The Dps bacterial nucleoid protein is associated with sugar metabolism

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Background

The DNA packaging is very important for bacterial growth upon adaptation to environmental changes. At various stages of growth, different nucleoid-associated proteins are prevailing. One of them, called Dps (DNA-binding protein from starved cells), is the main nucleoid protein abundant during stationary phase of *Escherichia coli* [1]. Dps is well known for its protective role in bacteria - it increases cells resistance to environmental stresses such as high temperature, oxidative damage, ultraviolet light, low or high pH [1]. Recently, it has been shown that Dps may selectively bind certain fragments of DNA [2]. Moreover, we used Electrophoretic Mobility Shift Assays (EMSA) to estimate the efficiency of Dps binding with regulatory regions of the *fliA* and *dps* genes, and found that presence of D-glucuronic acid stimulates formation of binary Dps-DNA complexes [3]. Keeping in mind that D-glucuronic

acid is an alternative carbon source metabolized via the Ashwell pathway, we assumed that Dps might be involved in control of sugar metabolism in *E. coli* and/or interaction with other metabolic regulators.

Materials and methods

DNA was from *Escherichia coli* str. K-12 substr. MG1655. Gene regulatory regions were PCR-amplified and used for EMSA to estimate the efficiency of Dps binding [3].Promoters were mapped by the PlatProm algorithm [2]. Potential regulators for the *dps* expression were found by comparative genomics and verified by qRT-PCR and LC/MS spectrometry. Multiple alignment was made with T-coffee (Pro-coffee, http://tcoffee.crg.cat).

Results and discussion

It was detected that *dps* expression reacts not only to oxidative stress and change of growth phases, but also to metabolic changes. Moreover, deletion of sugar regulator ExuR led to enhanced *dps*-mRNA level. Thus, we checked if there any other regulators with ability to control *dps* expression, in addition to well-known H-NS, MntR, Fis and OxyR. All binding sites for known regulatory proteins are located close to the Pdps promoter, but the regulatory region of this gene in *E. coli* is longer than average, and includes additional promoters P1, P1', P2 and P3 with stimulatory effect on Pdps [4]. Using different approaches, at these distal promoters possible binding sites for proteins controlling metabolic switches, such as CRP, ExuR, GntR and SdiA, were found.

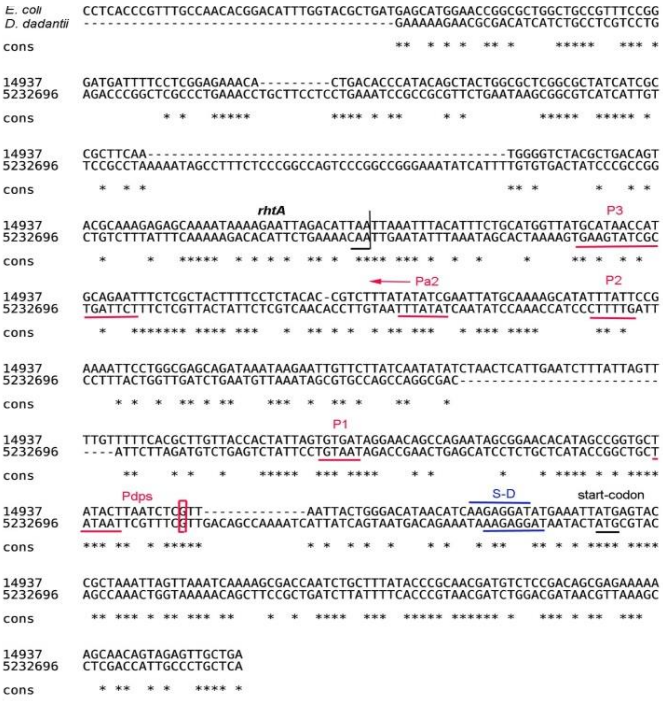

Fig.1 T-coffee multiple alignment of the *dps* gene regulatory region with distal promoters in *Escherichia coli* and *Dickeya dadantii*.

This regulatory region includes the same combination of additional promoters (P1, P1', P2, P3) and binding sites for carbon metabolism regulators GntR and SdiA. These metabolic regulators contribute to *dps*

expression and were verified by qRT-PCR. However, participation of carbon metabolism regulators in the expression of gene coding for nucleoid protein is not common. Multiple alignment of distal promoters revealed that all *Escherichiae* and related species have Pdps and P1 promoters, but not P1', P2 and P3. The same combination of additional *dps* promoters was found in *Dickeya dadantii*, a bacterial plant pathogen from the *Enterobacteriaceae* family (Fig.1). This allows us to make a guess that these additional promoters were horizontally transferred to the *E. coli* genome together with their own regulation mode. In *D. dadantii*, *dps* was strongly induced under oxidative stress applied during exponential phase which coincides with early stage of plant infection [5]. During this stage, *D. dadantii* grows exponentially and starts using simple sugars and small oligosaccharides dissolved in the apoplast, as the main carbon source. Thus, additional *dps* promoters in *D. dadantii* and *E. coli* may play a substantial role in sugar metabolism. We then assumed that Dps may control metabolism by cooperation or competition with some carbon metabolism regulators, such as ExuR. Using EMSA we found that in the absence of sugars Dps formed a diffuse complex with the *exuR* promoter, strongly enhanced and moved up when ExuR was added as the second protein (Fig. 2). Similar effect was registered for the *dps* regulatory region, where ExuR and Dps binding sites overlap. In this case, one more complex was observed (ExuR-Dps complex 2 in Fig. 2B), that might be formed due to the ExuR-Dps heterodimer formation.

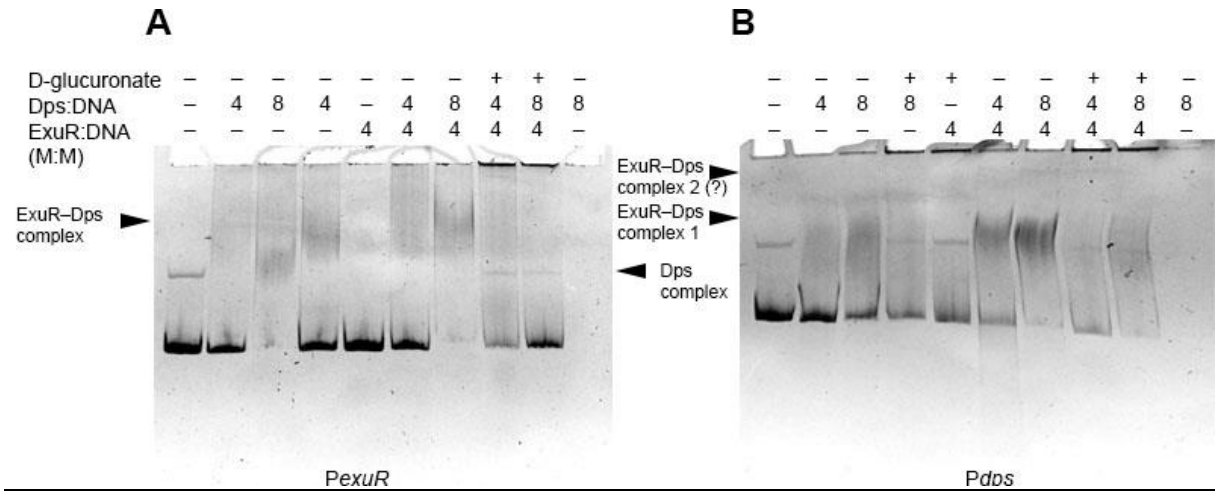

Fig.2 Dps and ExuR cooperatively bind the *exuR* (A) and *dps* (B) gene regulatory regions.

The most logical explanation for the observed phenomenon is that Dps dodecamer wraps the DNA in such a way that the binding sites for other proteins become more available. This suggestion is supported by the fact that if D-glucuronate is present in the sample, then the standard Dps-DNA binary complexes, observed earlier [3], are formed (Fig. 2). However, at

the moment there is no reason to think that dodecameric form of Dps interacts with DNA by wrapping, and our next aim is prove it or to find another explanation of these phenomenon.

Conclusion

The Dps protein functions not only as a protein of oxidative stress response and chromosome protection but also reacts to metabolic changes, and might play a key role in pathogenesis [6]. It may also be involved in metabolic control, assisting or preventing binding of its protein partners to their DNA targets at different growth phases.

Acknowledgements

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