

PREFACE

Two volumes of *Methods in Enzymology* devoted to methods in mitochondrial research were published in 1995 and 1996 under the editorship of the late Guiseppe Attardi and Anne Chomyn. The emphasis of the earlier volumes was on mitochondrial biogenesis. Nevertheless, they also contained several articles describing methods for examining the structure and function of inner membrane complexes that participate in electron transport and ATP synthesis. In the intervening years, high-resolution crystal structures have been obtained for complexes II, III, and IV derived from the mitochondrial inner membrane. However, a crystal structure has yet to be obtained for a eukaryotic complex I (NADH: quinone oxidoreductase). Thus, several chapters in this volume of *Methods in Enzymology* describe alternative methods to characterize the structure and function of complex I. Other chapters are focused on the location and function of mitochondrial iron-sulfur complexes and the characterization of reactive oxygen species that are formed during mitochondrial electron transport in mammalian and yeast mitochondria.

Because a crystal structure has not been obtained for complex I isolated from mammalian or yeast mitochondria, several chapters in this volume (Chapters 1, 2, 6 and 7) describe methods that have been developed to examine structural characteristics of complex I. Other articles describe methods that have been developed to examine electron transport through complex I (Chapters 3 and 4) and to assay complex I in human cells (Chapter 9). Methods to characterize type II NADH: quinone oxidoreductases isolated from the parasites *Plasmodium falciparum* and *Mycobacterium tuberculosis* are described in Chapter 17. Three chapters describe methods for the isolation and characterization of electron transport super complexes from yeast (Chapters 10 and 11) and mammalian mitochondria (Chapter 8). Another chapter describes methods to examine the assembly of subunits encoded by mitDNA and nuclear DNA in the mitochondrial inner membrane of mammalian cells (Chapter 18). The use of ruthenium ion photooxidation and photoreduction to examine electron transfer in mitochondrial complex II and complex IV, respectively, are described in Chapters 5 and 28, respectively. Chapter 16 describes methods for examining mitochondrial mobility and protein diffusion within the mitochondrial matrix.

Although they were discovered in the 1970s, the biosynthesis and assembly iron-sulfur proteins have been examined more recently. Three chapters (Chapters 12, 14 and 15) describe methods to examine the

location, function, and assembly of iron-sulfur complexes in the mitochondrial inner membrane, whereas Chapter 13 describes the isolation and characterization an iron-sulfur protein located in the mitochondrial outer membrane.

Although they probably play a beneficial role at low concentrations, reactive oxygen species (ROS), which include superoxide anion ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2), excessive ROS formation in mitochondria is invariably associated with pathologic conditions. Chapters 19 to 27 describe methods that have developed to detect, induce, or control formation of ROS during electron transport in mammalian and yeast mitochondria.

The methods described in this volume should provide investigators with techniques that can be used or modified to examine mitochondrial electron transport complexes, the location and function of iron-sulfur proteins, and the detection and control of reactive oxygen species that are formed during electron transport in mitochondria.

WILLIAM S. ALLISON AND IMMO E. SCHEFFLER