Integrative Flux Analysis of Eukaryotic Metabolism: Quantifying Carbon Traffic in Cells by Isotope Labeling and Mathematical Modeling

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Metabolic fluxes are the rates of carbon trafficking through various parts of an intracellular metabolic network. Quantifying fluxes in a systemwide manner is central to elucidating cell function and is valuable in the growing discipline of systems biology. Flux measurement in eukaryotic systems (e.g. plants and mammalian cells) is challenging due to their complex, incompletely known biochemistries and the occurrence of identical reaction networks in multiple subcellular compartments.

Stable isotope labeling is a promising methodology for flux quantification, wherein the organism of interest is fed a computationally designed mixture of different stable isotopes (e.g. $^{13}$C and $^{12}$C carbon sources), and fluxes are evaluated by decrypting the ensuing labeling data through computational techniques such as metabolic network modeling and isotopomer balancing. We developed an isotope labeling-based, computer-aided flux analysis tool that enables the concurrent evaluation of fluxes in several primary and intermediate metabolic pathways. Using this tool, we investigated carbon trafficking in two plant systems (soybean embryos and Catharanthus roseus hairy roots). Our flux measurements revealed several interesting insights into carbon allocation in these systems. Importantly, we were able to quantify fluxes of parallel pathways in separate subcellular compartments, which is essential in plant metabolism but had been challenging to implement previously.

Furthermore, we applied this flux analysis tool on a mammalian cell line to quantify the global metabolic effects of overexpressing glycerol kinase, an enzyme that plays a key role in lipid metabolism. Our results show that glycerol kinase causes substantial, unforeseen alterations of fluxes in several pathways, especially in the pentose phosphate pathway. These results highlight the multifunctional role of glycerol kinase, while contributing to unraveling the complexities of the associated genetic disease, glycerol kinase deficiency.