Emergent properties of metabolic systems and the effect of constraints on enzyme concentrations

Sicard Delphine, Dillmann Christine, Fiévet Julie, Talbot Grégoire, Grima Laure & de Vienne Dominique

UMR de génétique végétale, INRA/CNRS/INAPG/UPS, Ferme du Moulon, Gif sur Yvette, 91190, France, Phone : +33-01 69 33 22 42, Fax : +33-01 69 33 23 40, Email : sicard@moulon.inra.fr

Cell functioning and evolution rely on complex metabolic systems constituted of many components that communicate and interact with one another through networks. Several metabolic theories have been developed to predict the emergent properties of metabolic systems. However, the effects of constraints on the properties of such systems and on their evolution under selection have been poorly studied, whereas cell necessarily functions with limited resources. Using both theoretical and experimental approaches, we have studied the effect of constraints on enzymes concentrations and their consequences on metabolic fluxes and fitness.

The theoretical developments were based on the metabolic control analysis, which provides a framework linking enzymatic parameters, such as enzyme activities, to a macroscopic output of the system, the metabolic flux. We analysed the effect of competition for space and energy by introducing an overall cost for producing enzymes or by limiting the range of variation of the enzymes concentration in a pathway. In addition, we studied the effect of co-regulation by introducing correlation between enzyme concentrations. Under those conditions, our modelling revealed new emergent properties of metabolic fluxes. First, the total enzyme concentration allocated to a pathway, which is positively correlated with flux, can respond to selection. Second, competition leads to a distribution of enzyme concentrations within the pathway that maximizes metabolic flux: selection can act to increase low enzyme concentrations and to decrease high enzyme concentrations until an optimal level. Third, co-regulation leads to metabolic flux consistently lower than the one obtained with competition alone, suggesting that co-regulation may be costly. Finally, a biochemical model for hybrid vigour can be derived.

In vitro reconstruction of the first part of glycolysis and *in vivo* analysis of various *Saccharomyces cerevisiae* strains were carried out to test these predictions. *In vitro* experiments allowed us to estimate global enzymatic parameters and confirmed that a distribution of enzyme concentrations that optimizes flux can be predicted. “Test tube genetics” performed by varying *in vitro* enzyme concentrations allowed us to corroborate the metabolic mechanism for hybrid vigour. Finally, proteomics and biochemical analysis of a collection of *S. cerevisiae* strains showed that there is genetic variability at two levels of cell integration, enzyme concentrations and glycolytic fluxes, and that selection can act to increase or decrease flux.

In conclusion, taking into account constraints on enzyme concentrations allowed us to develop new modelling and *in vitro* tools for metabolic optimization, and gave new insight in the understanding of metabolic system evolution.