METHODS IN MOLECULAR BIOLOGY

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Metabolic Signaling

Methods and Protocols

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Preface

Metabolism is the biochemical reaction network that allows cells to convert nutrients into small molecules, called metabolites. Through these metabolite conversions, essential components needed for cell survival and proliferation are generated. For example, metabolite conversions allow the production of ATP, the energy currency of cells, as well as the production of amino acids, fatty acids, and nucleotides—the building blocks for proteins, membranes, and DNA/RNA. Moreover, dynamics in metabolite concentrations facilitate the crosstalk between cell signaling and the biochemical reaction network of metabolism. This bidirectional crosstalk is necessary to enable the response of cells to stimuli such as nutrient availability/limitations and growth factors. The central role of metabolism in integrating directly or indirectly (via cell signaling) various external and internal stimuli defines metabolic signaling.

In this book, we provide protocols to quantify metabolism (Chapters 1–10), to identify metabolic crosstalk (Chapters 11–14), and to set up and develop tools and models to gain a systems-level insight into metabolic signaling (Chapters 15–20). Quantifying metabolism is essential to describe and understand metabolic signaling. Metabolite concentrations, metabolic pathway activities, and metabolic fluxes constitute different functional readouts of metabolism. Different methods are required to determine these readouts of metabolism. Depending on the cellular system and the resolution level, variants of these methods should be applied. The most global readout of metabolism are metabolite concentrations. Changes in metabolite concentrations pinpoint the metabolite nodes within the biochemical reaction network that respond to a certain stimuli or perturbations. In Chapter 1, Wang and colleagues describe a protocol to determine the most suitable metabolomics method for broad metabolite coverage, and in Chapter 2, Langerborg and colleagues provide a method to specifically assess bioactive lipids. The activity of metabolic pathways and how they are fueled by nutrients is an important aspect of understanding the metabolic requirements of cells. In Chapters 3 and 4, Ogrodzinski et al. and van Gorsel et al. provide workflows for applying nutrients labeled with a stable isotope of carbon to determine metabolic pathway activity and nutrient contributions in vitro in adherent 2D and 3D spheroidal cell cultures, respectively. In Chapters 5 and 6, Broekaert and Fendt and Pinnick et al. provide protocols to extend the use of metabolites labeled with stable isotopes to analyze in vivo glucose and lipid metabolism in mice and humans, respectively. Metabolic fluxes are the most quantitative readout of metabolism, and several different and complementary approaches exist to measure absolute fluxes in cultured cells. Bird et al. and Newman and Maddocks describe in Chapters 7 and 8 methods to estimate ATP and amino acid synthesis rates, respectively, by estimating the steady state flux with flux inhibition. Veys and colleagues apply in Chapter 9 radioactive tracers to determine fluxes in central carbon metabolism of endothelial cells, while Nonnenmacher and colleagues estimate compartment-resolved metabolic fluxes in Chapter 10.

Metabolic signaling describes the bidirectional crosstalk between external stimuli and cell signaling pathways with metabolism. Chapters 11 and 12 from Guillaume et al. and Püschel and Munoz-Pinedo identify crosstalk between nutrient metabolism with autophagy and cell death pathways, respectively. Often, cellular functionality is directly linked to

metabolism. In Chapters 13 and 14, Liu and Ho and Fernandez Garcia and Fendt provide protocols to assess the regulation exerted by nutrient availability on macrophage polarization and T-lymphocyte metabolism, respectively.

To further understand and exploit metabolism in systems medicine, different model systems and cellular contexts can be considered. An important model system used in cancer research are patient-derived xenografts. Annibali and colleagues describe in Chapter 15 the setup of patient-derived xenografts, which can be used to determine in vivo tumor metabolism as described in Chapter 5. Moreover, it has been established that tumor metabolism changes during cancer progression. Therefore, it is important to closely follow cancer progression, as described in Chapter 16 by Stanchi and colleagues using microscopy. Further, external signals and stimuli are in crosstalk with metabolism. An important contributor hereby is visceral adipose tissue (VAT), an active endocrine organ producing hormones. To study the impact of VAT-produced hormones on metabolism, VAT can be surgically removed. A protocol to do so is described in Chapter 17 by Chakraborty and Bernard. Similarly, the secretome of other organs such as the bone is a function of health and disease. Potential interaction of this secretome with metabolism therefore requires quantitative methods to define bone status and its secretome. A method hereof is described by Lie and colleagues in Chapter 18. Moving from a whole-body physiology level to the cellular level, the relevance of organelle organization emerges, and it is tempting to speculate that there might be a crosstalk between organelle organization and metabolic fluxes within organelles (*see* Chapter 10). A protocol provided by Latge and Schauer in Chapter 19 allows determination of intracellular organelle organization. Finally, large-scale data often obtained when studying metabolism can be effectively displayed using heat maps. Fundamentals of constructing and interpreting heat maps are provided by Vacanti in Chapter 20.

With this book, we aim to provide researchers with methods to study, perturb and functionally interpret metabolic signaling from the subcellular to the whole-body level. Given the emerging importance of metabolism in sustaining health and metabolic deregulation in disease, we believe that applying methods described in this book will foster mechanistic understanding of metabolism and, in the long-term, prospectively support the development of innovative disease treatment strategies.

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