Transcriptome and Computational Analysis of the Regulatory Role of the *fur* in the H_2O_2 Stress Response of *Shewanella oneidensis*

H-067

Ting Li[1,2], Juntao Guo[1,2], Xiufeng Wan[1], Dawn Stanek[1], Liyou Wu[1,3], **Xueduan Liu[1]**, Tingfen Yan[1], Ying Xu[1,2], Alex Beliaev[1], Jizhong Zhou[1,3]

[1]Oak Ridge National Laboratory, Oak Ridge, TN, [2]University of Georgia, Atlanta, GA, [3]University of Oklahoma, Norman, OK

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Abstract

The upregulation of the iron-responsive genes in Shewanella oneidnesis represents an interesting divergence of the expression pattern from those of many other microorganisms. Iron is an essential nutrient with limited bioavailability. Due to its ability to react with and catalyze the generation of toxic radicals, iron when overloaded may pose a big threat to living cells and tissues; and so the acquisition of iron is usually tightly regulated in biological systems. In E.coli cells the iron regulator Fur serves as a repressor which suppresses the expression of many iron uptake genes; under iron starvation condition Fur is downregulated and the iron transport and metabolism genes are therefore upregulted. Fur is up-regulated by H2O2 treatment and its expression is activated by two oxidative stress regulators, oxyR and soxR. Therefore the iron inducible genes in E.coli are down regulated in the presence of H2O2, and similar regulation has been reported in many oxidative stress studies with various bacterial species. Microarray data of the wild type shewanella oneidensis show that the transcription level of fur is not significantly affected by H2O2, while many iron inducible genes show drastic up-regulation under H2O2 stress conditions. These genes include the TonB1 iron transport system genes, a bacterioferritin gene bfr, the ferrous iron transport genes, and some DNA repair and metabolism genes. Increased expression of some iron starvation-inducible genes has been observed in Pseudomonas aeruginosa and Rhodobacter sphaeroides when they are treated with H2O2; yet not much is known about the mechanism of the regulation. To investigate the H2O2-induced regulation of the iron responsive genes in shewanella oneidensis we included a fur deletion mutant in our microarray studies. Not surprisingly the fur deletion mutant of *Shewanela oneidensis* shows hypersensitivity to H2O2 stress. Further analysis by microarray study of the fur mutant under oxidative stress condition reveals interesting regulation pattern. In fur mutant the iron responsive genes are highly expressed when compared with the wild type strain, which is in agreement with the repressor role of fur. However in contrast to wild type strain fur mutant shows little or diminished induction of the iron inducible genes in response to H2O2, indicating that fur directly or indirectly mediates the upregulation of these genes by h2O2 treatment, and the regulation is apparently independent of the transcription of the fur gene since the expression level of fur does not show significant change under the stress condition. Interestingly we found that some genes with decreased expression in fur mutant cells in comparison with WT strain also show fur-dependent upregulation by H2O2 treatment, suggesting that fur may function as a positive regulator to these genes. More interestingly, many of the fur regulated iron responsive genes also exhibit unusually high expression in oxyR deletion mutant, and when H2O2 is applied to the oxyR mutant the transcription of the iron genes further increases, which coincides well with the regulation pattern of these genes in wild type strain; these data suggest that oxyR mutation may play a role in modulating fur activity. At the time being we are applying bioinformatics methods to identify the direct target genes of fur and oxyR in the transcriptional response to H2O2.

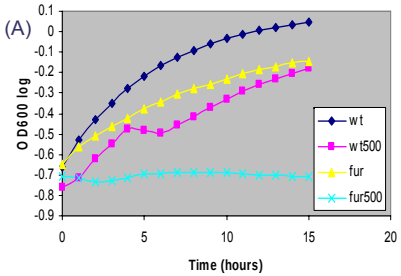

Fig.1 Phenotypic studies of Δfur mutant. (A) Comparison of the growth curves of WT and Δfur cells under oxidative stress. Various concentrations of H_2O_2 was added to log phase culture in LB medium. Growth kinetics was measured through optical density at 600 nm wavelength. Results represent the trend of multiple individual experiments. (B) Low aerobic tolerance of Δ fur. Log phase cells were serially diluted and spotted onto LB plates with or without catalase. Δfur cells show significant aero-sensitivity and the growth deficiency can be rescued by catalase.

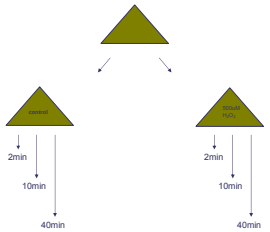

Fig.2 Experimental design. Early to mid-log phase aerobic cells were aliquot into two portions and treated with mock and 500uM H_2O_2 respectively. Cells were harvested in parallel to minimize handling differences. RNAs extracted from these samples were used in subsequent microarray hybridizations and RT-PCRs.

Acknowledgements

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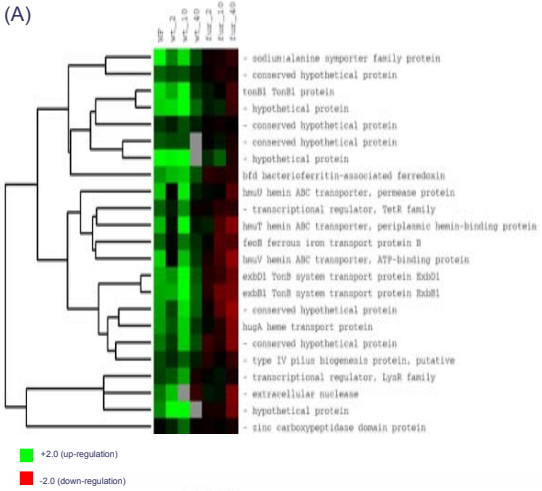
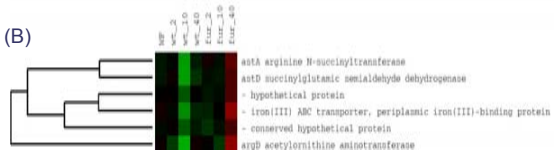
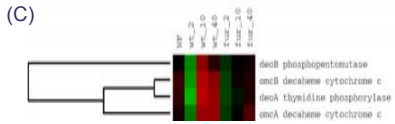


Orf No.	Predicted fur motif
SO3063	
SO2367	(527) CAAATGATATTGTTTATCATTTA
SO3670	(703) CAAATAGAAATCATTATCATTTG
SO3344	(756) TAAATGATAATGATTATCAGTTA
SO2711	
SO2642	
SO3062	(733) TTGTTGAGAACAATTCTCATCTA
SO0583	(745) CATTTGATAAGCGTTTTCATTTA
SO3674	
SO3684	
SO3673	
SO1784	
SO3675	
SO3672	(29) CTAATCCAATTGCAACGCATTTG
SO3671	(29) CTAATCCAATTGCAACGCATTTG
SO3407	(527) CAAATGATATTGTTTATCATTTA
SO3669	(631) CAAATGATAATGATTTCTATTTG
SO3408	(527) CAAATGATATTGTTTATCATTTA
SO3521	
SO4524	(707) TAAATGATAATAATTTTCAATAC
SO1066	(261) GGTATGTTAAAAAATATCATTAA
SO3406	(527) CAAATGATATTGTTTATCATTTA
SO2424	



SO0744 (423) AAACTGATAACTATTATCATTAA



SO1219	(145) CAAATATGTTGTATTAGTATTTA
SO1778	
SO1218	
SO1779	(181) CTAATGAGATTGTTCTTAATTTG

Table 1 orf numbers of the genes shown in the cluster images (left) and the predicted fur binding motifs. Genes highlighted in the same color are predicted to be in the same operon.

Fig.3 Hierarchical cluster analysis of microarray data reveal two possible regulation mechanisms by fur. Fur as a repressor: Cluster (A) represents genes that show higher expression in log phase fur than in WT cells, suggesting repression of these gene by fur gene during steady state growth in WT cells. When treated with H2O2, these genes are highly upregulated in WT cells, whereas in fur mutant the upregulation is either reduced or completely abolished, indicating that fur mediates the H2O2-induced changes in expression. Fur as an activator: Clusters (B) and (C) contain genes that exhibit similar H2O2 regulation by fur, but show no change or slightly decreased expression in log phase fur mutant cells when compared with WT cells, suggesting that fur is a positive regulator for these genes. (D) computationally predicted fur motif. WF, relative gene expression of WT strain to that of Δ fur. WT_2, 10,and 40, relative gene expression of WT cells treated with 500uM H2O2 for 2minutes,10 minutes, and 40 minutes respectively to that of the untreated control sample. fur_2, 10, and 40, relative gene expression of Δ fur cells treated with 500uM H2O2 for 2minutes,10 minutes, and 40 minutes respectively to that of the untreated control sample.

Gene	Gene	Fold change (oxyR/WT)	Fold change (oxyR-H2O2/ oxyR)	n
SO3670	tonB1	181.97	63.10	9
SO3672	exbD1	21.88	15.14	6
SO3671	exbB1	13.80	28.84	12
SO3669	hugA	6.31	6.46	10

Table 2 Higher expression of the fur target genes in oxyR mutant (microarray data). No oxyR motif has been found in the regulatory regions of these genes, strongly suggesting the upregulation is mediated by fur.

Summary

1. The fur gene plays an important role in regulating the transcription response of Shewanella oneidensis to H_2O_2 induced oxidative stress. It functions as both a positive and negative regulator in the presence of H2O2. By the use of computational analysis we identified the direct targets of the H2O2 responsive fur regulon.
2. The activity of fur is directly or indirectly modulated by oxyR gene.