Revealing and comparing regulons of homologues transcription factors UxuR and ExuR in *Escherichia coli*.

Maria N. Tutukina

Institute of Cell Biophysics RAS, Pushchino, Moscow region, 142290, Russia maria@icb.psn.ru

Inna A. Suvorova

Institute for Information Transmission Problems, Moscow, 127051, Russia inn1313@yandex.ru

Valery V. Panyukov

Institute of Mathematical Problems of Biology RAS, Pushchino, 142290, Russia panyukov@itaec.ru

Jeffrey A. Cole

University of Birmingham, Birmingham, B15 2 TT, UK j.a.cole@bham.ac.uk

Olga N. Ozoline

Institute of Cell Biophysics RAS, Pushchino, Moscow region, 142290, Russia ozoline@icb.psn.ru

Background.

Mounting evidence suggests that catabolism of hexuronic acids is important for colonization and motility of *Escherichia coli* (1). They are metabolized by the Ashwell pathway, which generates intermediates that are converted to pyruvate via the Entner-Doudoroff pathway. Two homologous proteins, UxuR and ExuR, were previously predicted to repress synthesis of enzymes required for hexuronic acid metabolism, but little is known about the relative roles of these proteins in gene regulation (2). The amino acid sequences of UxuR and ExuR are 45.5% identical. They are both members of the GntR family of transcription factors, with N-terminal helix-turn-helix DNA-binding domain and the C-terminal domain required for ligand binding and oligomerization. In earlier studies, UxuR was suggested to be a repressor for *uxuR*, *gntP*, *uxuAB*, *uidABC*, and the *yjjN* and *yjjM* genes (3, 4).The repressor function for ExuR has been confirmed only for *exuT*, *uxaB*, and the *uxaCA* operon (5). Bioinformatic analysis of the genes regulated by UxuR and ExuR suggested similar targets for their binding with promoter DNA (4). However, the consensus sequences recognized by UxuR and ExuR are not identical, assuming their differential interaction with individual promoters. The purpose of this study was to compare the UxuR

and ExuR regulons based on genome-wide analysis.

Materials and methods.

UxuR and ExuR proteins were purified by affinity chromatography (6) and then used to produce polyclonal antibodies in rabbit. ChIP was performed as described in (7) with minor modifications. *exuR* and *uxuR* deletion mutants of *Escherichia coli* K12 MG1655 (U00096.2) strain were constructed using recombineering. RNA was extracted using ZR-96 Quick-RNA kit (Zymo Research, USA) and quantified on NanoDrop 1000. Libraries were prepared exactly according to manufacturer's protocol (Illumina). Sequencing was performed on Illumina HiSeq (50nt single end) and data were then analyzed using FastQC, Matcher and PrSeqMatcher software. The profiles of sequence reads aligned on the genome for control and experimental data sets were normalized on the basis of corrected average. Peaks exceeding the background level for at least 3 Std were considered as significant.

Results.

Both *uxuR* and *exuR* expression was suggested to be dependent on the carbon source (4). Thus, all experiments were performed in two growth conditions – Minimal Salts medium supplemented with 5%LB and 0.2% of either D-glucose or D-glucuronic acid that induces expression of the most enzymes and transporters involved in hexuronate metabolism. Total of ~40 targets, with 10 highly overrepresented (Fig. 1), were found for UxuR. Most of them encode enzymes of sugar metabolism (*uxuAB*, *uxaB*, *uxaCA*, *uidA*, *yjjN*, *deoB*, *yeiQ*, *yfgD* and others). UxuR was also detected in the regulatory regions of several related transporters (*exuT*, *uidBC*, *ykgR*) and transcription factors (*uxuR*, *exuR*, *yjjM* and *crp*). It is likely that UxuR binding to its major targets is controlled by sugar ligands, as different carbon sources in the growth media significantly affect their occupancy. The most evident case is 10-fold glucuronate-induced binding to the *uxuAB* regulatory region accompanied by significant reduction of interaction with the *uxaB* and *uxaCA* promoters (Fig.1). Such "flipping" perfectly reflects complex metabolic changes taking place during growth of bacteria on different sugars. Interestingly, some of the targets were occupied by UxuR only in the presence of one or another sugar. For example, *lacZ*, *uidR-ABC* and *yjjM/N* were subjected to the UxuR regulation only during growth on glucuronate, while peaks corresponding to the *yfgD* and *deoB* regulatory regions appeared in the presence of glucose.

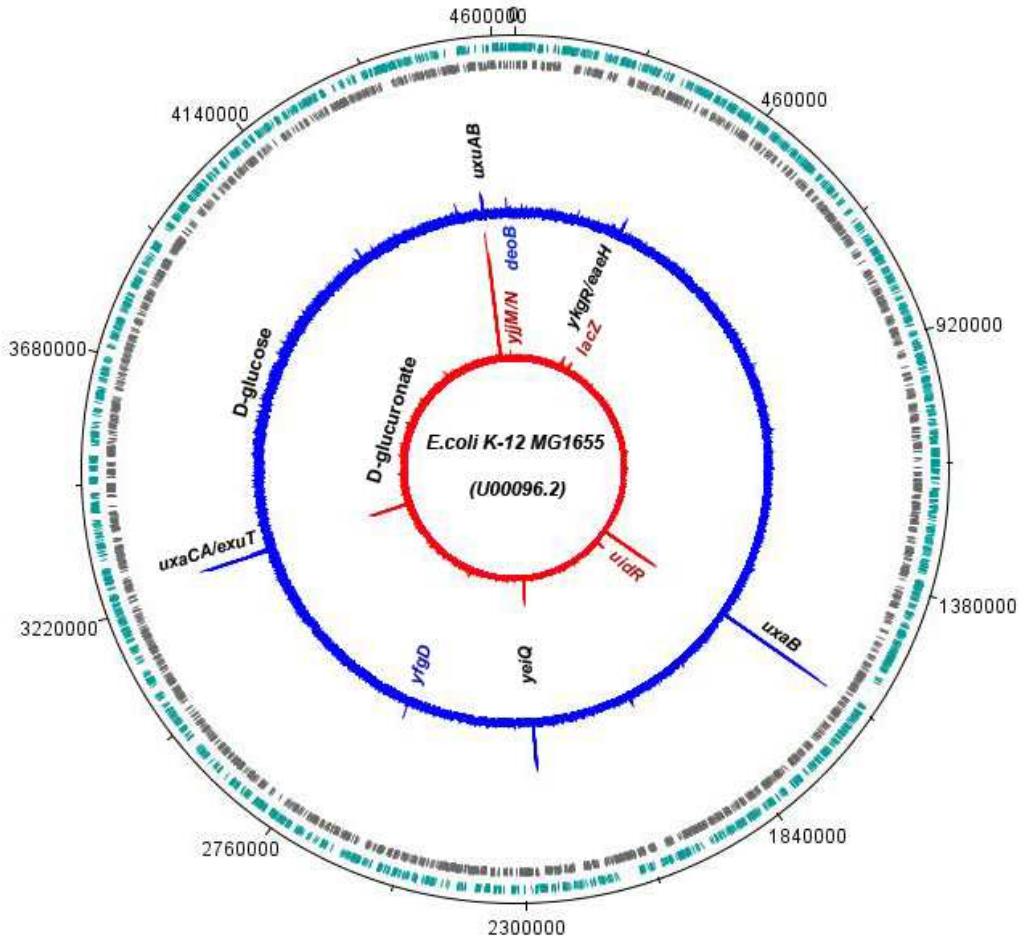

Fig.1 Distribution of the UxuR binding sites on the *E.coli* K-12 MG1655 choromosome (the third and the fourth circles). Carbon sources are indicated on the plot. The data were plotted with 10 bp running window.

ChIP-seq with anti-ExuR antibodies, oppositely, revealed more than 100 targets of moderate binding most of them representing genes encoding transporters (*exuT*, *aroP*, *cysA/cysW*, *mntH/nupC*, *putA/putP*, *ytfQ*, *fimC/D*, *oppA*, *manX*) and other transcription factors including *ompR*, *gcvA*, *gntR*, *nac*, *argR*, *bglG*, *fis* and *uxuR*. All of these targets were further confirmed by comparing the transcriptome of the wild type K-12 MG1655 and K-12 MG1655Δ*exuR* cells. The only one enzymatic system controlled by ExuR in our experiments was *uxaCA* that is in line with the previous report (5). ExuR binding to the targets was not as much dependent

on the carbon source as for UxuR. RNA-seq data indicated practically no changes in the *exuR* transcription, which is in contrast with 8-fold *uxuR* induction in the presence of glucuronate. ExuR is therefore much less dependent on the carbon source and may function as a more global regulator of bacterial metabolism. Taking together, our data suggest that UxuR and ExuR being structural homologues are far from identical in their functional employment. It seems possible that they are complementing each other function, and together with other sugar-dependent transcription factors (cAMP-CRP, GntR) participate in maintaining cell metabolism at the optimal level.

Acknowledgements. We are grateful to Mikhail Gelfand and Fyodor Kondrashov for the help with sequencing and useful suggestions. This work was supported by Russian Foundation for Basic Research (grants 12-04-01830, 13-04-0997, 15-07-05783 and 15-04-08716).

1. N. Peekhaus, T. Conway (1998) What's for dinner?: Entner-Doudoroff metabolism in *Escherichia coli*. *J. Bacteriol,* **180**:3495-3502.
2. D. A. Rodionov et al (2000) Transcriptional regulation of transport and utilization systems of hexuronides, hexuronates and hexonates in gamma purple bacteria. *Mol. Microbiol*., **38**:673-683.
3. C. Bates Utz et al **(**2004) GntP is the *Escherichia coli* fructuronic acid transporter and belongs to the UxuR regulon. *J. Bacteriol.,* **186:**7690-7696.
4. I. A. Suvorova et al (2011) Comparative genomic analysis of the hexuronate metabolism genes and their regulation in gammaproteobacteria. *J. Bacteriol.,* **193:**3956-3963.
5. M. Mata-Gilsinger, P. Ritzenthaler (1983) Physical mapping of the *exuT* and *uxaC* operators by use of *exu* plasmids and generation of deletion mutants *in vitro*. *J. Bacteriol.,* **155:**973-982.
6. A. V. Potapova et al (2014) Development of effective overproduction and purification approaches for ExuR transcription factor of *Escherichia coli*. *Sorption and chromatographic processes*, 14(3): 232-238.
7. D. C. Grainger et al (2005) Studies of the distribution of *Escherichia coli* cAMP-receptor protein and RNA polymerase along the *E. coli* chromosome. *Proc Natl Acad Sci U S A.*, **6,102**:17693-17698.