The Dps nucleoid protein from *E. coli*: is DNA protection accompanied by transcriptional regulation?

Maria N. Tutukina

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

Centre for Genomic Regulation (CRG) and Universitat Pompeu Fabra (UPF), Barcelona, 08003, Spain
masha.tutukina@crg.eu

Sergey S. Antipov

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

Immanuel Kant Baltic Federal University, Kaliningrad, 236041, Russia ss.antipov@gmail.com

Uliana S. Shvyreva

Institute of Cell Biophysics RAS, Pushchino, Moscow region, 142290, Russia
uliana.shvyreva@gmail.com

Tatiana A. Bessonova

Ural Federal University, Ekaterinburg, 620002, Russia

Institute of Cell Biophysics RAS, Pushchino, Moscow region, 142290, Russia
tatianabessonova66@gmail.com

Elena V. Preobrazhenskaya

Institute of Cell Biophysics RAS, Pushchino, Moscow region, 142290, Russia
elena.vl.preobrazhenskaya@gmail.com

Yuri A. Purtov

Institute of Cell Biophysics RAS, Pushchino, Moscow region, 142290, Russia yapurtov@yahoo.com

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

DNA structural remodeling plays an important role in bacteria, determining realization of almost all cellular processes. The task of packaging of 1.6 mm long DNA inside a tiny

bacterial cell ($1\mu M^3$) is accomplished by a family of nucleoid-associated proteins [1]. One of them, Dps, is the main architectural factor condensing DNA during stationary growth of *Escherichia coli* [2]. It is highly expressed upon starvation, and protects DNA from different stresses. According to the conventional point of view, Dps binds DNA without any sequence or structural specificity. However, deletion of *dps* changed the profile of cellular proteins and affected biofilm and fimbriae formation of *E. coli* [3]. Recently, a certain affinity of Dps for artificial branched molecules was detected by atomic force microscopy [4]. However, the question if Dps can participate in regulation of gene expression by interacting with particular sequence or structural elements still remains open.

Material and Methods

Promoters for *dps* were mapped with the PlatProm algorithm and confirmed *in vitro*. Novel regulators for the *dps* gene expression were predicted by comparative genomics and then confirmed by LC/MS spectrometry, reporter assays and qRT-PCR. Recombinant Dps was purified as described in [4]. Efficiency of Dps interaction with linear DNA was estimated by EMSA [5]. To reveal the distribution of the Dps binding sites on the *E. coli* chromosome, two slightly different ChIP-seq approaches with anti-Dps antibodies were used [6].

Results and conclusions

Under normal conditions, the *dps* expression is blocked by several regulators such as Fis, H-NS and MntR, with binding sites being located nearby the main P_{dps} promoter. However, four additional with low transcriptional activity but with strong stimulatory effect on the *dps* expression were found upstream of P_{dps}. They are conservative among *Escherichia* species, were found in the plant pathogen *Dickeya dadantii*, but are absent in most other bacteria. Using computational and experimental approaches we found new potential regulators that are associated with these additional promoters. Most of them represent regulators involved in cell division and colonization control (SdiA, NhaR), antibiotic resistance (EvgA), and metabolic responses (CRP, ExuR, GntR). Additional promoters can, therefore, mediate the Dps-driven antibiotic resistance and biofilm formation detected in [3]. Metabolic regulators may also be necessary to switch *dps* expression in changing environmental conditions as it takes place in the plant pathogen *Dickeya dadantii* during host invasion [7]. Thus, we concluded that Dps

plays not only the role of protective protein but also acts like a metabolic sensor. In this case, its functional state should be ligand-dependent. Given that Dps performs tight packaging of bacterial genome upon starvation, we assumed that this ligand should represent a nutrient that becomes deficient or available when a cell enters a new environment.

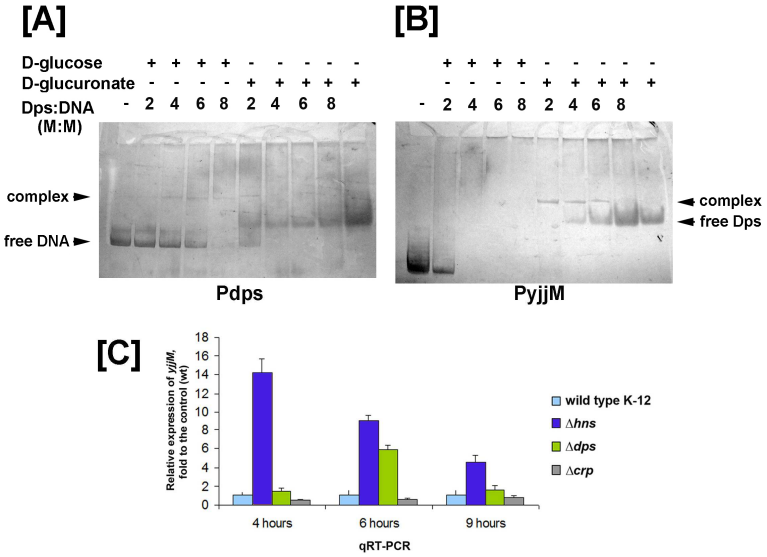

Fig. 1 Dps binds to the *dps* [A] and *yjjM* [B] promoter regions, and this binding is increased in the presence of glucuronate. In the case of yjjMp, additional complex was formed. Sample composition is indicated above the lanes.

Using EMSA (Fig. 1A and B), it was revealed that hexuronates, metabolized by the Ashwell pathway, can change the oligomeric form of Dps and affect its binding to linear DNA targets [5]. Using molecular docking we found that hexuronates bind Dps in the region of intersubunit contacts. Such destabilization of the bonding network can be the main factor provoking the protein decay to the smaller oligomers. We also found that Dps binds its DNA targets with different efficiency. One of the strongest effects was detected for the promoter region of *yjjM*, coding for a metabolic regulator (Fig. 1B). Reporter assays and qRT-PCR (Fig. 1C) confirmed dependence of *yjjM* expression on Dps, but significant effect was detected only after 6 hours of growth, when cell transition from swimming to colonization can occur. Such a time-scaled effect can not be explained by DNA packaging and assumes direct participation of Dps in regulatory events. To reveal preferred targets of Dps in a genome-wide scale, two ChIP-seq experiments were performed for mid-exponential cells (Fig. 2A and B). Except for the undoubted peaks indicative of non-random binding of Dps to different genomic loci, they revealed some difference in the registered patterns (look at the positions of rRNA operons), reflecting participation of Dps in chromatin remodeling. Five out of 9 selected targets were confirmed to be Dps-dependent [6].

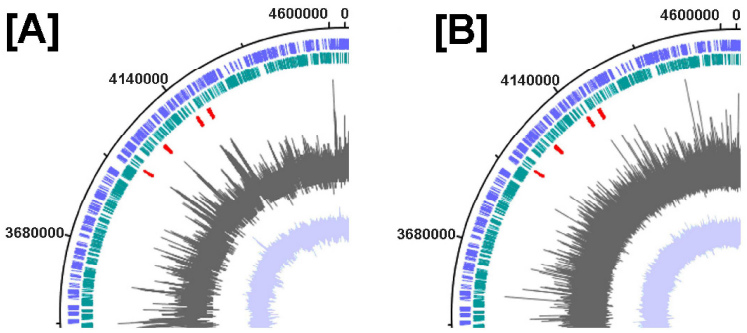

Fig. 2 Distribution of the Dps-binding sites in the *E. coli* MG1655 genome (ratio of read counts for immunoprecipitated and control libraries in 35 bp bins, circle 4). Two outer circles show gene map for two strands. rRNA operons are marked with red ticks. Circle 5 - distribution of reads in the control library.

Taking together, our data suggest that Dps may play not only the role of protective DNA-binding protein of stationary phase but also participate in targeted gene regulation in its different oligomeric forms during earlier stages of bacterial growth.

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