doi 10.18699/bgrs2024-8.3-09

How RNA free energy landscape of *ilvBNC* operon transcriptional attenuator drives *Corynebacterium glutamicum* valine production

Titov I.I.[1, 2]*, Ryabchenko L.E.[3], Leonova T.E.[3], Kalinina T.I.[3], Gerasimova T.V.[3], Khlebodarova T.M.[1, 2], Sheremetieva M.E.[3], Kolchanov N.A.[1, 2], Yanenko A.S.[3]

[1] *Department of Systems Biology, Institute of Cytology and Genetics, SB RAS, Novosibirsk, Russia*

[2] *Kurchatov Genomic Center of the Institute of Cytology and Genetics, SB RAS, Novosibirsk, Russia*

[3] *National Research Center "Kurchatov Institute", Kurchatov Genome Center, Moscow, Russia*

* *titov@bionet.nsc.ru*

Key words: *Corynebacterium glutamicum*; transcription regulation; attenuation; *ilvBNC* operon; acetohydroxy acid synthase AHAS

Motivation and Aim: The adaptation of bacteria to environmental changes has led to their ability to rapidly modulate gene expression in various ways, including RNA-dependent regulation. RNA free energy landscapes translate environmental signals into the language of RNA structural dynamics, which, due to the high specificity and speed of complementary interactions, ensures an immediate reaction of the bacterium. An example is an attenuator – a special RNA element, which frequently regulates bacterial transcription, in particular, of the *ilvBNC* operon of *C. glutamicum*, which then determines the biosynthesis of branched chain amino acids (L-isoleucine, L-leucine, L-valine). The goal of this work was to investigate the mechanism of transcriptional regulation of *ilvBNC* operon expression both experimentally and computationally.

Methods and Algorithms: The *C. glutamicum* strains were received from the Russian Collection Industrial Microorganisms (VKPM) of the NRC "Kurchatov Institute", the German Collection of Microorganisms and Cell Cultures (DSMZ) and Derbikov D. (NCR "Kurchatov Institute", Kurchatov Genome Center).

C. glutamicum strains with the mutations in the *ilvBNC* operon were constructed by replacing the native sequence of the regulatory region with a mutant copy using homologous recombination. The transcription level of the *ilvBNC* genes in the mutated strains was studied using real-time PCR with reverse transcription. The activity of AHAS in the cell-free extracts of strains was measured by the method [1].

We used the Vienna [2] and GArna [3] programs to calculate the secondary structure of the attenuator region of the *ilvBNC* operon and the thermodynamic parameters of [4, 5] to calculate the mutational perturbations in the hairpins free energies.

Results: Fig. 1A shows the organization of the *ilvBNC* operon and the structure of its regulatory region located upstream of the *ilvB* gene [6]. An interesting feature of the attenuator is the overlapping of Ter- and AT-hairpins which allows toehold formation and continuous transition between termination and antitermination states (Fig. 1B).

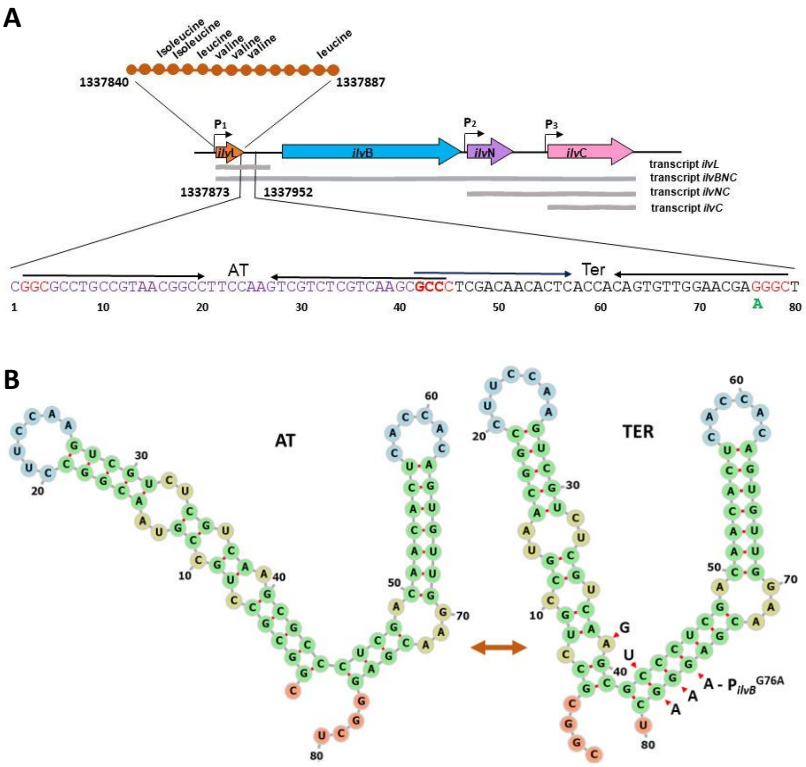

Fig. 1. Organization of the *ilvBNC* operon in *C. glutamicum* and alternative secondary structures of the transcription attenuator in the regulatory region of the *ilvBNC* operon of the *C. glutamicum*.
(A) The location of the sequences of the leader peptide IlvL, the structural genes *ilvB*, *ilvN* and *ilvC*, as well as the structure of the leader peptide and regulatory sequence upstream of the *ilvBNC* operon 80 bp long. Designations: P1, P2, P3 – promoters; the G76A mutation found during whole-genome sequencing of the valine-producing strain *C. glutamicum* VF is marked in green; the sequence GCCC (at positions 42–45) and its complementary GGGC (76–79) and GGC (2–4) are marked in red; the boundary trinucleotide GCC is marked in bold. (B) Alternative secondary structures of the transcription attenuator in the regulatory region of the *ilvBNC* operon of the *C. glutamicum*. Secondary structures responsible for free transcription (AT) and its arrest (TER) are shown on the left and right, respectively. Red arrows indicate mutations introduced into the attenuator structure

We created the strains with the mutations in the attenuator region and measured their characteristics (Fig. 2). A high correlation ($r^2 = 0.9443$, $p < 10^{-3}$) was observed between the level of valine production and the level of *ilvB* gene expression (Fig. 2C), indicating a key contribution of *ilvB* gene transcription to the control of valine production in *C. glutamicum*. The levels of *ilvB* gene expression and valine production also correlated well with the level of AHAS enzyme activity, $r^2 = 0.8926$ and $r^2 = 0.9421$, respectively. A significant correlation ($r^2 = 0.8027$, $p < 0.05$) was also observed between the expression of the *ilvBNC* operon in the *C. glutamicum* mutant strains and the difference in antitermination-termination states free energy according to the structural model (Fig. 1B).

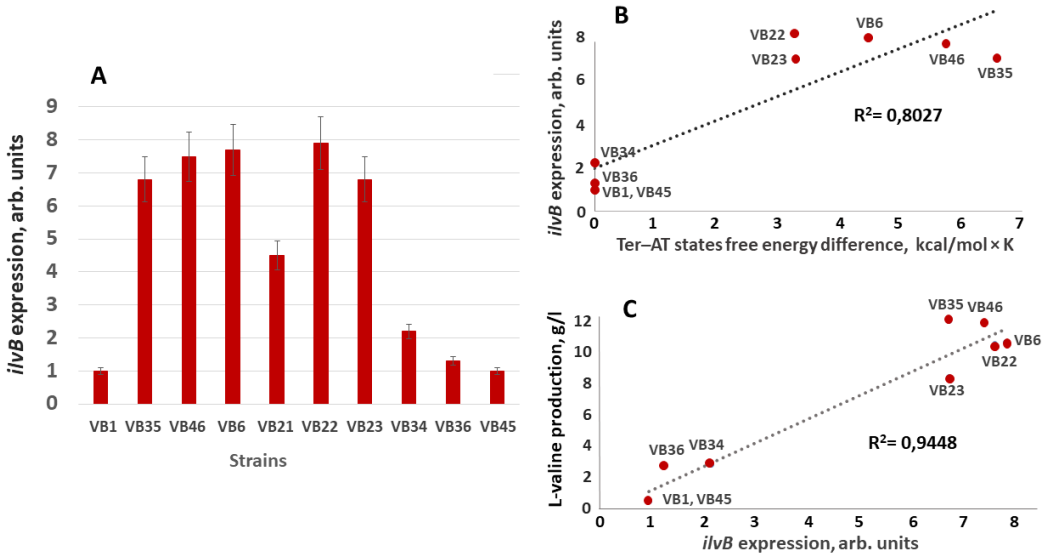

Fig. 2. Relative expression level of the *ilvBNC* operon in the *C. glutamicum* strains with mutations in the Ter-AT region (A), its dependence on changes in the free energy difference of Ter- and AT-hairpins (B), and the dependence of the level of valine production on the relative expression level of the *ilvB* gene in the mutant strains (C). The mutant strains are (see Fig. 1B): VB1 – ATCC13032, VB6 – G76A, VB22 – G76T, VB23 – G76C, VB34 – A39G, VB35 – G77A, VB36 – C43T, VB45 – C43T and G78A, VB46 – G78A

Conclusion: We develop a model of transcriptional regulation of *ilvBNC* operon expression, which explains our experimental data on single-nucleotide substitutions in the regulatory region. In this model, the hairpins of the terminator and the antiterminator partially overlap, so that the boundary trinucleotide GCC participates in the formation of both structures. As a result, in the free energy landscape, a valley is formed between the termination and antitermination states, and the attenuator is able to cross it using a sequence of low-energy transitions, thus controlling rapid changes in valine production.
Funding: The work was supported by the Ministry of Science and Higher Education of the Russian Federation (project No. 075-15-2019-1659) by the Program of Fundamental Studies of the Siberian Branch of the Russian Academy of Sciences (project No. FWNR-2022-0020).

References

1. Guo Y., Han M., Xu J., Zhang W. Analysis of acetohydroxyacid synthase variants from branched-chain amino acids-producing strains and their effects on the synthesis of branched-chain amino acids in Corynebacterium glutamicum. *Protein Expr Purif*. 2015;109:106-112. doi 471 10.1016/j.pep..02.006
2. Lorenz R., Bernhart S.H., Höner zu Siederdissen C., Tafer H., Flamm C., Stadler P.F., Hofacker I.L. ViennaRNA Package 2.0. *Algorithms Mol Biol.* 2011;6:26. 485 doi 10.1186/1748-7188-6-26
3. Titov I., Vorobiev D., Ivanisenko V., Kolchanov N. A fast genetic algorithm for RNA secondary structure analysis. *Russian Chemical Bulletin*. 2002;51:1135-1144
4. Davis A.R., Znosko B.M. Thermodynamic characterization of single mismatches found in naturally occurring RNA. *Biochem*. 2007;46:13425-13436. doi 10.1021/bi701311c
5. Zuber J., Schroeder S.J., Sun H., Turner D.H., Mathews D.H. Nearest neighbor rules for RNA helix folding thermodynamics: improved end effects. *Nucleic Acids Res*. 2022;50:5251-5262. doi 10.1093/nar/gkac261
6. Morbach S., Junger C., Sahm H., Eggeling L. Attenuation control of ilvBNC in Corynebacterium glutamicum: evidence of leader peptide formation without the presence of a ribosome binding site. *J Biosci Bioeng*. 2000;90, 501-507. doi 10.1016/s1389-1723(01)80030-491x