Discovery of a new conserved tandem motif upstream of outer membrane hemin transporters in alpha and beta-proteobacteria

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In many alpha-proteobacteria, the uptake of alternative iron sources such as hemin and siderophores is negatively regulated at the transcriptional level by the general iron response regulator RirA, while specific positive regulators involved in siderophore biosynthesis also exist in many cases. In our laboratory, a hemin outer membrane transporter, ShmR, was discovered in the alpha proteobacterium S.meliloti 1021 [1]. By an experimental screening we found that the small protein HmuP is a positive regulator of ShmR (Amarelle et al., unpublished). HmuP protein only affects expression of the shmR gene, and not of other genes involved in the uptake of hemin or other iron sources. By sequence alignment of HmuP homologues and comparison with the NMR structure of Rhodopseudomonas palustris CGA009 HmuP protein, we found that certain amino acids localized within predicted beta-sheets are well conserved. The structure of the HemP domain of the hmuP homolog of R. palustris is composed exclusively of three beta strands, which is an unusual domain for transcriptional regulators which bind DNA. In order to discover putative HmuP responsive sites in the promoter of shmR, we searched for conserved motifs in the upstream regulator region of shmR and other genes coding for similar outer membrane hemin transporters present in other bacteria. By using bioinformatic tools such as Gibbs Motif Sampler and MEME, we found a well conserved tandem motif that is only present upstream of hemin transporters in rhizobia, other alpha-proteobacteria and many beta-proteobacteria. Interestingly, this motif is not present upstream of other iron uptake related genes. Furthermore, this motif is only present in species that contain hmuP orthologs, but not in related species that do not contain this gene. This result suggests that this motif is involved in hemin uptake regulation, and that it could be the HmuP responsive sequence.


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