RESEARCH STATEMENT

Metabolism is fundamental for existence since it produces energy and synthesizes essential molecules of life. It is highly regulated and patterns in metabolic pathways have been conserved over evolution. A cell's response to environmental stimuli is governed by the coordinated action of three important components, namely, signaling, gene expression and metabolic reactions. Hence, it is important to understand how an organism's genotype can be bridged to a specific phenotype along with the underlying molecular mechanisms.

In the last decade, several formalisms have been developed to model the individual subsystems. However, integrated models that combine all the three into a coherent whole have been uncommon and the construction and analysis of such models pose several challenges. The usefulness of such integrated models is enormous as they help us understand cellular behavior under different environmental conditions as well as perturbations and enable us to map genotype to phenotype [1]. Further, the models help us generate hypotheses that can be experimentally validated.

Our main objective is "*Quantification, analysis and experimental validation of an integrated model comprising of signaling, gene expression and metabolic pathways, taking into consideration different control mechanisms and underlying mechanistic details enabling genotype-phenotype mappings for specific metabolic phenotypes under different environmental conditions in Escherichia coli*". With this, we aim to understand the exact mechanism of *how* the genotype translates to a particular metabolic phenotype under different environmental stimuli. We also hope to glean evolutionary insights of how control mechanisms have evolved in nature and the criteria for selection.

Escherichia coli is one of the simplest and well-studied prokaryotic organisms with a wealth of omics data. The enzymes responsible for catalyzing various reactions in the central metabolic pathway are controlled by genes, regulated by transcription factors which further get turned off or on depending upon various signals received by the organism from the environment. There are several distinct mechanisms of control of reaction rates in a pathway, namely, genetic control via transcriptional regulation [19], epigenetic control (via sRNAs [2]), enzymatic inhibition/activation and substrate limitation. We intend to study both transcriptional and sRNA control mechanisms. Bacterial sRNA regulators control gene expression in several pathways e.g., carbon utilization, carbohydrate metabolism, outer membrane composition and stress responses [20, 21].

A cell's response to external stimulus is governed by signal transduction pathways, where external stimulus such as presence of oxygen, change in pH or temperature can initiate the formation of chemical compounds that bind to the receptor proteins in the cell membrane and initiate a cascade of reactions that conduct/amplify the signals to activate signaling pathways further altering enzymatic gene expression that regulate metabolism [3]. Signaling pathways models include Boolean models [4] [5], stoichiometry based reversible biochemical reaction equations [6] simulated by ODEs and stochastic modeling [7]. Similarly gene expression can be modeled using Boolean models [8], Bayesian inference [9], ODEs [10], stochastic models [11] and time-dependent functions [12]. We have proposed a steady state modeling formalism for gene expression which includes molecular mechanisms that help in simulating microarray data with high accuracy and can be used to study the effect of both structural and parametric perturbation on gene expression [13, 14]. Metabolic network consists of a complex set of highly interconnected enzyme catalyzed reactions. A particular phenotype is characterized by a set of such reactions catalyzed by various enzymes present in its genomic capability. Flux balance analysis [17, 15], Extreme pathways [18] and Elementary modes [17] have gained popularity as it requires only stoichiometric information of the metabolic network. Our intention is to combine kinetic/steady state models for the three subcomponents and predict the behavior of the organism under perturbations and environmental stress. We aim to generate hypotheses by predicting the phenotype of the organism under perturbations such as deletion mutants, changes in environmental conditions and validate the predictions by experimentally

characterizing the organism under a given condition in the laboratory under a controlled environment, with the use of bioreactors and analyze metabolites using HPLC.

As a second objective, we are also interested in studying the *effect of selecting fittest generation based on growth rate of mutants of transcription regulators* which investigates the fitness advantage gained by low growing mutants of *E. coli* under a given condition, by successively selecting the fittest colony and propagating them in the laboratory. We intend to analyze the flux distributions and sequence the evolved mutant when sufficient growth advantage has been achieved and compare it with the original mutant and observe interesting mutations in gene loci that result in the increased growth rate.

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