raiA expression on the transcriptional level [5]. These metabolites have many different effects on cellular processes [6], including those carrying out through the modulation of gene expression [7]. Polyamines, along with indole, are involved in biofilm formation, antibiotic tolerance and others [8, 9, 10, 11]. In this work, the effects of polyamines on the *raiA* translation expression were investigated.

2 Materials and methods

2.1 Bacterial strains and growth conditions

We used E. coli strains BW25141 *(F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), Δ(phoB-phoR)580, λ- galU95, ΔuidA3::pir+, recA1, endA9(del-ins)::FRT, rph-1, Δ(rhaD-rhaB)568, hsdR514)* [12] and BW252141 *raiA::lacZ* (as BW25141, but *λRS45 raiA171::lacZ(Hyb)*). The cells of strain BW252141 *raiA::lacZ* were grown in M9 (+ 0.4% glucose) medium. Polyamines putrescine, cadaverine or spermidine (Sigma, USA) at 1 mM and 2 mM were added to cultures at 2 h of cultivation. The cultures unsupplemented with polyamines served as the control.

2.2 Construction of the translational *raiA::lacZ* reporter fusion

BW252141 strain with the chromosomal *raiA::lacZ* fusion was obtained by using pRS552/λRS45 system [13]. To construct the translational *raiA::lacZ* fusion, the oligonucleotides 5'-ATAATC**GGATCC**CGTTTGGTCCGTATT-3' and 5'-ACCAGA**GGATCC**TTAGGTGTATTGAT-3' were used to amplify 1018 bp fragment from genomic DNA. pRS552 plasmid contains the unique sites EcoRI and BamHI for molecular cloning. However, analysis of the sequence of *raiA* gene showed that EcoRI site is natively present in the promoter of *raiA* gene. In this regard, the molecular cloning was performed by using only the BamHI restriction endonuclease, while pRS552 plasmid was treated with the alkaline phosphatase. The insert was transferred from pRS552 plasmid into λRS45 phage by homologous recombination and finally integrated into the chromosome of BW252141 strain as in a single copy, so as a part of the prophage. The in-between and resulting genetic constructs were verified by PCR and sequenced. Sequencing was performed by Evrogen (Moscow, Russia). All used enzymes were purchased from Thermo Fisher Scientific (USA).

2.3 β-galactosidase assay

The gene expression in strain BW25141 *raiA::lacZ* was detected by the β-galactosidase activity [14].

2.4 mRNA secondary structure prediction

mRNA secondary structures were predicted by the RNAfold [15] and vsfold [16] programs.

2.5 Statistical analysis

Statistica for Windows 5.0 (StatSoft, Inc., 1995) software was used in the processing of experimental data, presented as mean values of 5 independent experiments ± standard deviation (Mean±SD). Statistical significance was evaluated with the *t*-test for independent samples. Means differences were considered to be statistically significant at $P \leq 0.05$ (* - notation in graphs).

3 Results and discussion

Polyamines are involved in a variety of cellular processes, including the regulation of gene expression. Genes, whose expression on the translational level is stimulated by polyamines, are combined into the polyamine modulon. There are various mechanisms, by which polyamines are able to stimulate the gene expression on the translational level, and, accordingly, there are specific features in the mRNA structures of such genes. First, these mechanisms include the stimulation of translation initiation of genes that have unusually long distance between start codon and Shine-Dalgarno sequence. Polyamines are able to shorten this distance by introducing «bend» in the mRNA structure in this region. Secondly, polyamines are able to stimulate the translation initiation of genes that have inefficient start codon. Thirdly, another mechanism is suppression and +1 frameshift on nonsense codon [7]. Moreover, these mechanisms include stimulation of translation initiation of genes that have the secondary structure «bulged-out region» between the start codon and Shine-Dalgarno sequence. Spermidine has been shown to be able to relax this structure, promoting translation [17]. We analyzed the structure of *raiA* mRNA, focusing on the presence of specific features characteristic for polyamine modulon genes, using GenBank and EcoCyc [18] databases. The *raiA* mRNA has the usual start codon AUG. The most similar to the consensus *E. coli* Shine-Dalgarno sequence (AGGAGGU) is the AAGAGGU sequence, located at a usual distance (7 nucleotides) from the start codon. In addition, there is no information about the presence of nonsense codons in the coding region of *raiA* mRNA. Using RNAfold [15] и vsfold [16] programs, we obtained the predicted secondary structures of *raiA* mRNA (Fig. 1). The results of both programs usage are similar in the specific region and showed the presence of the bulged-out region before the start codon at a distance of 3 nucleotides with a high probability. This gives an occasion for the assumption of polyamine-dependent manner of *raiA* expression, that has not been studied before.

We investigated the influence of polyamines on the *raiA* expression on the translational level, when putrescine, cadaverine or spermidine at 1 mM and 2 mM were added to cultures at 2 h of cultivation (Fig. 2). The results showed that *raiA* expression was low at the exponential phase, but significantly increased at 24 h of cultivation and remained at a high level throughout the stationary phase. At the same time, from 24 h to 96 h of cultivation, the stimulating effect of cadaverine at 1mM was observed. In contrast, putrescine at 2 mM positively influenced on the *raiA* expression only at 48 h of cultivation, while spermidine had no effect. Thus, cadaverine produced the most significant effect on the *raiA* expression. Moreover, this effect occurred at the stationary phase, when this polyamine is maximally accumulated in cells as a normal metabolite under natural conditions [11]. The obtained data are also in agreement with the function of RaiA, which is the inhibitor of costly translation under starvation and other stresses [1]. Although the mechanism of modulation of gene expression through the relaxation of bulged-out region was previously shown for spermidine [17], cadaverine remains unexplored from this point of view. Therefore, the data we obtained may serve as a prospect for further research.

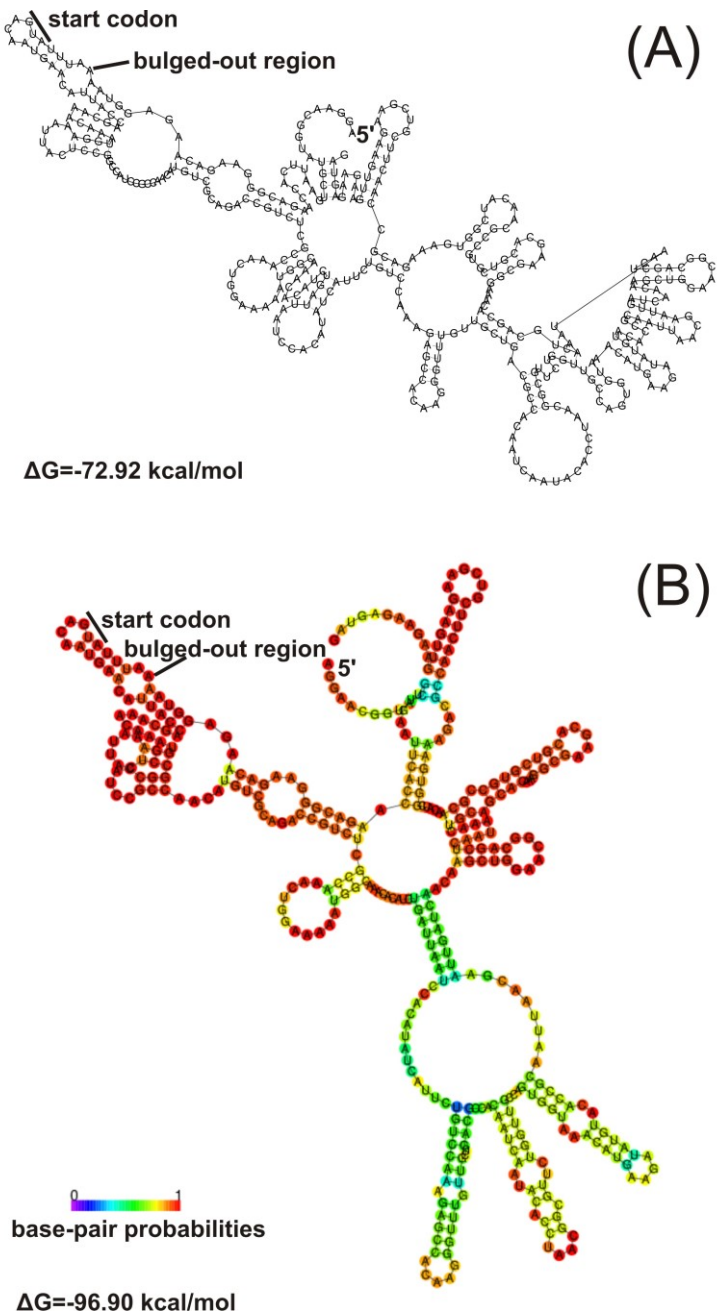

Fig. 1. Predicted secondary structures of *raiA* mRNA obtained by using vsfold (A) и RNAfold (B) programs.

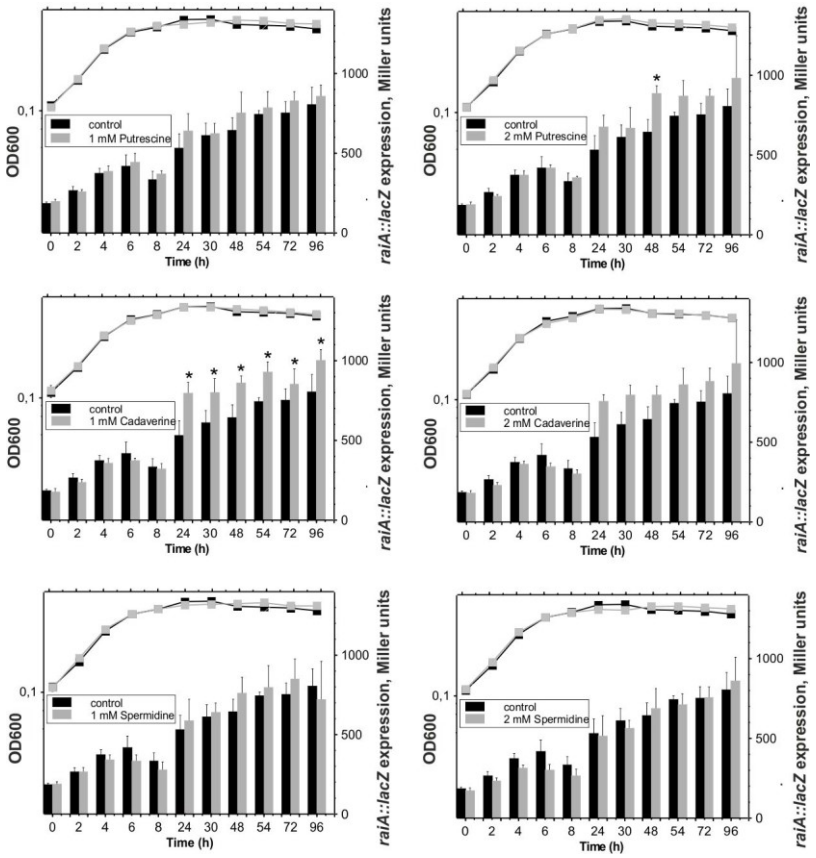

Fig. 2. The effects of putrescine, cadaverine and spermidine additions on the *raiA* expression on the translational level. Curves - OD600 (optical density), columns – *raiA::lacZ* expression.

4 Conclusion

The analysis of the predicted secondary structures of *raiA* mRNA showed with a high probability the presence of the bulged-out region before start codon at a distance of 3 nucleotides. Polyamines are able to relax this secondary structure [17]. The most significant stimulating effect on the *raiA* expression was produced by cadaverine. Moreover, this effect occurred at the stationary phase, when bacteria are affected by various stresses and an increase in the level of persisters occurs [4]. There are a few data on the involvement of both polyamines and RaiA in persistence [4, 11], which is one of the causes of recurrence of infectious diseases [4]. Thus, the effect of polyamines on the *raiA* expression on the translational level was shown for the first time. The results of this work can serve for further studies on the mechanism of stimulation of the *raiA* expression on the translational level by cadaverine and their cooperative role in persistence.

Acknowledgements

This work was supported by Russian Science Foundation [grant number 18-73-10156].

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