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Metabolic Flux Analysis in Eukaryotic Cells

Methods and Protocols

Edited by

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Preface

Recent advancements in metabolic flux analysis (MFA) are instrumental in revealing mechanistic underpinnings of cellular metabolism. Especially in the identification of metabolically vulnerable targets and the dissection of crosstalk between diseased cells and their microenvironment, MFA has been the backbone of new advancements in targeting several diseases. The chapters in this special issue feature state-of-the-art metabolic flux techniques that span analyses of cellular, organ-level, and whole-body metabolism.

Stable isotopes are commonly used as labels for analysis of metabolism of living cells. The uptake and utilization of isotopically enriched nutrients leads to the formation of enriched intracellular metabolites and lipids. The enrichment data obtained from mass spectrometry (MS) techniques is incorporated in metabolic models for metabolic flux estimation. Mairinger and Hann present the accurate and precise analysis of isotopologue and tandem mass isotopologue ratios in heavy stable isotope labeling experiments in the presence of measurement uncertainty. Dudek et al. describe a data processing workflow in non-targeted stable isotope labeling experiments to generate metabolite levels, mass isotopomer distribution, and similarity and variability analysis of metabolites. Damini et al. provide a detailed overview of methods for polar metabolite analysis in reverse phase ion pairing and hydrophilic interaction chromatography for ^{13}C MFA. To interrogate intracellular compartments, ^{2}H (deuterium) tracing approaches have gained popularity. Here, Lim et al. provide a detailed description of ^{2}H tracing applications for the interrogation of mitochondrial versus cytosolic NAD(P)H metabolism in mammalian cells. To broaden the scope of MFA from cells to whole body, ^{13}C-based in vivo flux analysis can be used. However, the complexity of handling mice in disease models had discouraged early in vivo tracing analysis. Recent advances in MS techniques and increased instrument sensitivity have however encouraged development of these methodologies. Altea-Manzano et al. fill an important gap by presenting a methodology for understanding the metabolism of metastases in vivo.

Biomass evaluation is an important component in MFA, and in the past generic data has been used to model this important flux. Széliová et al. present a detailed method for the determination of biomass flux from Chinese hamster ovary cells. Nitric oxide involvement in cancer and several other diseases has recently been uncovered. Still, there is a lack of methodologies for the measurement of NO flux. Sivaloganathan presents a detailed protocol, which includes experimental measurements and computational modeling, to estimate the NO flux distributions. To increase the accuracy and quantification of low-abundance metabolites, an accurate modeling workflow is needed. Jaiswal and Wangikar present a methodology called sequential windowed acquisition of all theoretical fragment ion mass spectra, which allows quantification of isotopic ^{13}C enrichment in a large number of cellular metabolites and fragments. To increase the scope of flux analysis to heterogeneous cellular systems, a methodology which can capture metabolic communication between different cell types is required. Achreja et al. present an integrated empirical and computational platform to quantify metabolic crosstalk between source and recipient cells. Their platform allows the estimation of contribution of source cell-derived extracellular vesicles to recipient cells. Garrity et al. describe a method to combine mRNA and

metabolomics data in a genome-scale metabolic model to curate a biologically feasible model for constrained MFA. Selivanov et al. present a software supporting a workflow of analysis of stable isotope-resolved metabolomics data obtained with MS and their use in a kinetic model based on ordinary differential equations for isotopomers of metabolites of the corresponding biochemical network to estimate a dynamic flux map. Campit and Chandrasekaran present a genome-scale modeling approach that uses time-course metabolomics to predict dynamic flux rewiring during transitions between metabolic states.

Metabolic flux approaches have also been garnering attention as a cost-effective platform to develop and test drugs for their efficacy toward specific metabolic targets. Rawls et al. present the application of metabolic flux approaches for drug development. Unraveling heterogeneity is difficult with respect to metabolic changes in living cells. Filippo et al. present a new computational framework called single-cell Flux Balance Analysis that aims to set up digital metabolic twins that also use laboratory patient cell models to unravel changes in heterogeneous populations. Toit et al. present an interesting application of MFA in autophagy, which is a cellular homeostasis process that maintains cellular nutrients. Peres and Fromion present a protocol for the integration of thermodynamic constraints in metabolic models to eliminate non-physical fluxes or inconsistencies in the metabolic system.

The chapters in this book will be of great interest to both experts in MFA techniques and researchers getting initiated in the role of quantitative studies to unravel the secrets of dysregulated pathways in human diseases. I would like to thank all the contributing authors for their valuable support in presenting their work and advancements toward making MFA an incisive and decisive technique to dissect metabolic states in diseases. Furthermore, this issue would not have been possible without support from my lab members, precious colleagues, and budding scientists, Dr. Abhinav Achreja, Anjali Mittal, Olamide Animasahun, and Noah Meurs. They dedicatedly helped me with editorial corrections and provided their input many times on short notice, and they are themselves looking forward to advancing the field of MFA. I would also like to thank Dr. John Walker and Anna Rakovsky (Springer Nature) for their editorial inputs and assistance.

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