RNA-Seq Transcriptional Profiling Of *Herbaspirillum seropedicae* SmR1 Reveals Roles For Fnr Orthologs In Controlling The Expression Of The Cytochrome c - Based Electron Transport Pathway

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**Introduction.** The Fnr protein plays an important role in transcriptional regulation of genes during the switch from aerobic to oxygen-limiting conditions. Analysis of *H. seropedicae* SmR1 genome revealed the presence of three *fnr* genes. We have used RNA-seq to compare global changes in the transcription profiles of a triple *fnr* mutant (MB231) against the wild-type strain of *H. seropedicae*. **Materials and Methods.** For RNA extraction we grew the wild-type and MB231 strains in aerobic conditions to an optical density of 0.4 and then switched the cultures to 2% oxygen for 1.5 hours. To validate the differential transcriptional profiles obtained from RNA-Seq, we performed β-galactosidase assays with transcriptional lacZ fusions of specific genes and also analyzed the cytochrome content spectroscopically and by heme protein staining. **Results and Discussion.** Global gene expression patterns revealed that potentially, 240 *H. seropedicae* genes were differentially expressed. Of these, 174 were down-regulated in the *fnr* triple mutant strain, indicating that these genes are activated by Fnr. 66 genes were up-regulated in the MB231 strain, implying that they are targets for Fnr-mediated repression. Large changes were observed in genes required for the biosynthesis and activity of the *cbb3*-type oxidase. The *petABC* operon, which encodes the cytochrome *bc1* complex was down-regulated 7-11 fold in the Fnr mutant compared with the wild-type control. In addition, differential expression of genes encoding c-type cytochromes and cytochrome c biogenesis was observed. Furthermore, deletion of all three *fnr* alleles resulted in a growth phenotype under microaerobic conditions. **Conclusions.** Our results imply that the *H. seropedicae* Fnr proteins activate the expression not only of the *cbb3*-type heme copper oxidase, but also of many other components of the cytochrome c branch of the respiratory chain. Probably the Fnr proteins enable *H. seropedicae* to exploit respiratory flexibility and optimize energy coupling in response to oxygen availability.

Key words: RNA-Seq, *Herbaspirillum seropedicae*, Fnr, cytochrome.

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