phuR intergenic mutation results in pleiotropic effects on global gene expression

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125

FUNTIONAL AND MECHANISTIC CHARACTERIZATION OF THE GLUTAMINE AMIDOTRANSFERASE 1-LIKE (GAT1-LIKE) SUBFAMILY OF TRANSCRIPTION REGULATORS IN PSEUDOMONAS AERUGINOSA

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One of the hallmarks of the genus Pseudomonas is its incredible metabolic diversity, which is reflected in both predicted metabolic genes and a large repertoire of transcriptional regulators. During our study of Pseudomonas aeruginosa detection and metabolism of host-derived compounds, we repeatedly identified transcription factors from a particular subfamily of AraC regulators called GAT1-like (glutamine amidotransferase 1-like). The transcription regulators that regulate metabolism of host-derived arginine, carnitine, and glycine betaine are members of the GAT1-like subfamily. Including previous work by others on the arginine regulator, we now know the activator specificity for three of the seven GAT1-like regulators in P. aeruginosa. We have been investigating this subfamily using genetics, bioinformatics, transcriptomics, and biochemistry to understand the ligand binding and DNA binding of the known members and use that information to inform characterization of the four uncharacterized members of the subfamily. Here we report on the identification and characterization of the sarcosine-specific regulator SouR (PA4184) including how it interacts with the known GAT1-like regulators GbdR and CdhR in metabolism of host-derived quaternary amines. These results suggest an interesting model for GAT1-like regulation that we are currently testing. Additionally, we have used this information and bioinformatics analyses to predict likely ligands for the remaining uncharacterized GAT1-like members and we are currently testing these predictions using genetic analyses. Based on our data, the GAT1-like regulators control metabolism of host-derived accessory nitrogen compounds where the nitrogen is secondary, tertiary, or quaternary bonded.

126

PHUR INTERGENIC MUTATION RESULTS IN PLEIOTROPIC EFFECTS ON GLOBAL GENE EXPRESSION

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We have previously found a positive selection for promoter mutations in Pseudomonas aeruginosa DK2 leading to increased expression of the phu (Pseudomonas heme utilization) system. By mimicking conditions of the CF airways in vitro, we experimentally demonstrated that increased expression of phuR confers a growth advantage in the presence of hemoglobin, thus suggesting that P. aeruginosa evolves towards iron acquisition from hemoglobin. Further analysis of the effect of this promoter mutation in P. aeruginosa lead to discovery of new additional phenotypes such as enhanced inhibition of Staphylococcus aureus and a clear change in pigmentation of P. aeruginosa from white to green/yellow. To begin to understand the underlying mechanism of these pleiotropic effects, we performed Affymetrix GeneChip DNA microarray analysis on isogenic strains of P. aeruginosa DK2 with (M2) and without (WT) the phuR promoter mutations. We find 163 gene expressions to be statistically different between the two strains, where the most significant difference was observed in the six local genes of the phu operon. Moreover, we see an apparent down-regulation of genes involved in other iron uptake system, possibly to compensate for the overexpression of the phu system. Interestingly, we find a number of stress related protein genes such as ibpA, grpE , hscB, clpV1 and clpX to be up-regulated in M2 compared to WT. We therefore propose

a model where significant overexpression of a membrane associated protein such as PhuR leads to a stress response that re-wires the transcription of certain genes. We are currently pursuing this model by further investigation of the target genes.

127

INVESTIGATION OF AZOREDUCTASES IN PSEUDOMONAS AERUGINOSA

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Azoreductases are a genetically diverse group of NAD(P)H dependent flavoenzymes found ubiquitously in nature and are produced by many pathogenic bacteria including P. aeruginosa. Responsible for catalysing cleavage of the azo bond found in azo dyes and azo-pro drugs they are primarily considered for their use in industrial bioremediation and treatment of inflammatory bowel disease. Recently, however, bacterial azoreductases have been indicated to play a key role in colonisation of both plant and mammalian hosts suggesting that they may be more important for bacterial survival that previously thought. They have been shown to reduce a wide range of reactive species including toxic quinones which are secreted by plants as a defence mechanism during bacterial invasion. Three azoreductases have so far been characterised in P. aeruginosa and through an extensive literature search a further seven putative azoreductases have been identified. Through techniques involving the generation of recombinant pure enzyme we have characterised the reductive abilities of these suspected azoreductases. We have determined their nicotinamide and flavin selectivity and have confirmed reductive activity against arrange of substrates including azo, quinone and nitroaromatic compounds. For the first time we have identified members of the modulator or drug activity B and the YieF families as azoreductases and NAD(P)H quinone oxidoreductases. We have confirmed the family of azoreductases in P. aeruginosa to be more extensive than previously thought, increasing the range of enzymes which can be engineered for bioremediation. We have also shown that these azoreductases can perform a two electron reduction of quinones to the less toxic quinol, implying an important function of the detoxification of xenobiotics.

128

REGULATION BY THE PRRF AND PRRH SMALL RNAS IN *PSEUDOMONAS AERUGINOSA*

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Pseudomonas aeruginosa is an opportunistic pathogen that causes a variety of life-threatening infections in compromised individuals. Iron is required for *P. aeruginosa* virulence and is obtained through siderophore, heme, and ferrous iron uptake systems. Excessive iron induces oxidative stress, thus, iron uptake is regulated by intracellular iron concentrations. Iron also represses the expression of two homologous small regulatory RNAs (sRNAs), PrrF1 and PrrF2. The PrrF sRNAs induce the degradation of mRNAs encoding non-essential iron-containing proteins, allowing *P. aeruginosa* to "budget" this nutrient when stores are limiting. The *prrF* genes are encoded in tandem in *P. aeruginosa*, allowing expression of a distinct sRNA named PrrH. PrrH expression is responsive to heme, representing a significant source of iron in the host. Previous studies demonstrated that heme regulates the expression of genes for nitrite reduction (*nirL*), heme acquisition (*phuS*), and virulence (*vreR*) in a *prrF* locus-dependent manner (Oglesby-Sherrouse *et al.*, 2010; Reinhart *et al.*, 2015). However, these studies were unable to determine whether the PrrF or PrrH sRNAs were responsible for heme-dependent regulation of these genes. In the current study, we have developed a complementation system that distinguishes