Branches in the plant world

It is more than 40 years ago that studies on the metabolism of microorganisms revealed the existence of isoenzymes in the allosteric regulation of branched pathways. Their role in the synthesis of amino acids derived from aspartate was especially well investigated in *Escherichia coli*, particularly from the point of view of their allosteric properties (Patte *et al.* 1964). The enzyme aspartokinase catalyses the phosphorylation of the amino acid aspartate, that being the first step in the biosynthesis of three different aminoacids, threonine, methionine and lysine, as well as of a fourth, isoleceucine (from threonine). Animals lack these pathways, which is what makes these amino acids 'essential' for us – we have to get them from our diet. At that time the global regulation of entire metabolic networks had not been clarified in a quantitative sense. Everyone seemed to take it for granted that there must be a logical reason behind the existence in *E.coli* for instance of three aspartokinases, each of them being regulated, meaning inhibited and/or repressed (at the level of gene activity), by its 'corresponding' amino acid.

This was subsequently also found in plants but with a more complex pattern of regulation. It was not clear whether the more complex regulatory pattern reflected the necessity of a multiplicity of metabolic responses or simply a 'stratification' of different regulations during evolution. In other words, could a simpler system of regulation offer the same wealth of metabolic responses? The pattern in the thale cress *Arabidopsis thaliana* has been painstakingly deciphered over a ten-year period by Curien and co-workers; all the steps, including their regulatory properties, are known in detail. As shown in their most recent publication (Curien *et al*. 2009), today such questions can be answered by building a mathematical model to simulate the global functioning of the network.

In order to obtain a steady state in the model as it occurs *in vivo*, it is necessary to add to the network the cellular demands of lysine, threonine and isoleucine in protein synthesis. Methionine is not a variable of the system because it is the precursor of S-adenosylmethionine (AdoMet) which in addition plays a regulatory role in the network (see below). The AdoMet concentration is set at its physiological value. The amino acid demand is adjusted in order to obtain a concentration of intermediate metabolites close to the ones measured *in vivo*. This steady state is called the reference state and is the starting point of all the other simulations. The authors use metabolic control theory (MCT, also known as Metabolic Control Analysis) (Kacser and Burns 1973; Heinrich and Rapoport 1974; Reder 1988) to identify the more sensitive steps and metabolites.

In metabolic networks, controls at the 'supply' and 'demand' ends usually act in opposition (Hofmeyr and Cornish-Bowden 2000); most trivially, an increase in substrate tends to increase the flux through a pathway whereas an increase in the product will tend to decrease the flux. In the present case, most of the control is on the 'demand' steps in the amino acids' biosynthetic pathways: that allows the network to respond directly to a change in protein synthesis. There is, however, a high control coefficient associated with the aspartate kinase isoform AK1, which is at the 'supply' end. The consequence is a significant contribution of AK1 to the maintenance of a threonine steady-state. This, according to the authors, is one of the reasons why the threonine level is less stable than those of the other amino acids – both in the model and *in planta.*

The first important result obtained from the model is the calculation of the flux through the different branches of the network and their detailed assignment to the different isoforms. It appears from figure 3 of the paper (which very clearly shows the different fluxes) that with regard to the first (aspartate kinase) step catalysed by the four aspartokinases isoforms in *A. thaliana*, the flux is mainly accounted for by one of them, namely AK1 (73%). The second important result is that to a large extent the different pathways behave independently. This means that when, for instance, the demand for threonine is increased, lysine production is not affected even though the two biosynthetic pathways share common steps in the beginning. This non-intuitive result is due to the special features of the regulation pattern, particularly due to the presence of the

Keywords. Allosteric regulations; amino acids derived from aspartate; isoenzymes; metabolic control theory; regulation of branched pathways

different isoforms with different effectors, mainly the respective terminal amino acids (as described in figure 3 of their paper).The explanation of the special role of AdoMet is the third important contribution of the model. This metabolite activates the terminal step in threonine synthesis, the threonine synthase (TS) step. Despite the fact that TS is activated by AdoMet, an inverse relationship between AdoMet and threonine concentrations was observed experimentally. This result is well predicted in the model, thereby highlighting the fact that even in a regulatory network that is not all that complex, intuitive predictions can be wrong.

The last point draws attention to the absolute necessity to develop a metabolic model to understand and predict the responses of a network to different (patho)physiological changes. Intuitive reasoning is not sufficient, and much of the time even leads to wrong predictions. It also emphasizes the fact that modelling relies on a huge amount of enzymological data; building and analysing a model network is the culmination of several years' work of collecting kinetic data in the traditional methodical way. However the work of Curien *et al*. (2009) should not be seen as an end. On the contrary, this model is likely to provide a basis for the interpretation of the changes that can be observed during the life of the plant when the amounts of the enzymes (concentrations) are changed. It will also provide a framework to extend similar simulations to other organisms including *E. coli* (Chassagnole *et al*. 2001).

We are probably at the beginning of a type of modelling in which the parameters come from a broad set of carefully amassed experimental data on the same organism (cell) and not from the fitting of some experimental results with an *a priori* model. In other words the model is, so to say, built from experimental data and the resulting simulations are confronted with the global functioning of the network under different physiological conditions. Discrepancies allow us to unveil particular unsuspected features of the network, for example previously unknown regulations. These can then be incorporated in a new model. This sort of dialogue between experiments and modelling is a powerful driving force for new discoveries. Contrast the underlying attitude with one that looks for a simple adjustment of the parameters of the model to 'fit' experimental results. The adjusted parameters could well have nothing to do with the reality. The V_{max}'s of all the enzymes in the network are examples of such parameters. Their experimental determination is crucial - and indeed much more informative – than their adjustment to a model . We can predict that the size of the metabolic networks studied in this way will progressively increase as the kinetic parameters (particularly the V_{max}'s) come to be more correctly assessed.

References

Chassagnole C, Fell D A, Raïs B and Mazat J-P 2001 Control of the threonine synthesis pathway in Escherischia coli: a theoretical and experimental approach; *Biochem. J.* **356** 433–444

Curien G, Bastien O, Robert-Genthon M, Cornish-Bowden A, Cárdenas M L and Dumas R 2009 Understanding the regulation of aspartate metabolism using a model based on measured kinetic parameters; *Mol. Sys. Biol*. **5** 271

Heinrich R and Rapoport T A 1974 A linear steady-state treatment of enzymatic chains. General properties, control and effector strength; *Eur. J. Biochem*. **42** 89–95

Hofmeyr, J-H S and Cornish-Bowden A 2000 Regulating the cellular economy of supply and demand; *FEBS Lett*. **476** 47–51

Kacser H and Burns J A 1973 The control of flux; *Symp. Soc. Exp. Biol*. **32** 65–104

Patte J C, Lovigny T and Cohen G N 1965 Interactions coopératives effecteur-effecteur chez deux enzymes allostériques inhibés de manière non-compétitive; *C. R. Acad. Sci. Paris* **259** 1255–1258

Reder C 1988 Metabolic control theory: a structural approach; *J. Theor. Biol*. **135** 175–201

Jean-Pierre Mazat* and Christine Nazaret**
Université Bordeaux 2,
146, rue Léo-Saignat,
33076 Bordeaux-cedex, France
**(Email, jpm@u-bordeaux2.fr) **(Email, nazaret@sm.u-bordeaux2.fr)*

*e*Publication: 17 August 2009