Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Biotechnology

Flux analysis in plant metabolic networks: increasing throughput and coverage

Björn H Junker

Quantitative information about metabolic networks has been mainly obtained at the level of metabolite contents, transcript abundance, and enzyme activities. However, the active process of metabolism is represented by the flow of matter through the pathways. These metabolic fluxes can be predicted by Flux Balance Analysis or determined experimentally by ^{13}C-Metabolic Flux Analysis. These relatively complicated and time-consuming methods have recently seen significant improvements at the level of coverage and throughput. Metabolic models have developed from single cell models into whole-organism dynamic models. Advances in lab automation and data handling have significantly increased the throughput of flux measurements. This review summarizes advances to increase coverage and throughput of metabolic flux analysis in plants.

Addresses
Institute of Pharmacy, Martin-Luther-University, Hoher Weg 8, 06120 Halle, Germany

Corresponding author: Junker, Björn H (bjoern.junker@pharmazie.uni-halle.de)

Current Opinion in Biotechnology 2014, **26**:183–188

This review comes from a themed issue on **Plant biotechnology**

Edited by **Birger Lindberg Møller** and **R George Ratcliffe**

For a complete overview see the Issue and the Editorial

Available online 21st February 2014

0958-1669/$ – see front matter, Published by Elsevier Ltd.

http://dx.doi.org/10.1016/j.copbio.2014.01.016

Introduction

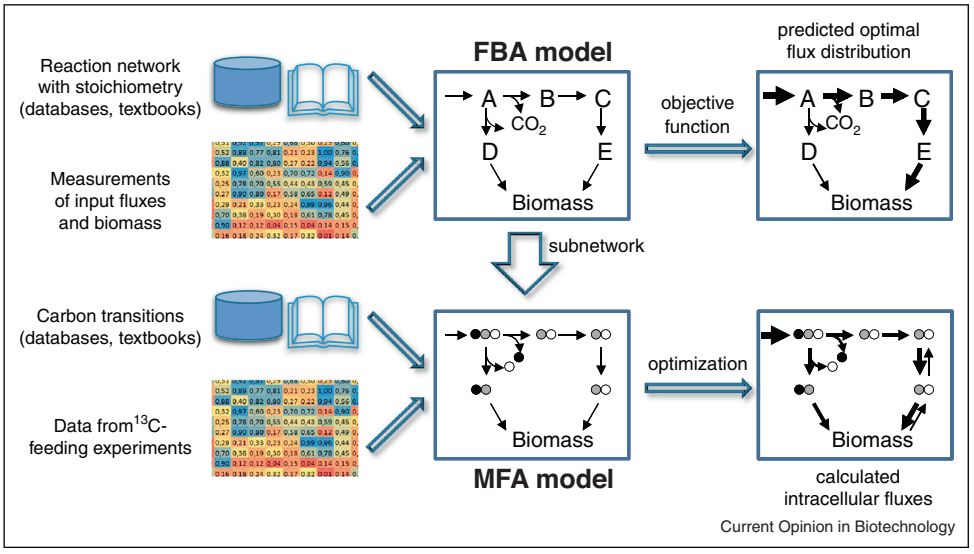
The manner in which biological research is conducted has undergone tremendous development throughout the last few decades. Bottom-up approaches, in which the interactions of single components are investigated in detail, have been in part replaced by top-down '-omics' approaches, which aim at a large-scale overview at a single level of cellular organization, for example, transcript, protein, metabolite contents or protein–protein interactions. Large-scale quantification of intracellular metabolic fluxes is on the wish list of many researchers in plant functional genomics [1–3]. The two main methods available for this purpose are Flux Balance Analysis (FBA) and Metabolic Flux Analysis (MFA) (Figure 1) [4–8]. Both perform stoichiometric simulations of the metabolic network under study, both need quantitative measurements of some input or output fluxes (e.g. carbon influx and biomass production, respectively), and in both methods optimization algorithms are used to numerically determine an optimal steady-state flux distribution in the metabolic network. While FBA relies on these optimizations to minimize or maximize an objective function (e.g. growth, carbon efficiency), MFA additionally takes into account carbon transition maps and data from stable isotope labeling experiments to minimize the difference between simulated and measured isotope distribution patterns. Thus, FBA is an underdetermined system, that is a prediction of one out of many possible flux distributions, while MFA is a 'model-assisted measurement' (or a 'data-enriched model', depending on the view) yielding a flux vector that can be assigned a certain statistical significance.

The general focus of this review is on developments that aim to increase the throughput and/or coverage of FBA (automated annotation, genome-scale models, multi-cell models, multi-organ models) or of MFA (automated sample processing and data evaluation, variations in the concept). As the number of papers in the field is quite limited, and the fields are relatively new, there is no restriction in the time period covered.

Increasing the coverage of Flux Balance Analysis

While the first genome-scale FBA models were developed for microorganisms in the early 2000s [9], in plants the first large-scale FBA model was published in 2009, on barley seed metabolism [10], soon followed by the first genome-scale model on heterotrophic Arabidopsis cell suspension cultures [11]. While these two models described the metabolic model of a single cell type, a coupled model describing each of the two central cell types of C4-photosynthesis in corn leaves was published soon afterwards [12••]. In coupled models, there is one distinct model for each cell type, and these models are coupled by the exchange of, typically, a small number of metabolites. The next logical step towards a full-plant model was to establish and connect organ-specific metabolic models, a task that was addressed by instituting a pipeline for the reconstruction of a collection of seven compartmentalized, tissue-specific models [13•]. However, these models were not connected in order to simulate a whole-plant metabolism. Recently, a dynamic FBA model of a barley plant was constructed that is capable of predicting the steady-state flux distribution of the metabolism of different organs throughout the entire plant development [14••]. This high temporal and spatial resolution was achieved by using the data

Figure 1



General workflow of Flux Balance Analysis (FBA) and steady-state ^{13}C Metabolic Flux Analysis (MFA). Usually MFA models are smaller than FBA models, so a subnetwork can be adopted. Note that some fluxes in the MFA results can be bidirectional.

derived from a dynamic functional–structural model, which was based mainly on biophysical phenomena and empirical derivation of model parameters, to parameterize connected FBA models. With models at this metabolic, structural, and temporal level of detail, it is now possible to connect macroscopic phenomena such as seed development or leaf senescence to intracellular metabolic fluxes. In this way, metabolic processes underlying structural and developmental observations can be much better understood, and, for example, by systematic prediction of the metabolic effect of gene deletions, biotechnological targets can be identified to obtain beneficial metabolic changes in specific plant organs or developmental stages.

Increasing the throughput of Metabolic Flux Analysis

The determination of intracellular metabolic reaction rates (i.e. fluxes) is a time-consuming task that usually combines isotope labeling experiments with biochemical analytics and mathematical models of metabolic pathways [15], and thus has so far been conducted with a relatively small number of genotypes or conditions. In plants, the current state-of-the-art is that typically no more than three genotypes or conditions are analyzed with ^{13}C-MFA per study for example, [16,17], with the exception of one study in which 19 maize genotypes were analyzed with MFA, however it was limited to a low resolution [18].

As in many cases, the microbial research community is more advanced in this issue. By 2005, a study was published in which 137 *Bacillus subtilis* null mutants were analyzed with stable isotope labeling studies [19]. The labeling patterns were compared, but no full set of metabolic fluxes or flux maps were generated for each line. More recently, full steady state flux vectors of 91 *E. coli* strains with mutations in transcriptional regulators were determined [20••].

Large-scale metabolic flux analysis in plant systems would open up a number of scientific, biotechnological and agronomical applications. First of all, MFA significantly improves the level of understanding of metabolic behavior, and, with increased throughput, MFA could be used more routinely to understand metabolism of a larger number of genotypes. Furthermore, genetic populations for association mapping, mutant collections, or large-scale treatments with chemical agents could be screened for desirable changes of intracellular metabolic fluxes. However, this scale would require MFA protocols with decreased complexity and increased level of automation in the following fields: firstly, cell or tissue culture for the labeling experiment; secondly, biochemical fractionation and measurements; and thirdly, computational methods for data extraction, data correction, flux determinations, statistical examination, and flux visualization.

The first field is undoubtedly the most problematic and currently the limiting step of flux analysis. MFA requires the feeding of defined concentrations of ^{13}C-labeled carbon chains, which can only be achieved in a cell or organ culture system. This requires isolation and cultivation of the organs under sterile conditions, which is time-consuming work and cannot easily be automated. The only way to face this challenge is to organize the