Summary

The different elements of the arginine biosynthetic system of *Escherichia coli* and the principal regulatory mechanisms operating at genetic and enzymatic levels are identified. Arginine is synthesized from glutamate in eight enzymatic steps. The synthesis of all enzymes is subject to repression by arginine, mediated by the repressor ArgR. In addition, the first enzyme of the pathway, N-acetylglutamate synthase (NAGSase) is subject to feedback inhibition by arginine. In *E. coli*, a single carbamyl phosphate synthase (CPSase) provides the intermediate carbamyl phosphate (CP) for both the arginine and the *de novo* pyrimidine biosyntheses. Reflecting this dual function of CPSase, its synthesis is subject to cumulative repression by arginine and pyrimidines and its activity is subject to a complex network of metabolic regulations which ensure a balanced distribution of CP between the arginine and pyrimidine biosyntheses. Such complex regulations cannot be described solely by an intuitive approach. More generally, in order to understand and describe arginine biosynthesis in quantitative terms, a simple mathematical model integrating all existing data and also giving further insight into the control of this highly regulated biological system needed to be developed. This was the major objective of this work.

As a prerequisite to the building of a model of arginine biosynthesis, including its dynamics, it was necessary to redefine the limits of the arginine regulon by analyzing the cellular response to arginine, and quantifying this response in order to obtain a set of coherent values for key parameters of the system. A transcriptome analysis using hybridization to microarrays and real-time quantitative PCR defined the amplitude of transcription regulation and the time course of arginine mediated repression of all *arg* genes (chapter 2). This study identified new members of the regulon: the arginine-specific transport (*artPIQM* and *artJ*) and the LAO uptake systems (*hisJQMP*). The control regions of *artPIQM*, *artJ* and *hisJQMP* contain ARG boxes, the core elements of arginine operator loci. The functionality of these ARG boxes was demonstrated. A corollary of this analysis was to emphasize the importance of the feedback inhibition of NAGSase in controlling the arginine biosynthetic flow in the period following the onset of repression. To accurately model this important regulation, the kinetic parameters of NAGSase (V_{max}, K_m and K_i) were determined after purification of the enzyme (chapter 3). One of the experimental perturbations of the system, used for the validation of the model, is the cancellation of the feedback inhibition regulation (chapter 4). Therefore, a feedback-resistant (fbr) mutant of NAGSase was generated. After purification, we determined kinetic and regulatory properties

of the fbr NAGSase, including its arginine inhibition constant, in parallel with those of the wild-type enzyme (chapter 3).

To model arginine biosynthesis, the pathway was divided into three modules connected through a tightly regulated branch point, a simplified representation derived in part from the transcriptome analysis (chapter 2). Each module synthesizes a key metabolite and comprises one key regulated enzymatic step, the other steps within the module being considered as non-limiting for arginine synthesis and non-involved in other biological processes. The rigid branch point is the synthesis of CP and the network of regulations controlling its distribution between the arginine and pyrimidine pathways. The modules could be described as follow: (I) the synthesis of ornithine from glutamate, (II) the synthesis of arginine from ornithine and CP and (III) the utilization of CP to ultimately produce UTP and CTP. The simplified system was mathematically described by a set of five differential equations describing the evolution of the key metabolites involved in arginine biosynthesis or in its regulation, along with the appropriate kinetic expression for each key enzyme. These equations were numerically integrated using an appropriate software (XPPAUT).

To calibrate the model, two steady-states of arginine biosynthesis in wild-type *E.* coli were used: partial repression due to endogenous free arginine, and full repression, where the intracellular arginine concentration is augmented by the uptake of arginine from the medium. The calibration process showed that the relationship between CP availability for pyrimidine synthesis and the size of pyrimidine nucleotides pools is not simple: the maintenance of pyrimidine nucleotides pools must depend mainly on the nucleotide recycling and salvage pathways.

To validate the model, steady-states were simulated for three different perturbed conditions of the system, each corresponding to the abolition of one regulatory mechanism. The simulation results were compared to the experimental observations. The conditions were: genetic derepression, cancellation of feedback inhibition by arginine, and a very low and fixed rate of CPSase synthesis which will bring CP concentration below its wild-type repressed level. Both simulation and measured data suggested that, on a quantitative scale, genetic repression and metabolic regulation of the first enzyme of the pathway contribute equally to the control of the metabolic flux through the pathway. In addition, a significant decrease of intracellular arginine concentration was predicted and observed when a fixed low rate of CP synthesis is imposed, showing that in such extreme conditions CP synthesis can be rate-limiting for arginine synthesis.

The validated model provides an accurate description of arginine biosynthesis in *E. coli* and it was applied to describe the evolution of metabolite pools in response to simulated parameter changes or to predict arginine production in strains combining different alterations of regulatory circuits. The validity of these predictions was proven experimentally.

The model presented can be used for the rational design of arginine overproducing strains. This approach can be advantageous when compared to random mutagenesis, a method that has been frequently used for the design of the production strains.

Surprisingly, in a feedback-resistant mutant of NAGSase, where the arginine concentration is 6 times higher than in the wild-type, the levels of various enzyme associated with arginine biosynthesis remain nearly unchanged as compared to the wild-type. This prompted a study of the dynamics of arginine repression. It was observed that in the wild-type as in the mutant, a threshold intracellular arginine concentration of about 0.90 mM needs to be achieved to establish repression. This phenomenon cannot be explained solely by the autoregulation of repressor synthesis and it indicates the existence of an as yet unidentified regulatory loop.