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NEW INSIGHT INTO THE MECHANISM OF ACTION OF ε-ELEMENT ENHANCER IN *E. COLI*

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ABSTRACT

Epsilon element (ε-element) is a complex translational enhancer capable of promoting the prokaryotic translation efficiency in combination with the Shine-Dalgarno (SD) sequence. However, the detailed mechanism of action of ε-element is unknown, and there is no single hypothesis to explain the available evidence. We here propose a new hypothesis for the mechanism of action of ε-element from the 5′-untranslated region (UTR) of the 10 gene of phage T7 during translation initiation in *Escherichia coli*. The mechanism of action was considered to be driven by the complementary interaction of ε-element (and its variation εII) with the 3′-end of *E. coli* 16S rRNA, specifically with the 45th hairpin region. This hypothesis was then applied to currently available data of the influence of ε-element on protein expression in bacterial cells. Several 5′-UTR sequences were constructed containing variations of ε-element, and their properties were predicted according to this new hypothesis and confirmed by measuring the protein expression level. We further applied temperature stresses (cold and heat shock) to these variants to examine their influence on protein expression controlled by various enhancers. Overall, the εII_SD and ε_SD enhancers could significantly increase the protein expression level in *E. coli* cells under cold shock, representing a 6–9-times increase relative to that obtained with the classical SD sequence.

Key words: ε-element, Shine-Dalgarno, prokaryotic translation initiation, 16S rRNA, protein expression

INTRODUCTION

The epsilon element (ε) as a translational enhancer was first described in the late 80s by the *Olins* group [1]. This element is a nine-nucleotide sequence (5'-UUAACUUUA-3') located in the 5'-UTR of T7 phage gene *10* immediately before the Shine-Dalgarno (SD) sequence. The 'epsilon' notation is derived from the abbreviation EPSIlon (Enhancer of Protein Synthesis Initiation) and acts as an allusion to Ω-element described by *Gallie* [2]. Initially, the ε-element showed an extremely great enhancing effect on translation: more than two orders of magnitude compared with the construction with single SD sequence not supplemented by ε-element [1]. Over the next fifteen years, the ε-element was actively studied by several groups of scientists, however, the data obtained were contradictory both in relation to the actual activity of this element and in relation to the possible mechanism of its action.

The *Olins* group considered the ε-element as a translation enhancer capable of manifesting its enhancing function only in the presence of the SD sequence. Also, this group first suggested that the ε-element directly interacts with 16S rRNA, forming complementary bonds with the 458 - 466 b region located in its 17-th hairpin [3]. Thenadays this assumption was advanced, complete three-dimensional models of ribosomal subunits had not yet been built, and it was not obvious how far the 17-th hairpin was from the region of the "neck" and the decoding center. And till now this has been the only hypothesis that explained the possible mode of action of the ε-element.

The same hypothesis was adhered to by the

Golshani group [4], which postulated the presence of an enhancing effect of the ε-element on translation through the formation of complementary bonds with the region 458 - 466 b of the 17-th hairpin. This group supposed an increase in the enhancer effect of the ε-element with an increase in the complementary to the 17-th hairpin: thus, the εII enhancer was obtained [4]. This enhancer had an increased complementarity to the region of the 17-th hairpin (451 - 466 b), thus bringing the number of complimentary bases to sixteen. The resulting element enhanced translation regardless of the presence of the SD sequence in the 5'-UTR, that is, it acted as an independent enhancer; its efficiency was 80% of that of the SD sequence.

At the same time, the *Sprengart* group [5] investigated the full-length leader of the T7 phage *10* gene and showed the absence of the enhancing effect of the ε-element on translation. In a number of constructs obtained by this group, the ε-element was removed and its absence did not affect the level of mRNA translation compared to those constructs where it was presented. Also, in this study, it was noted that "the stimulating effect of the ε-sequence ... may be partially associated with the functional stabilization of *lacZ* mRNA, and not with the specific interaction between ε and 16S rRNA".

In further works of the *Golshany* group, the positional importance of the ε-element location relative to the start codon and the SD sequence was demonstrated [6]. The natural one was admitted as optimal for translation enhancement – 16 b before the start codon and in two nucleotides before SD. They also showed the enhancing effect of the ε-element in combination with the SD sequence on translation in *A. tumefaciens* [7, 8] and at the same time, the lack of enhancer activity of the εII-element in agrobacterial cells.

O□Connor and*Dalberg* [9] showed that the model of the ε-element's functioning through a complementary interaction with area 460 (the 17-th hairpin) was completely inconsistent. They obtained strains of *E. coli* in which either the sequence of the 17-th hairpin was completely replaced by that of *Salmonella enterica*, or the 458 - 466 b 16S rRNA region was mirrored, so all the ribosomes of the obtained strains were unable to compliment with the sequences ε and εII by forming a bond with the 17-th hairpin. Nevertheless, the effect on the translation of εII and ε / SD constructs was the same as for *E. coli* cells with unmodified ribosomes. Thus, this group confirmed the presence of an enhancer effect for the εII element and the enhancing effect of the ε-element in combination with SD, but the model of their interaction with the 460 16S rRNA region was refuted. The data obtained by *O'Connor* and *Dalberg* led to the fact that, for lack of a possible functioning model, studies on the ε-element by other scientific groups were stopped, leaving open the question of the essence of the observed effect.

In this paper, we propose a new model for the interaction between ε-element and *E. coli* 16S rRNA 3'-terminus (the 45-th hairpin). Computer analysis of *E. coli* 16S rRNA regions located closest to the decoding center on the three-dimensional model revealed the sequences of the 44-th and 45-th hairpins as possible sites of ε-element binding. Subsequent analysis of complementarities between the last 150 nucleotides of 16S rRNA (hairpins 44 and 45) and the ε-element showed a high probability of the complementary bonds formation in the region of the 45-th hairpin. We have obtained plasmid constructions of the β-glucuronidase gene, containing ε and εII elements as leaders, but containing nucleotides, which give additional complementarity to the region of the 45-th hairpin. The relative expression level of these constructs was higher than that shown by the works of previous researchers. The level of β-glucuronidase expressed in *E. coli* cells subjected to cold temperature stress for all constructs containing variations of ε-elements was 1.1 - 12.15 times higher than that for construct bearing the Shine-Dalgarno sequence.

Materials and methods

Cloning. The leader sequences were cloned into the pET23c plasmid before the β-glucuronidase gene at *Xba*I and *Nco*I restriction sites according to standard methods. Adapters for cloning were obtained from "Macrogene".

Protein expression was performed in *E. coli* BL21 (DE3) cells in TB medium (Terrific Broth) with 1% glucose. Optical density of night cultures was adjusted to OD600 = 0.1. Expression was induced by addition of IPTG to the final concentration of 1 mM. Incubation of cell cultures with IPTG was carried out in 1.5 ml of media in 24-well immunological plates for 1 hour at various

temperatures and with 120 rpm shaking.

Total cellular protein was extracted according to [10] directly in immunological plates by the repeating (3 times) cycles of freezing at -80°C and thawing at 37°C. The concentration of total cellular protein was measured by the Bradford method [11] on EL311 microplate reader ("Bio-Tek").

The β-glucuronidase activity measurement. PNPG substrate (Paranitrophenyl β-D-glucuronide) was used for GUS activity measurement according to recommendations [12]. Spectrophotometric measurements were carried out on EL311 microplate reader ("Bio-Tek") at 405 nm and a reference wavelength of 630 nm. The unit of enzyme activity was determined as 1 nM of substrate converted into the product per 1 minute per 1 mg of total protein.

Protein SDS-electrophoresis was performed according to [13] in 8% PAAG in a tris-tricin system.

Western blot was performed on a NitroBind nitrocellulose membrane ("SantaCruz") with rabbit anti-GUS antibodies ("AgriSera") at 1:3000 dilution and anti-rabbit IgG antibodies ("Sigma") conjugated with alkaline phosphatase at 1:3000 dilution. The membrane was stained with BCIP / TMB substrate.

Statistical analysis was carried out in Microsoft Excel. To determine the significance degree of differences between the means of control and experimental data, the Student's t-test was used; 5% level of significance was accepted ($\alpha \leq 0.05$). The actual t-test for each compared data sets was calculated as independent two-sample t-test with unequal variances and two-sided distribution using the Microsoft Excel function «TTEST». The statistical test power was calculated in STATA 10 by the "test power" operator. We calculated the effect size for all tests performed. For comparison of two mean values, we used Cohen's *d* as an effect size measurement (*d* value of 0.2 indicated a small effect, 0.5 indicated a medium-sized effect, and 0.8 indicated a large effect) [14].

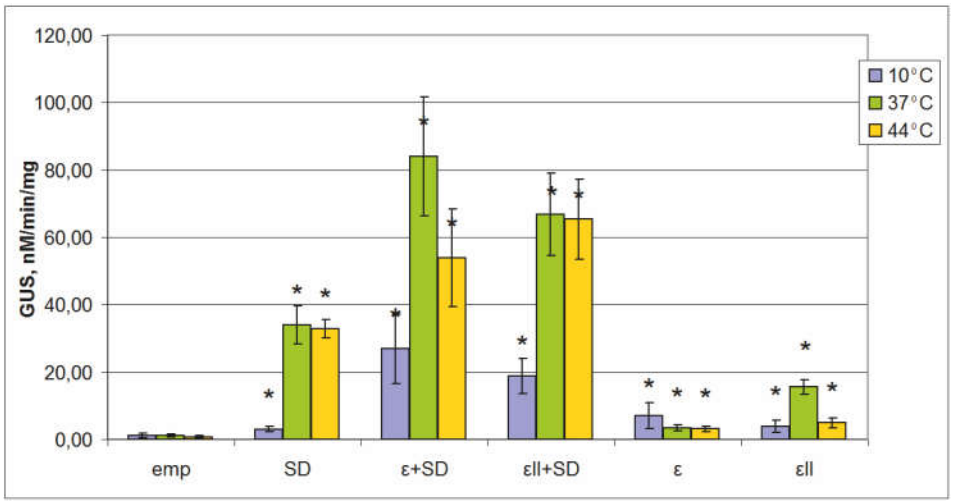
RESULTS

We've obtained genetic constructs based on pET23c plasmid encoding the β-glucuronidase (GUS) gene under the control of T7-phage transcription promoter and terminator, and differing only in their 5′-UTR (table 1). As a positive control, we used the Shine-Dalgarno (SD) sequence, canonical for *E. coli*, located in 8 nucleotides before the start codon. As a negative control was used the 'emp' spacer sequence which was a part of the plasmid between restriction sites.

Table 1. Leader sequences of expression constructions

Construction	Sequence
emp	5'-TCTAGC**ATG-**3'
SD	5'-TCTAAGGAGGTCTATTCC**ATG-**3'
εI	5'-TCTAGATTTAACTTTATCTATTCC**ATG**-3'
εII	5'-TCTAGATTTAACTTTATTTACCTTATCTCTATTCC**ATG**-3'
ε_SD	5'-TCTAGATTTAACTTTAACTAAGGAGGTCTATTCC**ATG**-3'
εII_SD	5'-TCTAGATTTAACTTTATTTACCTTATCACTAAGGAGGTCTATTCC**ATG**-3'
Note: the sequences of interest are shaded in gray; start codon is shown in bold	

Cells of *E. coli* expression strain BL-21 (DE3) were cultured in TB medium with 1% glucose to prevent spontaneous expression from T7-promoter. In overnight non-IPTG-induced cell culture the level of GUS expression was measured – not a single construct showed it greater than the level of negative control. After dilution and induction, cell suspensions were incubated at different temperatures (10°C, 37°C, 44°C) for one hour. The cultivation temperature of 37°C was taken as the physiological optimum for *E. coli* cells, 10°C – cold stress, 44°C – heat shock [15]. The isolated total cellular protein was a water-soluble gross lysate, for which the protein concentration and β-glucuronidase activity was measured. The results of GUS activity measurement are presented in the diagram (fig. 1).



The asterisk indicates the mean values significantly differing from the control ($p \leq 0.05$) values of the flempffi-construction for each of the cultivation temperatures.

Fig. 1. β-glucuronidase activity at different *E. coli* cells cultivation temperatures.

The data obtained were statistically processed separately for each temperature regime. The expression level of each studied construction was compared with the expression level of the negative (emp) and positive (SD) controls using the Student's test. Also, a measure of the effect, which was calculated using statistical power, was measured for each of the constructs. The results of statistical analysis are presented in table 2.

Table 2. The relative level of β-glucuronidase expression at different cell culture temperatures of incubation

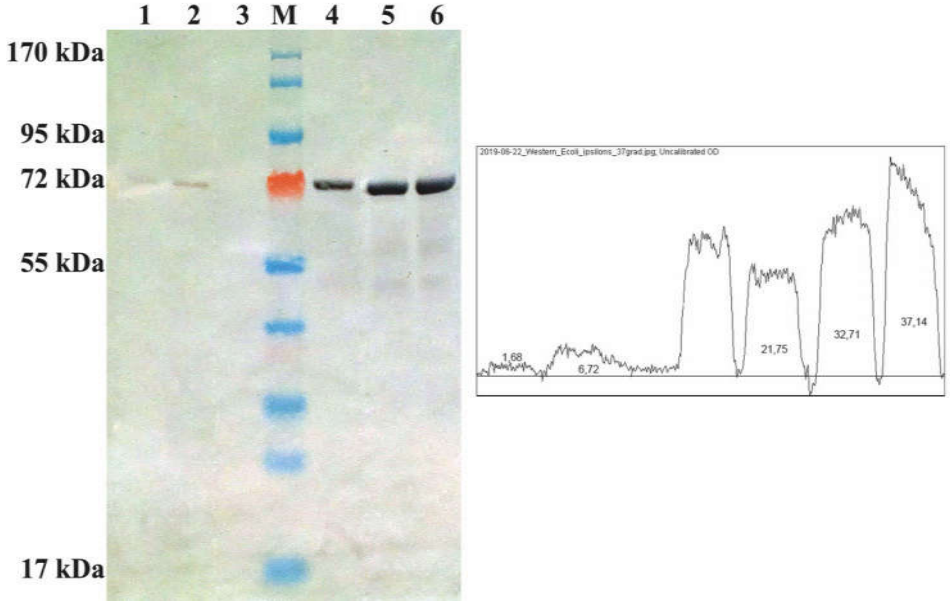
5'-UTR	Cultivation temperature 10ºC		Cultivation temperature 37ºC		Cultivation temperature 44ºC	
	Expression level to emp, %	Expression level to SD, %	Expression level to emp, %	Expression level to SD, %	Expression level to emp, %	Expression level to SD, %
emp	100	-	100	-	100	-
SD	250* (0,43)	100	2965* (1,00)	100	4164* (1,00)	100
εI	590* (0,57)	236 (0,40)	296* (0,51)	10* (1,00)	390* (0,69)	9* (1,00)
εII	326* (0,40)	130 (0,14)	1355* (0,91)	46* (1,00)	622* (0,67)	15* (1,00)
ε_SD	2278* (0,93)	909* (0,91)	7317* (0,99)	247* (0,73)	6850* (0,96)	164 (0,56)
εII_SD	1597* (1,00)	638* (0,99)	5827* (1,00)	197* (0,63)	8302* (1,00)	199* (0,72)

Notes: * – the difference between samplings is significant ($p \leq 0.05$). The significance level corresponding to the actual t-criterion was calculated in Microsoft Excel as a two-sample t-test with unequal variances and two-sided distribution; for each of the constructions the number of repeat measurements was 10 or more. In parentheses is indicated the measure of the effect in the form of Cohen's *d*, which was calculated as the test power in STATA 10. The gray marker indicates cells which value is significant with respect to statistical errors of the first and second kind.

Based on the data obtained, it can be concluded that the ε-element itself is a very weak enhancer, and its lowest effect was shown for the optimum temperature of bacterial cultivation. At the same time, under the stressful conditions, the enhancer effect of ε element compared to 'emp' construction was ~6-times greater in the cold shock and 4-times greater in the thermal shock. The εII sequence also proved to be an enhancer, and stronger one than εI: at 37°C its enhancing effect was 46% of that of SD sequence. The constructions ε_SD and εII_SD showed a significant excess of the enhancer effect of a single SD sequence at the cold shock condition (9.1 times and 6.4 times, respectively) and at standard cultivation temperature

(2.5 times and 2.0 times, respectively). As for the heat shock condition only εII_SD showed a 2-times excess over the SD sequence expression level.

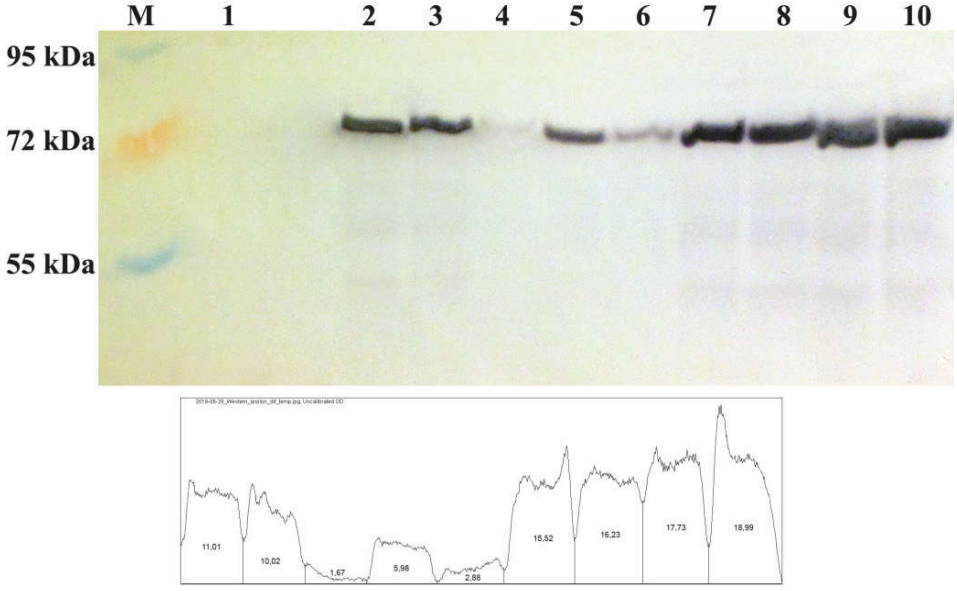
Protein SDS-electrophoresis of bacterial extracts from the standard cultivation temperature and subsequent Western blotting confirmed the data obtained by β-glucuronidase activity measurement. The results of Western blotting with GUS-specific antibodies, as well as the results of membrane densimetric analysis are presented in figure 2.



On the left side of the figure is a photograph of a nitrocellulose membrane; 10 μg of total soluble protein were applied to the tracks: 1 ć εI cell extract; 2 ć εII cell extract; 3 ć emp cell extract; 4 ć SD cell extract; 5 ć ε_SD cell extract; 6 ć εII_SD cell extract; M ć PageRuler Plus Prest. Protein Ladder (flThermo Scientificffi). On the right side of the figure is the densimetric analysis of the membrane from the left side of the figure in ImageJ 1.42q program.

Fig. 2. Western blot of β-glucuronidase in bacterial extracts cultivated at 37°C.

Figure 3 shows the results of Western blotting with GUS-specific antibodies for ε_SD and εII_SD constructions cultivated at different temperature regimes.



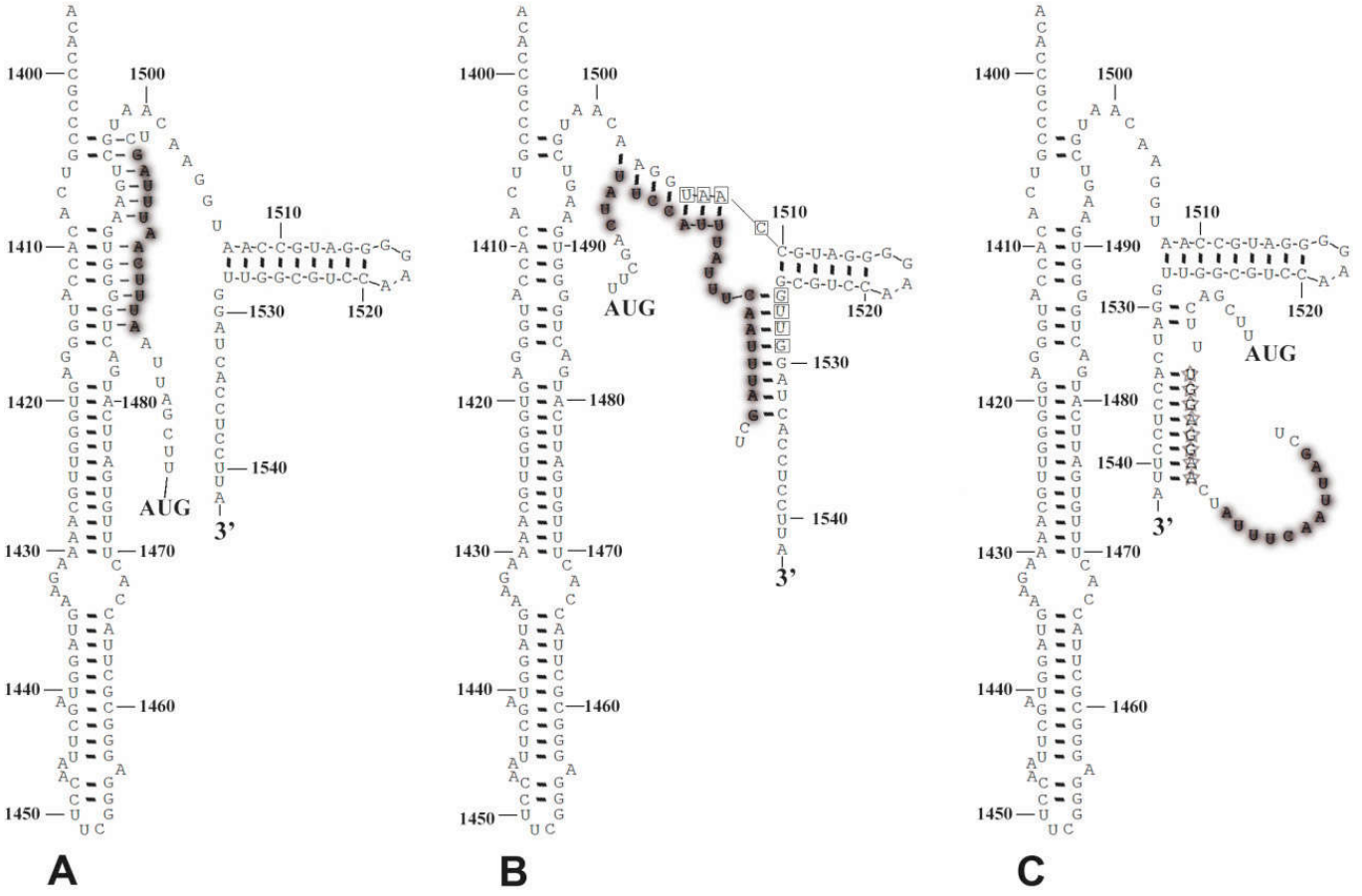
At the top of the figure is a photograph of a nitrocellulose membrane; 10 μg of total soluble protein were applied to the tracks: 1 ć emp cells extract from 37°C; 2 ć SD cells extract from 44°C; 3 ć SD cells extract from 37°C; 4 ć SD cells extract from 10°C; 5 ć ε_SD cells extract from 10°C; 6 ć εII_SD cells extract from 10°C; 7 ć ε_SD cells extract from 37°C; 8 ć εII_SD cells extract from 37°C; 9 ć ε_SD cells extract from 44°C; 10 ć εII_SD cells extract from 44°C; M ć PageRuler Plus Prest. Protein Ladder (flThermo Scientificffi). The bottom part of the figure shows the densimetric analysis of the membrane from the top of the figure in the ImageJ 1.42q program.

Fig. 3. Western blotting of β-glucuronidase in extracts of bacteria cultured at different temperatures.

DISCUSSION

At the initial stage of the study, we performed a computer analysis of *E. coli* 16S rRNA sites located on the three-dimensional model closest to the decoding center using Swiss-PdbViewer 3.7. The stretches of the hairpins 44-th and 45-th were selected as possible targets for ε-element binding. Subsequent analysis of possible interactions between sequences of different ε-element variations and the 3'-terminal part of 16S rRNA (the 44-th and 45-th hairpins) showed a high probability of the complementary bonds formation between them.

We analyzed the sequences studied in the works of *Dalberg* and *Golshani* groups [6, 8, 9], as ones where the ε-element positive effect on translation was shown and the most methodologically valid. In addition, the studied sequences in both groups were identical up to the cloning sites, since *Dalberg* and *O'Connor* received constructions from the *Golshani* group [9]. At figure 4 are shown two-dimensional structures of complementary interactions between 16S rRNA 3'-terminus and sequences of ε, εII, and ε_SD obtained in the "RNAstructure 5.4". All presented structures have a minimum free energy in a number of their variations and a maximum number of variants for the indicated complementary nucleotides.



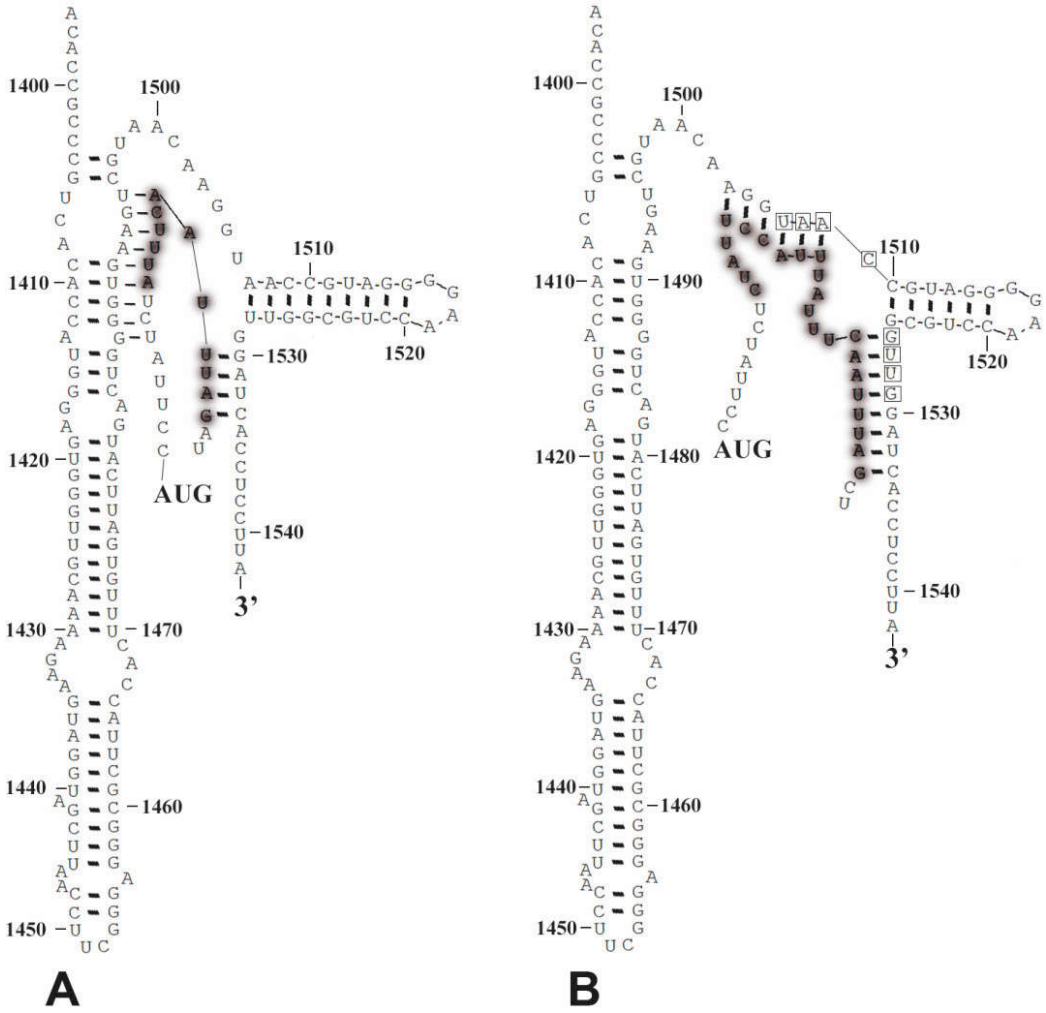
A- the initial sequence of the ε-element. B - εII sequence. C - ε_SD sequence. The sequences of ε and εII are indicated by a gray marker, the SD sequence ć by stars. The nucleotides bound complementary to 5'-UTR, disrupting their binding inside the 45-th hairpin, are framed. The models were created in RNA structure 5.4.

Fig. 4. Complementary interactions between the *E. coli* 16S rRNA 3'-terminus and 5'-UTRs containing the ε-element.

The sequence of the ε-element or εI, as *Golshani* refers to it [4], formed complementary bounds only with 44-th hairpin region from nucleotides 1485 to 1497. Since this 44-th hairpin is a very long and very stable structure, such a bond formation is unlikely when considering the entire 16S rRNA molecule. Thus, we accepted that the εI sequence does not form complementary bonds with the 16S rRNA 3'-terminal region during translation initiation step. At the same time, the εII sequence was simultaneously complementary to two sections of the 45-th hairpin, slightly "untwisting" it (figure 4B): 1526 - 1533 b and 1502 - 1508 b. The total number of complementary bases between the two sequences was 15 b (7 nucleotides before and 8 after the hairpin), thus compensating the loss of interaction within the hairpin. It should be noted that these interaction schemes can explain the data obtained by

the *Golshani* and *Dalberg* groups when εI expression level was 2-4% of that of SD, and εII – 73%. The construction ε_SD was characterized by the formation of bonds exclusively between the Shine-Dalgarno and anti-Shine-Dalgarno regions (figure 4C) without involving the sequence of ε-element in the process of complementary interactions. However, two additional pairs of complementary bonds were formed in 2 b before the anti-Shine-Dalgarno sequence with nucleotides -6 – -7 from the start codon. These two additional complementary bounds could be considered as an explanation for enhanced expression from this construction, which amounted to 147% [9] and 174% [7] relative to SD.

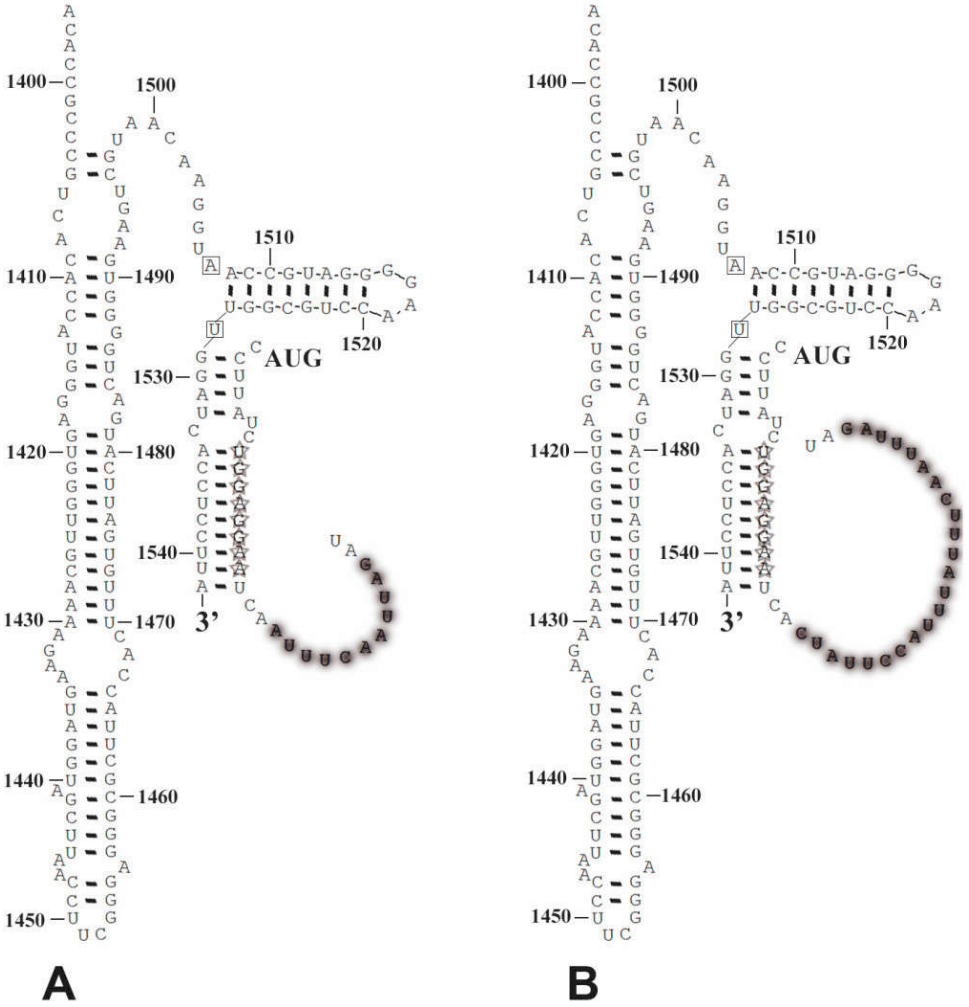
In order to show the possibility of ε-element action through the interactions shown above with the 16S rRNA 3'-terminal part, we decided to obtain a number of constructs in which complementarity to this region will be enhanced or weakened. The sequences of εI, εII and ε_SD proper remained unchanged; we've modified only nucleotides preceding the start codon. Using "RNAstructure 5.4" program, we've selected sequences which interactions with the 16S rRNA 3'-end are shown in figure 5. For εI, the 5'-UTR sequence was changed in such a way that part of the ε-element formed complementary bonds with nucleotides 1530 - 1533 lying above anti-Shine-Dalgarno (figure 5A), which positively affected the expression level of this construct – 10% relative to SD expression level. For εII sequence, on the contrary, by increasing by three nucleotides the length of the sequence before the start codon, the probability of complementary bonds formation with 16S rRNA 3'-end was reduced (figure 5B). The expression efficiency of this construct was 46% of that of SD, which was significantly less than the enhancer effect of 73% for the initial εII.



A -The sequence of the ε-element with changed complementarity. B - The sequence of the εII with changed complementarity. The sequences of ε and εII are indicated by a gray marker. The nucleotides bound complementary to 5'UTR, disrupting their binding inside the 45-th hairpin, are framed. The models were created in RNA structure 5.4.

Fig. 5. Complementary interactions between the E. coli 16S rRNA 3≥-terminus and 5≥-UTRs containing the ε-element and examined in this study

Constructions containing both SD and ε-element were obtained in two versions with εI and εII – ε_SD and εII_SD (figure 6).



A -The sequence of ε_SD with changed complementarity. B -The sequence of εII_SD with changed complementarity. The sequences of ε and εII are indicated by a gray marker, the SD sequence ć by stars. The nucleotides bound complementary to′5 UTR, disrupting their binding inside the 45-th hairpin, are framed. The models were created in RNAstructure 5.4.

Fig. 6. Complementary interactions between the E. coli 16S rRNA 3≥-terminus and 5≥-UTRs examined in this study and containing both ε-element and SD sequence

For both designs the number of additional complementary bonds above anti-Shine-Dalgarno was increased to 4. The expression level of these constructions was 247% and 197% relative to SD expression level, respectively, which exceeded the maximum level of 174% obtained by *Golshani* [7]. Thus, it could be stated that the complementarity increase between 5′-UTRs and 45-th hairpin promotes the level of protein expression, and a decrease of complementarity accordingly depress it. Moreover, it should be noted that the nucleotide composition of the studied sequences ε, εII, SD remained unchanged, but due to the variation of spacer nucleotides (8 b before the start codon), we varied the probability of complementary bonds formation with 16S rRNA 3'-terminus. And the obtained data on protein expression were in accordance with what we've expected, confirming the likelihood of complementary bonds formation with 16S rRNA 3'-terminus.

Having obtained the above data, we decided to check the effect of temperature stress on the level of protein expression from the studied constructions. Since it's known that temperature affects the conformation of any RNA, we suggested the possibility of different interactions between rRNA and 5'-UTR, which would be expressed in different levels of protein expression at different temperatures.

We summarized data on protein expression in figure 1, where the absolute activity of GUS is indicated, and in table 2, where its comparative level is shown. A statistical comparative analysis of the data on the temperature dependences of protein expression, carried out separately for each construct, showed the absence of a statistically significant difference between the expression level under standard conditions, cold and heat shock for emp, ε, and εII constructions. While for the SD, ε_SD and εII_SD constructions, a statistically significant difference was shown in the expression level obtained under cold shock and under standard conditions. No sig-

nificant difference between heat shock and standard conditions was shown for any 5'-UTR. Thus, heat shock did not have a statistically significant effect (compared to standard conditions) on the expression level of any construction.

At the same time, cold shock significantly decreased the protein expression level for all structures containing the Shine-Dalgarno sequence: for SD, it dropped to 8% of the expression level under standard conditions, for ε_SD – to 40%, for εII_SD – to 28%. Probably this phenomenon is due to the fact that during cold shock, conformational changes in the bacterial ribosome occur, which impede the interaction of mRNA 5'-UTR with the anti-Shine-Dalgarno sequence at the 3′-end of 16S rRNA. Most probably, these changes do not affect the ability of mRNA 5'-UTR to bind to the ribosome in other regions, in particular, located above the anti-Shine-Dalgarno or behind the 45-th hairpin. This assumption could explain the fact that the expression level of other constructions is not susceptible to cold shock. It should also be noted that the decline of expression level for ε_SD and εII_SD was much less dramatic than for the single Shine-Dalgarno sequence. These data could be interpreted in such a way that, if it's difficult to use the interactions between anti-Shine-Dalgarno and Shine-Dalgarno, an alternative mechanism involving the sequences ε and εII is used in the process of translation initiation. And this mechanism, quite possibly, is realized through interaction with the region of the 45-th hairpin located above the anti-Shine-Dalgarno.

CONCLUSION

Thus, summarizing the results of the study, it can be noted that, firstly, the advanced hypothesis of the ε-element (and its variations) mechanism of action through interaction with the 16S rRNA 45-th hairpin region does not contradict the previously obtained data. Secondly, the enhancer activity of the 5'-UTR investigated in this work corresponded to that predicted by us within the bounds of the advanced hypothesis, both in the direction of increasing and decreasing activity. And thirdly, we would like to note the practical significance of the obtained data, since the use of complex enhancers εII_SD and ε_SD for protein expression at low cultivation temperatures could significantly (6 and 9 times respectively) increase the yield of the target protein compared to the classical Shine -Dalgarno.

Acknowledgments

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REFERENCES

1. Olins P.O., Devine C.S., Rangwala S.H., Kavka K.S. The T7 phage gene 10 leader RNA, a ribosome-binding site that dramatically enhances the expression of foreign genes in *Escherichia coli*. *Gene (Amst.)*, 1988, vol. 73, pp. 227-235.

2. Gallie D.R., Sleat D.E., Watts J.W., Turner P.C., Wilson T.M.A. A comparison of eukaryotic viral 5'-leader sequences as enhancers of mRNA expression *in vivo*. *Nucleic Acids Research*, 1989, vol. 15, no. 21, pp. 8693-8711.

3. Olins P.O., Rangwala S.H. A novel sequence element derived from bacteriophage T7 mRNA acts as an enhancer of translation of the *lacZ* gene in *Escherichia coli*. *J. of biological chemistry*, 1989, vol. 264, no. 29, pp. 16973-16976.

4. Golshani A., Golomehova V., Mironova R., Ivanov I.G., AbouHaidar M.G. Does the Epsilon Sequence of phage T7 function as an initiator for the translation of CAT mRNA in *Escherichia coli. Biochemical and biophysical research communications*, 1997, vol. 236, pp. 253-256.

5. Sprengart M.L., Fuchs E., Porter A.G. The downstream box: an efficient and independent translation initiation signal in *Escherichia coli*. *The EMBO Journal*, 1996, vol.15, no. 3, pp. 665-674.

6. Golshani A., Kolev V., AbouHaidar M.G., Ivanov I.G. Epsilon as an initiator of translation of CAT mRNA in *Escherichia coli*. *Biochemical and Biophysical Research Communications*, 2000a, vol. 273, pp. 528-531.

7. Golshani A., Kolev V., Mironova R., AbouHaidar M.G., Ivanov I.G. Enhancing activity of ε in *Escherichia coli* and *Agrobacterium tumefaciens* cells. *Biochemical and Biophysical Research Communications*, 2000b, vol. 269, pp. 508-512.

8. Golshani A., Xu J., Kolev V., AbouHaidar M.G., Ivanov I.G. Inability of *Agrobacterium tumefaciens* ribosomes to translate in vivo mRNAs containing non-Shine-Dalgarno translational initiators. *Z. Naturforsch.*, 2002, vol. 57c, pp. 307-312.

9. O'Connor M., Dahlberg A.E. Enhancement of translation by the epsilon element is independent of the sequence of the 460 region of 16S rRNA. *Nu-*

cleic Acids Research, 2001, vol. 29, no. 7, pp. 1420-1425.

10. Cervera M. Histochemical and fluorometric assays for *uidA* (GUS) gene detection. *Methods in Molecular Biology*, 2002, vol. 286, pp. 203-212.

11. Bradford M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 1976, vol. 72, pp. 248-254.

12. Aich S., Delbaere L.T.J., Chen R. Continuous Spectrophotometric Assay for b-Glucuronidase. *BioTechniques*, 2001, vol. 30, pp. 846-850.

13. Schägger H. Tricine–SDS-PAGE. *Nat. Protocols*, 2006, vol. 1, pp. 16-22.

14. Nakagawa S., Niimura Y., Gojobori T. Comparative genomic analysis of translation initiation mechanisms for genes lacking the Shine-Dalgarno sequence in prokaryotes. *Nucleic Acids Research*, 2017, vol. 45, no. 7, pp. 3922-3931.

15. Ron E.Z., Davis B.D. Growth rate of *Escherichia coli* at elevated temperatures: limitation by methionine. *J. Bacteriol*, 1971, vol. 107, pp. 391-396.

E. COLI ЖАСУШАЛАРЫНДА ε-ЭЛЕМЕНТТІ КҮШЕЙТЕТІН ӘСЕР ЕТУ МЕХАНИЗМІНЕ ЖАҢА КӨЗҚАРАС

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ТҮЙІН

E. coli-ге **трансляция кезінде 10 фага T7 генінің 5'-ТР-тен ε-элементтің әсер ету механизмі үшін жаңа гипотеза ұсынылды. Эпсилон элементі (ε) Шайн-Далгарно (SD) тізбегімен үйлесіп, прокариоттық трансляция тиімділігін арттыруға қабілетті күрделі трансляция күшейткіш ретінде белгілі. ε-элементтің әсер ету механизмі белгісіз; сондай-ақ, қазіргі уақытта қолда бар фактілерді түсіндірудің бірде-бір гипотезасы жоқ. Бұл зерттеу ε-элементтің (және оның өзгеруі εII) *E. coli* 16S рРНҚ-ның 3'-соңымен, дәлірек айтқанда, 45-ші түтікшенің аймағымен өзара әрекеттесуіне негізделген әрекет ету механизмін ұсынады. Осы гипотезаны қолдана отырып, қазіргі кезде қолжетімді ε-элементтің бактериалды жасушалардағы ақуыздар құрамына әсер етуі туралы мәліметтер түсіндірілді. Осы жаңа гипотезаға сәйкес қасиеттері болжанатын ε-элементтің вариациялары бар бірқатар 5'-ТР құрылды. Болжамдар ақуыздың экспрессия деңгейін өлшеу арқылы расталды. Температуралық кернеулер (суық және жылу соққысы) олардың әр түрлі жақсартқыштармен бақыланатын ақуыздың көрінуіне әсерін зерттеу үшін де қолданылды. εII_SD және ε_SD күшейткіштері суық соққы жағдайында E. coli жасушаларында өрнек деңгейін (классикалық Шайн-Далгарно тізбегіне қарағанда 6 - 9 есе).едәуір арттыра алатындығы көрсетілді**

Негізгі сөздер: ε-элемент, Шайн-Далгарно, прокариоттық трансляция бастамасы, 16S рРНҚ, ақуызды экспрессия.

НОВЫЙ ВЗГЛЯД НА МЕХАНИЗМ ЭНХАНСЕРНОГО ДЕЙСТВИЯ ε-ЭЛЕМЕНТА В КЛЕТКАХ *E. COLI*

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АБСТРАКТ

В работе предложена новая гипотеза механизма действия ε-элемента из 5'-НТП гена 10 фага Т7 при инициации трансляции в *E. coli*. Эпсилон элемент (ε) известен как комплексный трансляционный энхансер, способный усиливать эффективность прокариотической трансляции в сочетании с последовательностью Шайн-Далгарно (SD). Механизм действия ε-элемента неизвестен, более того в настоящее время нет ни одной гипотезы, предложенной для объяснения имеющихся фактов. В этом исследовании представлен механизм действия ε-элемента, основанный на комплиментарном взаимодействии ε-элемента (и его вариации εII) с 3'-концевым участком 16S рРНК *E. coli,* точнее с районом 45-й шпильки. Используя эту гипотезу, объясняются имеющиеся к настоящему моменту данные по влиянию ε-элемента на экспрессию белка в бактериальных клетках. Основываясь на представленной гипотезе, конструируется ряд 5'-НТП вариаций ε-элемента с предполагаемыми в рамках гипотезы свойствами, которые подтверждаются при проверке уровня экспрессии белка. Также проведено исследование влияния на экспрессию белка, контролируемую различными энхансерами, температурных стрессов – холодового и теплового шока. Показано, что при холодовом шоке энхансеры εII_SD и ε_SD способны достоверно повышать уровень экспрессии в 6 и 9 раз относительно классической последовательностью Шайн-Далгарно.

Ключевые слова: эпсилон элемент, Шайн-Далгарно, прокариотическая инициация трансляции, 16S рРНК, экспрессия белка