DOI 10.18699/SBB-2022-333

The secondary structure model of the transcriptional terminator of the *C. glutamicum* inbc operon proved by mutational analysis of the operon gene expression and valine production

Titov I.I.[1, 2]*, Ryabchenko L.E.[3, 4], Leonova T.E.[3, 4], Kalinina T.I.[3, 4], Gerasimova T.V.[3, 4], Kolchanov N.A.[1, 2], Yanenko A.S.[3, 4]**

[1] *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] *NRC Kurchatov Institute-Gosniigenetika, Kurchatov Genomic Center, Moscow, Russia*

[4] *NRC Kurchatov Institute, Kurchatov Genomic Center, Moscow, Russia*

** titov@bionet.nsc.ru, ** yanenko@genetika.ru*

Key words: attenuator, transcriptional terminator, RNA secondary structure, bacteria, valine

Motivation and Aim: L-Valine is an essential branched-chain amino acid for humans and animals: it is widely added in the products of food, medicine, and feed. L-leucine is predominantly produced through microbial fermentation by using *Corynebacterium glutamicum* as host bacteria. In recent years, continuing efforts have been made in revealing the mechanisms and regulation of L-valine biosynthesis in *C. glutamicum* with the goal for genetic engineering the industrially competitive L-valine-producing *C. glutamicum* strains.

It is known that the *ilv*BNC operon of *C. glutamicum* encodes acetohydroxy acid synthase and isomeroreductase, which are key enzymes of L-valine synthesis. Morbach et al. [1] have shown that the *ilv*BNC transcription is controlled by an attenuation mechanism involving antitermination. In this study, we have built the computer model of *ilv*BNC attenuator secondary structure and examined it by introducing the mutations into the attenuator region and measuring the *ilv*B transcription level and valine production of the mutant strains.

Results: The RNA secondary structures of the attenuator region were calculated using the unafold [2] and GArna [3] programs. The attenuator consists of two hairpins that compete for pairing with GCC triplet (Fig. 1). It is important that low-barrier GCC-pairing transition between these hairpins allows fast switching between terminator and antiterminator modes thus providing fluent regulation of *ilv*B transcription. Using this model and mainly focusing on the triplet critical region, we suggested five single and one double mutations and predicted their effect on the level of *ilv*B transcript. Some of these mutations were expected to increase the valine levels while others would retain it. Then we experimentally tested our predictions and found that they are confirmed by experiment. In particular, we observed that two of the mutations drastically change valine production as much as increase it up to 28 times compared to the wild type.

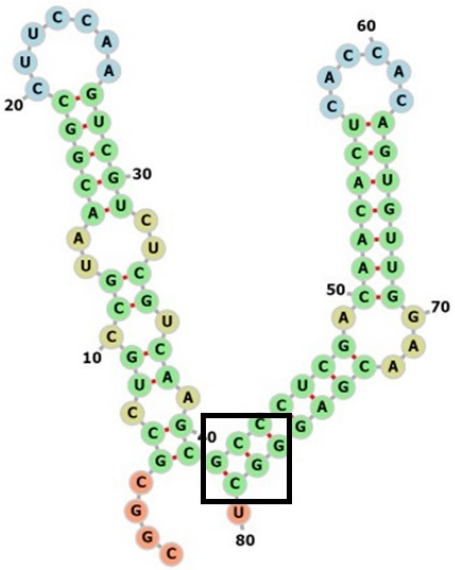

Fig. 1. Predicted RNA secondary structure of the wild type of the *ilv*BNC attenuator of *C. glutamicum.* The antiterminator and terminator hairpins are left and right, correspondingly. The rectangular shows the critical region for attenuator regulation

Conclusion: In this work, we built the model of the *ilv*BNC attenuator of *C. glutamicum* and supported it with the measurement of *ilv*B transcription level and valine production of the mutant strains. This provides one of the key elements for modelling the regulation of L-valine biosynthesis and for engineering the efficient L-valine-producing *C. glutamicum* strains.

Acknowledgements: The work of I.I.T. and N.A.K. was supported by the Kurchatov Genomic Center of the Institute of Cytology and Genetics, SB RAS (075-15-2019-1662).

The work of L.E.R., T.E.L., T.I.K., T.V.G. and A.S.Y. was supported by the Ministry of Science and Higher Education of the Russian Federation (Grant No. 075-15-2019-1658).

References

1. Morbach S., Junger C., Sahm H., Eggeling L. Attenuation control of *ilv*BNC in *Corynebacterium glutamicum*: Evidence of leader peptide formation without the presence of a ribosome binding site. *J. Biosci. Bioeng*. 2000;90(5):501-507.
2. Markham N.R., Zuker M. UNAFold: Software for Nucleic Acid Folding and Hybridization. In: Keith J. (Ed.). Data, Sequence Analysis, and Evolution, Bioinformatics: Volume 2, Chapter 1. Humana Press Inc., 2008;3-31.
3. Titov I., Vorobiev D., Ivanisenko V., Kolchanov N. A fast genetic algorithm for RNA secondary structure analysis. *Russ Chemical Bulletin.* 2002;51:1135-1144.