

Synopsis

The *bgl* operon of *E. coli* encodes for the proteins involved in the uptake and catabolism of plant-derived aromatic beta-glucosides, normally associated with the soil environment. The silent state of the operon seen predominantly in gut isolates has posed an evolutionary puzzle in terms of its maintenance in the genome in a potentially functional form. Mutational activation of the operon, in addition to enabling the hydrolysis of aromatic beta-glucosides, has been shown to confer additional metabolic capabilities in stationary phase, providing a strong selective force for its retention in the genome. The activated operon has also been shown to be involved in bacterial predator evasion strategy. The ability of the *bgl* operon to confer accessory benefits to cells makes it an important system to study its effect on downstream genes. A comparison of the proteome profiles of Bgl⁺ and Bgl⁻ strains could identify over 12 genes that are differentially expressed in Bgl⁺ strains in stationary phase. The detailed analysis of the regulation of one of these proteins OppA that encodes an oligopeptide transporter implicated a role for BglG, the antiterminator encoded by the *bgl* operon, in the regulation. The studies presented in Chapter 2 of this thesis were aimed at elucidating the mode of regulation of another downstream target gene of the operon, *ridA*. The RidA protein which was found to be over-expressed in Bgl⁺ strains in stationary phase is a deaminase that acts on the toxic metabolites produced during the catabolism of amino acids serine and threonine. Conversion or deamination of these metabolites by RidA also imparts it a special function as a metabolic scavenger making it an important target to be investigated. Analysis of the *ridA* promoter suggested that Lrp is one of the potential regulators of the gene. The physical binding of Lrp to *ridA* promoter *in vitro* was confirmed by Electrophoresis Mobility Shift Analysis (EMSA). Deletion of *lrp* leads to reduced *ridA* expression, indicating a positive role for Lrp in the regulation. These studies also indicate an indirect role of BglG on the expression of *ridA* by modulating the regulators of the glycine cleavage system GcvA and the small regulatory RNA *gcvB* that in turn regulates Lrp.

The results presented in chapter 2 also highlight the physiological significance of *ridA* up-regulation in a Bgl⁺ background. The deaminase activity of RidA has been shown to be essential to eliminate the toxic intermediates generated upon breakdown of peptides containing serine/threonine in stationary phase. The loss of RidA function in Bgl⁺ strains results in a higher level of sensitivity to the presence of serine in the medium compared to Bgl⁻ strains due to increased transport of serine and serine-containing peptides during stationary phase. This is consistent with the up-regulation of serine transporter genes *sstT* and *dppA* seen in Bgl⁺ strains by RT-PCR analysis.

The role of BglG in the regulation of *oppA* and *ridA* during stationary phase and its possible involvement on the expression of several other genes suggest that the BglG regulon might be larger than presumed till now. In Chapter 3 of this thesis, the results of a comparative transcriptome analysis of a *bglG* wild type versus a *bglG* deletion strain by RNA sequencing are presented. The RNA Sequencing data highlights important stationary phase regulators such as *bolA* and *gadE* that show a strong down-regulation upon loss of *bglG* suggesting that *bglG* might be involved in their regulation. These stationary phase regulators maintain the homeostasis of the bacterial cells as well as provide them defence against various kinds of stress. The involvement of BglG in the regulation of these genes indicates its role as a regulator of stress response. The results of the comparative transcriptome analysis provide an array of genes belonging to pathways such as biofilm formation, LPS biosynthesis, and folate metabolism that are affected in terms of expression upon *bglG* deletion. Phenotypic analysis of biofilm formation, motility, and cell permeability revealed that the *bglG* mutant displayed higher biofilm formation, defective motility, permeability and folate metabolism. The Epilogue section summarises the broader implications that can enhance our understanding of gene regulation dynamics going on in the cells during stationary phase and discusses possible directions for future investigations.

