Talk

ECF sigma factor SigX: caracterization and transcriptional regulation in Pseudomonas putida.

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ABSTRACT

Motivation: Extracytoplasmatic function (ECF) sigma factors play a key role in bacteria response to the environment. ECF σ factors are commonly inactivated by an anti-σ factor, which can detect an external signal and release the σ factor. Then, the activated σ factor can bind the RNA polymerase and redirect transcription toward specific response promotors. Pseudomonas putida is a ubiquitous Gram-negative bacterium capable of surviving in a broad range of natural environments which has 19 different ECF σ factors. SigX is an ECF σ factor without an identified anti-σ factor, but there is a putative upstream anti-σ factor (CfrX). In this study we made a characterization of SigX, investigating if there is read-through transcription from cfrX-cmpX unit and analysing the regulation of sigX expression from three different promoters (P1, P2 and P3), also testing if CbrAB two-component system has a control over sigX transcription.

Methods: The read-through transcription of sigX has been studied by RT-PCR by amplification of the intergenic region between cmpX and sigX. Growth of a sigX insertion mutant has been studied in LB with different salt concentrations (from 0-500 mM NaCl) and in M9 medium with succinate. Expression was measured by β-galactosidase assays or by fluorescence in transcriptional fusions of the three putative promoters P1, P2 and P3 to lacZ and gfp, respectively.

Results: There is read-through transcription from cfrX-cmpX unit into sigX. The growth of a SigX mutant is not affected in the absence of salt in comparison with KT2442, but the promoters P2 and P3 are downregulated in a sigX background, thus suggesting they may be activated by SigX. P1 does not respond to SigX. Also, P1, P2 and P3 seem to be activated by the CbrAB control system in a medium containing succinate as carbon source. The expression of P3 is always higher than P2 suggesting there might be a regulatory element between them.

Conclusions: sigX is transcribed from its own promoter and also from an upstream promoter containing cfrX-cmpX, which may contain a regulatory element of the sigma factor. SigX is involved in the response to osmotic stress and is also controled by the CbrAB regulatory system. There is a regulatory element upstream P2 which increases the expression of sigX.

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