Methods in ENZYMOLOGY

Volume 457

Mitochondrial Function, Part B: Mitochondrial Protein Kinases, Protein Phosphatases and Mitochondrial Diseases

Edited by

William S. Allison

Anne N. Murphy

VOLUME FOUR HUNDRED AND FIFTY-SEVEN

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Mitochondrial Function, Part B: Mitochondrial Protein Kinases, Protein Phosphatases and Mitochondrial Diseases

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METHODS IN ENZYMOLOGY

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PREFACE

The field of mitochondrial research broadened significantly in the 1990s with the discovery of the critical role that mitochondria play in cell death signaling and apoptosis. Participation in the field has continued to expand at a rapid pace as interest in mitochondrial research expands from other disciplines. A diverse array of topics has come to the forefront of mitochondrial research, including signaling via reversible phosphorylation, organellar trafficking, involvement in secretory events, and the pathogenesis of prevalent diseases including chronic forms of neurodegeneration and diabetes, each of which is addressed in this volume of *Methods in Enzymology*. Here we highlight some of the advances in methodology that have allowed further identification of components and posttranslational modifications of the mitochondrial proteome, characterization of the dynamic changes in structure and movement of the organelles, as well as assessment of the functional changes of mitochondria both in health and disease.

Specifically, advancements in bioinformatics and mass spectrometry that have provided more accurate prediction and identification of components of the mitochondrial proteome are described in Chapters 1–3. It is now recognized that the functions of certain proteins located in the inner membrane, the outer membrane, and the mitochondrial matrix are controlled by posttranslational modifications. Methods to detect protein phosphorylations are the focus of Chapters 4–7 and the detection of protein acylations are the focus of Chapters 8 and 9.

Experimental approaches arising from advances in proteomics include the characterization of functional changes of key mitochondrial enzymes induced by signaling proteins and posttranslational modifications. These approaches are addressed by techniques described in the second section of this volume. Methods for the study of mitochondrial kinases and phosphatases and their effects on mitochondrial function, morphology, and the control of cell death are the focus of Chapters 10–14. The important topic of determining submitochondrial localization of specific enzymes is addressed in Chapter 15, which is critical to identify relevant substrates for these signaling enzymes.

Other significant advances in mitochondrial research have risen from techniques that allow visualization of individual mitochondria within intact cells, revealing the highly dynamic nature of mitochondrial morphology, localization, and turnover. Imaging methods, including the use of photoactivateable GFP as well as other approaches, are described to monitor

mitochondrial fusion and fission (Chapters 13 and 16), mitochondrial autophagy in yeast (Chapter 17), and mitochondrial movement in neuronal processes (Chapter 18). A method to establish a hybrid cell line containing exchanged mitochondrial-DNA is described in Chapter 19.

The third section of this volume describes methods developed to examine ATP production in human tissues and the participation of mitochondria in pancreatic insulin secretion and insulin responsiveness of various tissues. Chapter 20 describes a bioluminescence approach to measuring rates of ATP production in biopsies of human muscle and Chapter 21 describes the use of non-invasive magnetic resonance spectroscopy to examine modulation of ATP production in human muscle. Recent developments in mitochondrial research have revealed the importance of uncoupling proteins in both insulin secretion and insulin action. Methods to assess uncoupling protein 2 (UCP2) expression in pancreatic islets and the hypothalamus (Chapter 22) and the effects of UCP2 expression on respiratory function in monolayers of INS1 cells (Chapter 23). The importance of mitochondrial anaplerosis and the redox state of pyridine nucleotide coenzymes for insulin secretion have also been demonstrated recently. Consequently, Chapter 24 provides methods for the study of the mitochondrial isoforms of malic enzyme in insulin secretion, and Chapter 25 describes methods to examine the effects of deletion of the nicotinamide nucleotide transhydrogenase on insulin secretion.

The chapters in this volume of *Methods in Enzymology* describe useful approaches in mitochondrial research that can be used directly or modified to investigate emerging areas of mitochondrial research. We wish to thank all the contributors to this volume and hope that the methods described will be useful in facilitating future studies in this rapidly evolving and exciting field.

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