Analysis of a Ferric Uptake Regulator (Fur) Mutant of *Desulfovibrio vulgaris* Hildenborough

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While iron is an essential element for most prokaryotic metabolism, an excess of Fe^{2+} in an aerobic environment can lead to the formation of reactive oxygen species via Fenton chemistry. Thus the global transcriptional regulator Fur (ferric uptake regulator) controls genes involved in iron metabolism by responding to available Fe^{2+} concentrations. However, little is known about the iron metabolism of strict anaerobes. In anaerobic environments, Fe^{2+} is believed to dominate over the more oxidized Fe^{3+} species. In fact the anaerobic sulfate reducer, *Desulfovibrio vulgaris*, appears to lack recognizable genes for the synthesis of Fe^{3+} transporters such as siderophores. To study the metabolism of iron in *D. vulgaris*, a method for deleting the *fur* gene was developed. This scheme utilized a cloning without restriction enzymes procedure to create a construct containing a Kan^r gene flanked by 800 bp of up- and downstream regions of the *fur* gene.

Following the introduction of the mutagenic construct by electroporation, two G418 resistant *D. vulgaris* transformants were isolated. PCR and Southern analysis indicated that the *fur* gene was replaced by the Kan^r gene in both transformants. When compared to the wildtype, the mutants also showed a four-fold increase in resistance to $MnCl_2$ treatment (a method used to isolate *E. coli fur* mutants). Under normal iron replete growth conditions, transcriptional analysis of the mutant and wildtype indicated mRNA for *fur* only in the wildtype, confirming the deletion. Genes such as *feoAB* (encoding an Fe^{2+} transporter), which are predicted to be repressed by Fur and its corepressor Fe^{2+}, exhibited an expression increase in the mutants. Transcription of a flavodoxin gene was also increased in the mutants. This result was counterintuitive since flavodoxins are suggested to compensate for the absence of iron-containing electron transport components. However, in the absence of the Fur repressor, one would predict plentiful iron in the cell. The consequences of the deletion of this regulator in *D. vulgaris* continue to be explored.