Summary

This thesis is concerned with the study of the regulatory processes involved in the adaptation of metabolic systems to environmental and genetic changes. The study of regulation is an endeavor unique to biology. It addresses systems of a complexity that is unparalleled in the inanimate realm. More importantly, these systems are adaptive: living cells modulate their system properties in response to environmental changes. These modulations are governed by yet unknown drives and constraints.

In the introductory chapter (Chapter 1) it is argued that our understanding of regulatory processes is hindered by the lack of a precise definition of the term regulation and of appropriate methodologies to describe regulatory process in an unambiguous and quantitative manner. Chapters 2 to 5 report the implementation, evaluation and further development of Regulation Analysis, a method that enables the quantitative description of the regulation of enzyme rates and their catalytic capacities. This method was implemented, tested and elaborated in a series of investigations upon the regulation of *Saccharomyces cerevisiae*’s glycolysis to nutrient starvation, oxygen deprivation, increased free-energy dissipation by addition of benzoic acid, or deletion of the gene *HXK2* encoding hexokinase II. The experimental findings and analyses reported in this thesis yielded new insights into the complexity of the regulation of metabolic fluxes and the catalytic capacities of the enzymes catalyzing their reactions.

In the past, several efforts have been made to devise a quantitative framework for the study of metabolic regulation. Of these, Regulation Analysis stands out as a method suitable for the experimental study of regulatory processes. Regulation Analysis quantitatively dissects the contributions of changes in enzyme capacities ($V_{\text{max}}$—called hierarchical regulation) and changes in the way enzymes interact with the rest of metabolism (called metabolic regulation) to the local regulation of enzyme rates. This dissection is based on a property of most enzyme-catalyzed reactions: the rate of catalysis is directly proportional to the amount of active enzyme. Regulation Analysis introduces the possibility of making unambiguous and quantitative descriptions of the regulation of fluxes through individual enzymes embedded in biochemical networks of any complexity, in response to any number or kind of simultaneous perturbations.

Regulation Analysis is used throughout this thesis to describe the regulation of fluxes through individual glycolytic and fermentative enzymes in the yeast *Saccharomyces cerevisiae* when it adapts to a variety of environmental and genetic changes. Chapter 2 gives a detailed description of the method and introduces precise biochemical interpretations for all possible numerical outcomes of the analysis. Further, it refines the original interpretation of both hierarchical and metabolic regulation so
as to accommodate the common feature of differential expression of isoenzymes with different kinetic properties.

In Chapter 3, Regulation Analysis is used to formulate testable predictions of three hypotheses on the global regulation of pathway fluxes in terms of the local regulation of the enzyme rates in that pathway. These hypotheses were: exclusive metabolic regulation, single-site modulation and multi-site modulation. The first hypothesis predicts that all enzyme rates are regulated metabolically (i.e., the absence of hierarchical regulation); the second hypothesis, predicts that a single “key” metabolic step is regulated hierarchically while all other steps are regulated metabolically. The third hypothesis, predicts exclusive hierarchical regulation of all the enzymes rates in the pathway so as to ensure the homeostasis of metabolite concentrations. Chapters 3, 4 and 5 describe the regulation of fluxes through individual glycolytic and fermentative enzymes when cultures of *Saccharomyces cerevisiae* adapted to: nitrogen or carbon starvation, oxygen deprivation, increased free-energy dissipation by addition of benzoic acid and the deletion of the gene *HXK2* encoding hexokinase II. These studies taken together allow the following conclusions: (i) metabolic regulation is often an important contributor to the local regulation of enzyme rates, (ii) living yeast cells use all possible combinations of hierarchical and metabolic regulation to modulate the rates of individual enzymes, (iii) fluxes through enzymes in a common pathway are regulated in different ways, suggesting that they play different regulatory roles in the regulation of the pathway’s flux, (iv) the same metabolic step is often regulated differently when cells adapt to different perturbations, (v) the suggested hypotheses on the global regulation of metabolic pathway fluxes were falsified for the conditions tested, implying that they are not general, and (vi) the regulation of glycolytic and fermentative fluxes is often regulated by changes within as well as without the pathway’s enzymes. These findings suggest that pathway fluxes are regulated in a subtle way with different enzymes playing different regulatory roles and show that the regulation of pathway fluxes need not to be governed by single drives or constraints. They also urge the formulation of new hypotheses on the global regulation of pathway fluxes.

An extension of the scope of Regulation Analysis to quantify the regulation of enzyme amounts and catalytic capacities in terms of the contributions of changes in mRNA concentration, translation and protein degradation rates, and posttranslational modifications is developed in Chapter 4. The analysis is based on the assumptions that protein concentrations are at steady-state and that the rates of translation and degradation of individual protein species are directly proportional to the corresponding concentrations of mRNA and protein. While the former assumption is likely to be warranted by the use of chemostat cultures, the latter two still require experimental verification, which will require increased precision of the available analytical techniques. The assumptions of direct proportionality of translation and protein degradation rates with respect to the concentrations of the corresponding mRNA and protein are based on the expectation that the machineries involved (ribosomes and proteasome) are unspecific and that the concentration of any single mRNA or protein species represents a minority in the population of all other mRNA or protein species.

In Chapter 4 this extended Regulation Analysis is applied to study the regulation of glycolytic enzyme amounts and capacities when *S. cerevisiae* adapts to anaerobiosis or to the presence of benzoic acid. Experiments showed that mRNA concentration
changes correlate poorly with the changes of enzyme amounts and capacities. The analysis suggests that mRNA changes account for less than 50% of the regulation of the glycolytic enzyme amounts and capacities and that changes in the rates of translation and/or protein degradation are the main regulators of protein amounts. Attempts to quantify the contributions of posttranslational modifications to the regulation of enzyme capacities highlighted the need for more accurate and reproducible proteomics. Although the standard errors of the mean were too large to be decisive, the occurrence of posttranslational modifications affecting the catalytic capacities of triosephosphate isomerase and phosphoglycerate kinase is suggested.

The methodologies used and developed in this thesis provide a quantitative framework with which experimental testing of hypotheses on the drives and constraints governing regulatory processes is made possible. Application of these methodologies to describe real regulatory processes in living cells has provided insights into a previously undescribed complexity of metabolic regulatory processes. It has shown that processes that have received relatively little attention such as metabolic regulation of fluxes, translation and protein degradation rates, are likely to play a major role in the regulation of metabolic systems.