NOVEL STABLE ISOTOPE METHODS TO IDENTIFY FLUX BOTTLENECKS IN PHOTOSYNTHETIC HOSTS

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Engineering host cell metabolism to promote high yield and specific productivity is a major goal of the biotech industry. 13C metabolic flux analysis (MFA) provides a rigorous approach to quantify host metabolic phenotypes by applying isotope tracers to map the flow of carbon through intracellular biochemical pathways. In particular, transient measurements of isotope incorporation following a step change from unlabeled to labeled CO_2 can be used to estimate photosynthetic carbon fluxes by applying isotopically nonstationary MFA (INST-MFA) [1]. We have previously developed a package of MATLAB routines called INCA [2] that automates the computational workflow of INST-MFA. INCA is the first publicly available software package that can perform INST-MFA on metabolic networks of arbitrary size and complexity. We have recently applied INCA to model the photoautotrophic metabolism of cyanobacteria that have been engineered to produce isobutyraldehyde (IBA) [3]. The flux analysis identified an alternative three-step route from PEP to pyruvate, which supplied the majority of carbon for IBA synthesis. Based on these results, we overexpressed each single enzyme involved in this pathway and identified strains with significant improvements in IBA production.

We next adapted our INST-MFA modeling approach to a terrestrial plant system[4]. We performed in vivo isotopic labeling of Arabidopsis thaliana leaves with $13CO_2$, measured the transient labeling of 37 metabolite fragment ions using mass spectrometry, and estimated fluxes throughout leaf photosynthetic metabolism using INCA. Leaves were acclimated to either 200 (LL) or 500 (HL) µmol/m2/s light intensity. Approximately 1,400 independent mass isotopomer measurements were regressed to estimate 136 fluxes under each condition. Despite a doubling in the carboxylation rate, the photorespiratory flux increased from 17% to 28% of net CO_2 assimilation in HL acclimated plants. Photorespiration is considered a wasteful metabolic process that results in losses of energy and fixed carbon, and the ability to precisely quantify photorespiratory flux with INST-MFA is now being leveraged to guide metabolic engineering efforts to improve photosynthetic efficiency in plants. These studies have established 13C INST-MFA and the INCA software package as a comprehensive platform to map carbon fluxes in cyanobacteria, plants, and other photosynthetic host organisms.

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