H-NS nucleoid protein binds to the *oppA-oppB* intergenic region of the *E. coli oppABCDF* operon and controls *oppB* expression

Nataliia A. Sukharicheva
Institute of Cell Biophysics RAS, Pushchino, Moscow region, 142290, Russia
natasha-sukharicheva@yandex.ru

Maria N. Tutukina
Institute of Cell Biophysics RAS, Pushchino, Moscow region, 142290, Russia
masha.tutukina@crg.eu

Olga N. Ozoline
Institute of Cell Biophysics RAS, Pushchino, Moscow region, 142290, Russia
ozoline@rambler.ru

Irina S. Masulis
Institute of Cell Biophysics RAS, Pushchino, Moscow region, 142290, Russia
masulis@hotbox.ru

Background

Bacterial transport systems are essential cellular components providing functioning and survival in varying environmental conditions. ABC transporters responsible for uptake of oligopeptides are encoded by operons composed of genes of periplasmic sensor, channel proteins and ATPases [1]. The present work is focused on the *oppABCDF* operon representing a typical ABC transporter [2, 3]. Nevertheless, the precise mechanisms of transcriptional regulation of individual genes from this operon are not completely clear. This operon is transcribed from the σ^{28} promoter [4], and at the level of transcription it is regulated by two repressors, Fur-Fe^{2+} and Lrp [5, 6], while small regulatory RNA GcvB affects protein biosynthesis [7]. The first two genes of this operon, *oppA* and *oppB*, are separated by 86 bp, containing potential terminator in the *oppA* proximal region (RegulonDB [8]). That assumes a possibility of independent transcription from the remaining part of the operon, and a mode of its regulation becomes of a particular interest.

Materials and methods

Potential starts of transcription initiation in the *oppA-oppB* intergenic region were predicted using universal promoter search algorithm PlatPromU [9]. Nucleotide sequences of homologous regions including *oppA-oppB* loci were analyzed for the following species: *Rhizobium etli* (*Rh. etli), Bacillus subtilis* (*B. subtilis*), *Yersinia pseudotuberculosis* (*Y. pseudotuberculosis*), *Clostridium botulinum* (*Cl. botulinum*), *Salmonela enterica* (*S. enterica*), *G. thermodenitrificans*, *Escherichia coli (E. coli), Chlamidophila felis* (*Chl. felis*), *Shigells flexneri* (*Sh. flexneri*), *Bifidobacterium dentium (B. dentium), Corynebacterium glutamicum* (*C. glutamicum*), *Corynebacterium pseudotuberculosis* (*C. pseudotuberculosis*), and

Propionebacterium acnes (*P. acnes*). Potential binding sites for the H-NS nucleoid protein upstream to and within *oppB* were predicted by the Internet resource "Virtual Footprint" [10]. The homogenous and functionally active H-NS was obtained after overproduction of the recombinant protein from the pGEM_HNS_his plasmid [11] in BL21*(DE3) cells with subsequent purification by affine chromatography on a column with Ni-IDA agarose. Efficiency of H-NS interaction with linear DNA was estimated by EMSA. Involvement of H-NS in transcriptional regulation of *oppB* was testified in the *hns* deletion mutant using qRT-PCR.

Results and conclusion

oppABCDF is arranged according to a typical scheme of an operon with sequential order of functionally related genes. Although polycystronic transcription is usually proposed for such cases, additional intra-operonic promoters may enhance expression of internal genes or confer them another mode of regulation. Such promoter was predicted by PlatPromU (Fig. 1) within *oppA* terminatory module and its activity was confirmed by RNA-seq.

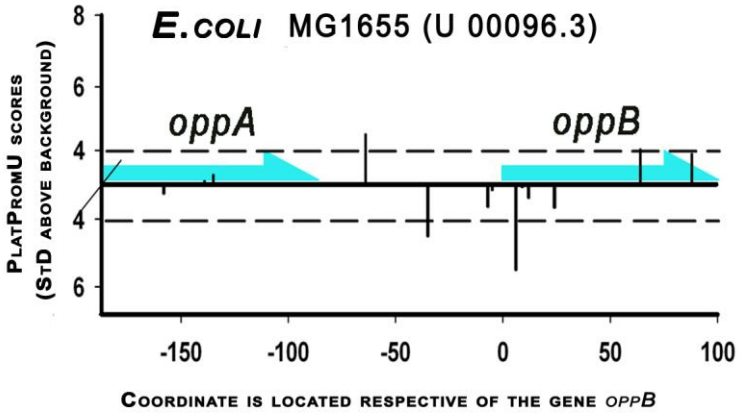

Fig.1. Potential start for independent *oppB* transcription was predicted by PlatPromU in the intergenic *oppA-oppB* region of the *Escherichia coli oppABCDF* operon.

Similar pattern of transcriptional signals distribution within *oppA-oppB* was shown using universal promoter search algorithm PlatPromU [9] for at least 13 evolutionary distant genomes. In the case of *B. dentium* the *oppA-oppB* genomic region exhibits extremely high density of the promoter-like signals allowing to consider this intergenic space as “promoter island”, primarily described in the genome of *E. coli*.

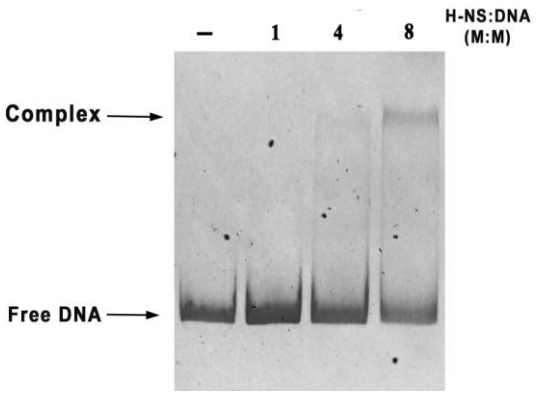

Fig.2. H-NS binds to the intergenic fragment *oppA-oppB* of *oppABCDF*

"Promoter islands" usually possess high affinity to H-NS protein [12, 13, 14], and Virtual Footprint Internet resource [10] predicted several potential H-NS binding sites in the *oppA-oppB* intergenic region. Thus, it was reasonable to verify the involvement of H-NS in the *oppB* transcriptional regulation. Electrophoretic mobility shift assay (Fig. 2) confirmed the propensity of DNA fragments containing intergenic space to form stable complexes with purified H-NS protein, while the involvement of H-NS in the control of OppB mRNA biosynthesis was detected by qRT-PCR: deletion of the *hns* gene activates the synthesis of OppB mRNA approximately 10 fold. Therefore, this nucleoid protein plays an important role in the expression of structural component of oligopeptide transport system.

The work has been supported by RFBR № 16-34-01138 mol_a

References

1. Doeven M. K., Bogaart G. v. d., Krasnikov V., Poolman B. Probing Receptor-Translocator Interactions in the Oligopeptide ABC Transporter by Fluorescence Correlation Spectroscopy. Journal Biophysical. 2008., V. 94, P. 3956–3965

2. Hogarth B.G., Higgins C.F. Genetic organization of the oligopeptide permease (opp) locus of Salmonella typhimurium and Escherichia coli. J. Bacteriol. 1983. V. 153. P. 1548–1551.

3.Pearce S.R., Mimmack M.L., Gallagher M.P., Gileadi U., Hyde S.C., Higgins C.F. Membrane topology of the integral membrane omponenets, OppB and OppC, of the oligopeptide permease of *Salmonella typhimurium*. Molecular Micribiology. 1992., V 6 (1), P. 47-57.

4. Gama-Castro S1, Jiménez-Jacinto V, Peralta-Gil M, Santos-Zavaleta A, Peñaloza-Spinola MI, Contreras-Moreira B, Segura-Salazar J, Muñiz-Rascado L, Martínez-Flores I, Salgado H, Bonavides-Martínez C, Abreu-Goodger C, Rodríguez-Penagos C, Miranda-Ríos J, Morett E, Merino E, Huerta AM, Treviño-Quintanilla L, Collado-Vides J. RegulonDB (version 6.0): gene regulation model of Escherichia coli K-12 beyond transcription, active (experimental) annotated promoters and Textpresso navigation. Nucleic Acids Research. 2008., V 36, P 120-124.

5. Fraenkel YM1, Mandel Y, Friedberg D, Margalit H. Identification of common motifs in unaligned DNA sequences: application to Escherichia coli Lrp regulon. CABIOS. 1995., V 11 (4), P 379-387.

6. Chen Z1, Lewis KA, Shultzaberger RK, Lyakhov IG, Zheng M, Doan B, Storz G, Schneider TD. Discovery of Fur binding site clusters in Escherichia coli by information theory models. Nucleic Acids Research, 2007, V 35 (20), P 6762–6777

7. Urbanowski ML1, Stauffer LT, Stauffer GV. The gcvB gene encodes a small untranslated RNA involved in expression of the dipeptide and oligopeptide transport systems in Escherichia coli. Molecular Microbiology. 2000. 37(4), P 856-868

8. RegulonDB. URL: http://regulondb.ccg.unam.mx

9. Kiselev S.S., Ozoline O.N. Structure-Specific Modules as Indicators of Promoter DNA in Bacterial Genomes. Mat. Biolog. Bioinform., 2011, V. **6**(1), P. 39-52.

10. Virtual Footprint. http://prodoric.tu-bs.de/vfp.

11. Tutukina M.N., Shvyreva U.S., Ozoline O.N. Architectural factor of bacterial nucleoid, H-NS: overproduction and verification of its functional activity. Sorption and Chromatographic Processes. 2015. V. 15 (3), P. 435-442.

12. Panyukov V.V., Ozoline O.N. Promoters of *Escherichia coli* versus promoter islands: function and structure comparison. *PLoS ONE*. 2013. V. 8, Article № e62601.

13. Shavkunov K.S., Masulis I.S., Tutukina M.N., Deev A.A., Ozoline O.N. Gains and unexpected lessons from genome-scale promoter mapping. Nucl. Acids Res. 2009. V. 37, P. 4919–4931.

14. Grainger D.C. Structure and function of bacterial H-NS protein. Biochemical Society Transactions. 2016., V 44, P. 1561-1569.