Antisense transcription may contribute to a fine-tuned expression of sugar regulators in *Escherichia coli*

Nataliya Yu. Markelova

Institute of Cell Biophysics RAS, Pushchino, Moscow region, 142290, Russia

Pushchino State Institute for Natural Sciences, Pushchino, Moscow region, 142290, Russia

markelova.n.y@gmail.com

Maria N. Tutukina

Institute of Cell Biophysics RAS, Pushchino, Moscow region, 142290, Russia

Skolkovo Institute of Science and Technology, Skolkovo Innovation Center, build. 3, Moscow 143026, Russia

Centre for Genomic Regulation (CRG) and Universitat Pompeu Fabra (UPF), Barcelona, 08003, Spain

masha.tutukina@crg.eu

Irina S. Masulis

Institute of Cell Biophysics RAS, Pushchino, Moscow region, 142290, Russia

masulis@hotbox.ru

Fyodor A. Kondrashov

Centre for Genomic Regulation (CRG) and Universitat Pompeu Fabra (UPF), Barcelona, 08003, Spain

Institució Catalana de Recerca i Estudis Avançats (ICREA), 23 Pg. Lluís Companys, 08010 Barcelona, Spain.

fyodor.kondrashov@crg.eu

Olga N. Ozoline

Institute of Cell Biophysics RAS, Pushchino, Moscow region, 142290, Russia

ozoline@rambler.ru

Background

Evolution of whole-genome techniques allowed discovering thousands of potential non-coding RNAs, including those transcribed in antisense direction. *In E. coli*, their ratio to the total amount of annotated genes can reach 22%. The reasons of such a widespread antisense transcription and possible mechanisms for asRNAs' action are not yet understood. In 2000s, it was found that transcription initiation starts in pro- and eukaryotes are often associated with

divergent starts in antisense direction, and the idea was proposed that such promoter-associated RNAs represent a mechanism for RNA polymerase pausing [1]. Later, studying the genomes of gamma-proteobacteria *Buchnera aphidicola* and two *Mycoplasma* species, the authors found that a number of produced untranslated RNAs correlated with a number of AT-pairs in the genome, that are capable of forming motifs similar to the -10-elements of promoters [2]. Thus, they concluded that antisense transcription occurs stochastically, and that low levels of random production of asRNA from these spurious -10 boxes would not affect the levels of the sense mRNA. In line with this, in [3] it was found that potential promoters of asRNAs, indeed, usually have well-pronounced -10-elements that are not very conservative among bacterial species, even closely related. However, earlier findings made in our lab indicated that asRNAs transcribed *in cis* to mRNAs of transcription factors often affect their intracellular level in *E. coli* [4, 5]. The aim of this work was to validate this observation on a genome-wide scale via analyzing the profile of asRNAs within the genes coding for transcription factors in *E. coli* strains growing on different carbon sources.

Material and Methods

E. coli K-12 MG1655 wt, *exuR* and *dps* mutants were grown aerobically on M9+0.2% glucose/glucuronate for 4/8 hours. RNA was isolated with Ambion RNAqeous kit. cDNA libraries were prepared and then sequenced on Illumina HiSeq2000 as 50nt single end reads. Data were analysed using Matcher GeneCount. Promoters for antisense transcription were predicted by PlatProm [6]. Reverse transcription was performed with 20μg of total RNA, 4pmol of corresponding ^{32}P-labeled primers and M-MulV. Samples were then loaded on a 6% denaturing gel, and bands were visualized on PMI Molecular Imager.

Results and conclusions

First, we analysed RNA-seq profiles of 160 *E. coli* MG1655 genes coding for transcription factors (76% from all annotated regulators). In line with previous *in silico* and *in vitro* studies [4], antisense transcription was registered within most loci, being absent only in 18 genes (11%). Both constitutive asRNAs and those responding to environmental changes, such as carbon source and growth phase, were registered. Transcripts detected *in vivo* nicely corresponded to promoter prediction, though most of them were found with relatively low

scores. In most cases, the main contribution to the value of scores was from -10-elements, confirming the observations made in [2, 3]. We then chose most representative examples, where both the levels of sense and antisense transcription were responding to environmental changes, and tested the profile of asRNAs synthesized using primer extension *in vivo*. One of such examples is shown in Fig. 1A.

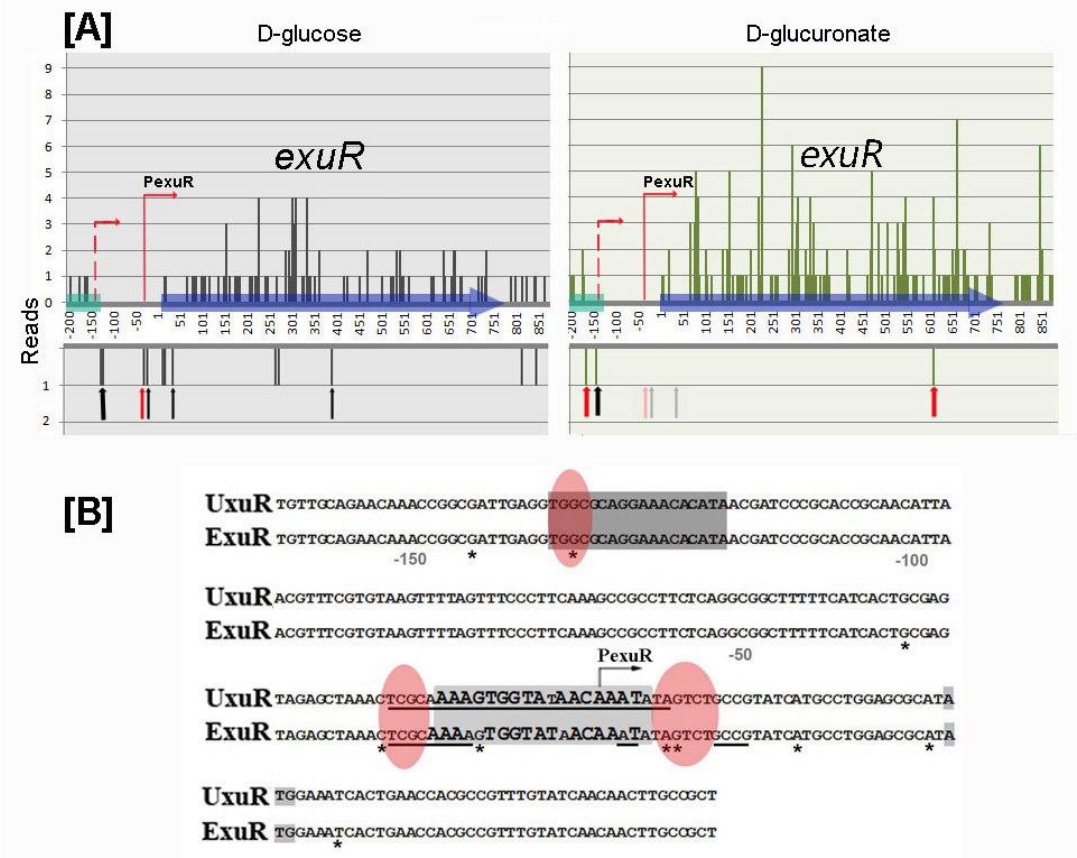

Fig. 1 [A] Profiles of sense (columns up) and antisense (columns down) transcription within the *exuR* locus as revealed by RNA-seq. Red vertical arrows indicate transcripts confirmed by both promoter prediction and primer extension. Black arrows – by primer extension. Coordinates relative to the start of *exuR* translation are shown on X axis [B] Base pairs near *exuR* promoter protected by UxuR or ExuR from DNAse I digestion [7] are underlined, hypersensitive positions are marked by asterisks. CRP binding sites are shaded gray. Positions of antisense starts overelapping with the ExuR and CRP binding sites are marked with red ellipses.

The *exuR* gene codes for a transcriptional regulator involved in control of hexuronate metabolism [7], and the level of its transcription during growth on D-glucuronate is predictably higher than that during growth on glucose, while the level of asRNAs becomes

lower, suggesting a possibility of interference between sense and antisense transription. We also noticed that some asRNAs were dependent on the presence of a transcriptional regulator, and their profile near the gene transcription start point was changed in the absence of ExuR and/or Dps. This observation allowed suggesting that antisense transcription may interfere not only with RNA polymerase, as it was proposed earlier [1], but also with transcription factors. Indeed, asRNAs initiated nearby the *exuR* transcription start point overlap with binding site for ExuR which, in the presence of D-glucuronate, serves as a self-activator, and for cAMP-CRP [Fig. 1B, 7]. During growth on glucose, *exuR* is repressed by UxuR and CRP, and RNA polymerase works in the antisense direction, while during growth on glucuronate, *exuR* promoter becomes relieved, and RNA polymerase turns to the sense direction. The same was observed for the *crp* gene, coding for global regulator of sugar metabolism during growth on glucose. During growth with D-glucuronate, *crp* expression decreased ~3 fold, while asRNAs appeared nearby its transcription starts. Thus, being in line with a concept of widespread antisense transcription, our findings do not support the idea that bacterial asRNAs are the products of transcriptional noise, and suggest their role in fine tuning expression of transcription factors. The same idea has recently been proposed for yeast [8], and our findings allow extending it for bacterial species.

Funding: RFBR 15-04-08716 and 16-34-01115.

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