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Mitochondrial Medicine

Volume II, Manipulating Mitochondrial Function

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Preface

Mitochondrial Medicine is an interdisciplinary and rapidly growing new area of biomedical research comprising genetic, biochemical, pathological, and clinical studies aimed at the diagnosis and therapy of human diseases which are either caused by or associated with mitochondrial dysfunction. The term “Mitochondrial Medicine” was probably used for the first time by Rolf Luft [1] who is widely accepted as the father of Mitochondrial Medicine. Over 50 years ago, it was he who described for the very first time a patient with clinical symptoms caused by malfunctioning mitochondria [2].

The beginning of mitochondria-related research dates back to the end of the nineteenth century. During the 1890s, early cytological studies revealed the existence of bacteria-resembling subcellular particles in the cytosol of mammalian cells. Robert Altman termed them bioblasts, and he hypothesized that these particles were the basic unit of cellular activity. The name mitochondrion, which means thread-like particles, was coined in 1898 by Carl Benda. During the 1940s, progress was made in the development of cell fractionation techniques which ultimately allowed the isolation of intact mitochondria from cell homogenates, thereby making them more accessible to biochemical studies. Subsequently, by the end of the 1940s, activities of a variety of enzymes needed for fatty acid oxidation, the Krebs cycle, and other metabolic pathways were found to be associated with mitochondrial fractions.

Human mitochondrial DNA was discovered in 1963 [3], and Mitchell’s disputed chemiosmotic theory [4] of ATP synthesis became generally accepted in the early 1970s. In 1972, Harman proposed the Mitochondrial Theory of Aging, according to which aging is the result of the cumulative effects of mitochondrial DNA damage caused by free radicals [5, 6]. In 1986, Miquel and Fleming published their hypothesis about the involvement of mitochondria-originated free radicals in the process of ageing [7]. By 1981, mitochondrial DNA was completely sequenced [8], and, 5 years later, its entire genetic content had been described [9, 10]. Obviously, research on and with mitochondria has been conducted for over 120 years continuously and with steady success. Nevertheless, the last decade of the twentieth century saw another significant boost of interest in studying mitochondrial functions. First, in 1988, two papers, one published in *Science* and the other in *Nature* [11, 12], revealed for the very first time deletions and point mutations of mitochondria DNA to be the cause for human diseases. Second, by around 1995, mitochondria well known as the “powerhouse of the cell” have also been accepted as the “motor of cell death” [13] reflecting the organelle’s key role in apoptosis. It is nowadays recognized that mitochondrial dysfunction is either the cause of or at least associated with a large number and variety of human disorders, ranging from neurodegenerative and neuromuscular diseases, obesity, cardiovascular disorders, migraine, liver and kidney disease to ischemia-reperfusion injury and cancer. Subsequently, increased pharmacological and pharmaceutical efforts have led to the emergence of mitochondrial medicine as a new field of biomedical research [1, 14]. Future developments of techniques for probing and manipulating mitochondrial functions

will eventually lead to the treatment and prevention of a wide variety of pathologies and chronic diseases, “the future of medicine will come through mitochondria” [15].

Our book is dedicated to showcasing the tremendous efforts and the progress that has been made over the last decades in developing techniques and protocols for probing, imaging, and manipulating mitochondrial functions. All chapters were written by leading experts in their particular fields. The book is divided into two volumes. Volume I (*Probing Mitochondrial Function*) is focused on methods being used for the assessment of mitochondrial function under physiological conditions as well as in healthy isolated mitochondria. Volume II (*Manipulating Mitochondrial Function*) describes techniques developed for manipulating and assessing mitochondrial function under general pathological conditions and specific disease states.

Volume I

Stefan Lehr and coworkers critically evaluate in a review chapter a commonly used isolation procedure for mitochondria utilizing differential (gradient) centrifugation and depict major challenges to achieve “functional” mitochondria as basis for comprehensive physiological studies. The same authors provide in a protocol chapter an isopycnic density gradient centrifugation strategy for the isolation of mitochondria with a special focus on quality control of prepared intact, functional mitochondria. The isolation of interorganellar membrane contact sites is described by Alessandra d’Azzo and colleagues. They outline a protocol tailored for the isolation of mitochondria, mitochondria-associated ER membranes, and glycosphingolipid-enriched microdomains from the adult mouse brain, primary neurospheres, and murine embryonic fibroblasts. The analysis of single mitochondria helps uncovering a new level of biological heterogeneity and holds promises for a better understanding of mitochondria-related diseases. Peter Burke and colleagues describe a nanoscale approach for trapping single mitochondria in fluidic channels for fluorescence microscopy. Their method reduces background fluorescence, enhances focus, and allows simple experimental buffer exchanges. Stephane Arbault and colleagues describe the preparation and use of microwell arrays for the entrapment and fluorescence microscopy of single isolated mitochondria. Measuring variations of NADH of each mitochondrion in the array, this method allows the analysis of the metabolic status of the single organelle at different energetic-respiratory stages.

Deep resequencing allows the detection and quantification of low-level variants in mitochondrial DNA (mtDNA). This massively parallel (“next-generation”) sequencing is characterized by great depth and breadth of coverage. Brendan Payne and colleagues describe a method for whole mtDNA genome deep sequencing as well as short amplicon deep sequencing. In another chapter, the same group provides a method for characterizing mtDNA within single skeletal muscle fibers. This approach allows the detection of somatic mtDNA mutations existing within individual cells which may be missed by techniques applied to the whole tissue DNA extract. The authors also apply single-cell mtDNA sequencing for analyzing differential segregation of mtDNA during embryogenesis. They demonstrate how to study this phenomenon by single-cell analysis of embryonic primordial germ cells. Next-generation sequencing (NGS) as an effective method for mitochondrial genome sequencing is also the subject of Shale Dames’ chapter. He and his group describe an mtDNA enrichment method including library preparation and sequencing on “Illumina NGS platforms” and provide also a short command line alignment script for downloading via FTP.

Conventional methods for mitochondrial DNA (mtDNA) extraction do not yield the level of mtDNA enrichment needed for direct sequencing, and the necessary subsequent long-range PCR amplification may introduce bias into the sequence results. Alexander Maslov and colleagues provide a protocol involving a paramagnetic bead-based purification step for the preparation of mtDNA-enriched samples ready for direct sequencing. Lars Eide and coworkers give a detailed protocol for the use of real-time qPCR to analyze the integrity of mitochondrial DNA and RNA quantitatively. Their method has low material requirement, is low cost, and can detect modifications with high resolution.

Mitochondria in species ranging from yeast to human have been found to import a small number of nucleus-encoded RNAs. With the advent of high-throughput RNA sequencing, additional nucleus-encoded mitochondrial RNAs are being identified. Michael Teitell and his group describe both an *in vitro* and *in vivo* import system for studying mitochondrial RNA import, processing, and functions.

In the last decade an increasing number of studies have been conducted aimed at quantifying acquired changes in the concentration of circulating mitochondrial DNA (mtDNA) as an indicator of mitochondrial function. Afshan Malik and colleagues provide a protocol for accurately measuring the amount of human mtDNA in peripheral blood samples which is based on the use of real-time quantitative PCR (qPCR) to quantify the amount of mtDNA relative to nuclear DNA. Their protocol is suitable for high-throughput use and can be modified for application to other body fluids, human cells, and tissues. The characterization of mtDNA processing at the single-cell level is poorly defined. Laurent Chatre and Miria Ricchetti describe a mitochondrial transcription and replication imaging protocol which is based on modified fluorescence *in situ* hybridization and which allows the detection of qualitative and quantitative alterations of the dynamics of mtDNA processing in human cells undergoing physiological changes.

William Sivitz and colleagues describe a highly sensitive and specific nuclear magnetic resonance-based assay which allows the simultaneous quantification of ATP and reactive oxygen species using small amounts of mitochondrial isolates or permeabilized cells. Their novel assay also avoids the problem of changing mitochondrial membrane potential while ADP is converted to ATP, as occurs in conventional assays. Accurate detection of mitochondrial superoxide especially in living cells remains a difficult task. Werner Koopman and coworkers describe a live-cell microscopy-based method for detecting superoxide in both mitochondria and the entire cell using dihydroethidium. Boronate-based probes were developed over the last decade for detection of hydrogen peroxide and peroxynitrite in biological systems. However, most boronates lack specificity needed to distinguish between hydrogen peroxide and peroxynitrite within a complex biological system. Jacek Zielonka and colleagues describe how a newly developed mitochondria-targeted phenylboronic acid can be used to detect and differentiate peroxynitrite-dependent and independent probe oxidation. Time-resolved fluorescence spectrometry can be used to detect and characterize mitochondrial metabolic oxidative changes by means of endogenous fluorescence. Alzbeta Marcek Chorvatova and coworkers describe the detection and measurement of endogenous mitochondrial NAD(P)H fluorescence in living cells *in vitro* using fluorescence lifetime spectrometry imaging after excitation with a 405 nm picoseconds laser. Quantifying the mitochondrial membrane potential is essential for understanding mitochondrial function. Most of the current methodologies are based on the accumulation of cation indicators. Roger Springett describes a new methodology which allows calculating the membrane potential from the measured oxidation states of the b-hemes. To better understand the impact of oxygen on cellular function, James Hynes and Conn Carey outline the procedure

for measuring in situ oxygenation of cells in 2D and 3D cultures. These authors also illustrate how the impact of drug treatment on cell oxygenation can be assessed and how the link between oxygenation and glycolytic metabolism can be examined. Egbert Mik and Floor Harms have developed a method called Protoporphyrin IX—Triplet State Lifetime Technique as a potential tool for noninvasive monitoring of mitochondrial function in the clinic. In their chapter they describe the application of mitochondrial respirometry for monitoring mitochondrial oxygen tension and mitochondrial oxygen consumption in the skin of experimental animals. The selective monitoring of mitochondria-produced hydrogen peroxide inside living systems can be challenging. Alexander Lippert and colleagues describe the synthesis of the small molecular probe MitoPY1 and its application for measuring hydrogen peroxide in vitro and in live cells. The authors also provide an example procedure for measuring mitochondrial hydrogen peroxide in a cell culture model of Parkinson's disease. Erich Gnaiger and colleagues describe how the Amplex Red assay can be used to detect hydrogen peroxide production in combination with the simultaneous assessment of mitochondrial bioenergetics by high-resolution respirometry. They have optimized instrumental and methodological parameters to analyze the effects of various substrate, uncoupler, and inhibitor titrations (SUIT) on respiration versus hydrogen peroxide production. The authors also show an application example using isolated mouse brain mitochondria as an experimental model for the simultaneous measurement of mitochondrial respiration and hydrogen peroxide production in SUIT protocols. Andrey Abramov and Fernando Bartolome describe a strategy for assessing NADH/NAD(P)H and FAD autofluorescence in a time course-dependent manner. Their method provides information about NADH and FAD redox indexes both reflecting the activity of the mitochondrial electron transport chain. Their analysis of NADH autofluorescence after induction of maximal respiration can also offer information about the pentose phosphate pathway activity where glucose can be alternatively oxidized instead of pyruvate. Coenzyme Q10 (CoQ10) is an essential part of the mitochondrial respiratory chain. Outi Itkonen and Ursula Turpeinen describe an accurate and sensitive liquid chromatography tandem mass spectrometry method for the determination of mitochondrial CoQ10 in isolated mitochondria.

Assessing bioenergetic parameters of human pluripotent stem cells (hPSCs), including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), provides considerable insight into their mitochondrial functions and cellular properties, which allows exposing potential energetic defects caused by mitochondrial diseases. Alessandro Prigione and Vanessa Pfiffer describe a method that facilitates the assessment of the bioenergetic profiles of hPSCs in a noninvasive fashion, while requiring only small sample sizes and allowing for several replicates.

Due to the complexity of the interactions involved at the different levels of integration in organ physiology, current molecular analyses of pathologies should be combined with integrative approaches of whole organ function. By combining the principles of control analysis with noninvasive ^{31}P NMR measurement of the energetic intermediates and simultaneous measurement of heart contractile activity, Philippe Diolez and colleagues have developed MoCA (Modular Control and Regulation Analysis), which is an integrative approach designed to study in situ control and regulation of cardiac energetics during contraction in intact beating perfused isolated heart. In their review chapter the authors present selected examples of the applications of MoCA to isolated intact beating heart, and they also discuss wider application to cardiac energetics under clinical conditions with the direct study of heart pathologies.

Mitochondrial proteins encoded on the cytosolic ribosomes carry specific patterns in the precursor sequence needed for mitochondrial import. Rita Casadio and colleagues discuss the feasibility of utilizing computational methods for detecting such mitochondrial targeting peptides in polypeptide sequences. These authors also introduce their newly implemented web server and demonstrate its application to the whole human proteome for detecting mitochondrial targeting peptides. Fabiana Perocchi and Yiming Cheng describe evolutionary biology approaches for studying mitochondrial physiology. One strategy, which they refer to as “comparative physiology,” allows the *de novo* identification of mitochondrial proteins involved in a physiological function. Another approach known as “phylogenetic profiling” allows predicting the function of uncharacterized proteins as well as functional interactions by comparing phylogenetic profiles of uncharacterized and known components. Besides DNA mutations, faulty posttranslational modifications can also cause malfunction of mitochondrial proteins. Suresh Mishra and colleagues describe procedures for the isolation of mitochondria from cells and for separating the mitochondrial proteins by two-dimensional gel electrophoresis. The employment of antibodies specific to each posttranslational modification allows them to assess posttranslational modifications of mitochondrial proteins. Posttranslational protein glutathionylation regulates protein function in response to cellular redox changes and is involved in carbon monoxide-induced cellular pathways. Helena Viera and Ana S. Almeida describe a technique for the assessment of mitochondrial protein glutathionylation in response to carbon monoxide exposure.

High-resolution melting (HRM) allows detecting homozygous or heterozygous point sequence variants and small deletions within specific PCR products. Marketa Tesarova and colleagues provide an updated HRM-based protocol for routine variant screening of nuclear genes encoding assembly factors and structural subunits of cytochrome c oxidase (COX). Their general recommendations given for HRM analysis are applicable for examining any genetic region of interest. Anton Vila-Sanjurjo and colleagues have designed a computational approach named Heterologous Inferential Analysis or HIA for making predictions on the disruptive potential of a large subset of mt-rRNA variants. The authors demonstrate that in the case of certain mitochondrial variants for which sufficient information regarding their genetic and pathological manifestation is available, HIA data alone can be used to predict their pathogenicity.

Mitochondria play a key role in apoptosis. Vladimir Gogvadze and coworkers describe how to evaluate the release of intermembrane space proteins during apoptosis, alterations in the mitochondrial membrane potential, and oxygen consumption in apoptotic cells. Fluorescent lifetime imaging microscopy-Förster resonant energy transfer (FLIM-FRET) is a high-resolution technique for the detection of protein interactions in live cells. David Andrews and colleagues provide a detailed protocol for applying this technique to assess the interaction between BclXL and Bad at the mitochondrial outer membrane in live MCF7 breast cancer cells. Mitochondrial Ca^{2+} uptake is essential for regulating mitochondrial function. Markus Waldeck-Weiermair and colleagues analyze the benefits and drawbacks of various established old and new techniques to assess dynamic changes of mitochondrial Ca^{2+} concentrations in a wide range of applications.

Untargeted lipidomics profiling by liquid chromatography-mass spectrometry (LC-MS) allows the examination of lipids without any bias towards specific classes of lipids. Bruce Kristal and group describe a workflow including the isolation of mitochondria from liver tissue, followed by mitochondrial lipid extraction and the LC-MS conditions used for data acquisition. The authors also highlight how, in this method, all ion fragmentation can

be used for the identification of species of lower abundances, which are often missed by data-dependent fragmentation techniques.

Mitochondrial dynamics, i.e., mitochondrial location, number, and morphology, has an essential function in numerous physiological and pathophysiological phenomena in the developing and adult human heart. Elizabeth Lipke and colleagues describe the application of a computer-based tool (MATLAB, MQM) to quantify mitochondrial changes, in particular number, area, and location of mitochondria, during human pluripotent stem cell differentiation into spontaneously contracting cardiomyocytes. Helena Bros and coworkers present an *ex vivo* method for monitoring the movement of mitochondria within myelinated sensory and motor axons from spinal nerve roots.

Volume II

The development of mitochondria-targeted pharmaceutical nanocarriers began at the end of the 1990s with an accidental discovery of the vesicle-forming capacity of dequalinium chloride. Volkmar Weissig describes a detailed protocol for the preparation, characterization, and application of dequalinium-based nano vesicles called DQAsomes. Whether small molecule xenobiotics (biocides, drugs, probes, toxins) will target mitochondria in living cells without the assistance of any mitochondria-targeted delivery system can be predicted using an algorithm derived from QSAR modeling and is described in detail by Richard Horobin. Small molecules can be physicochemically targeted to mitochondria via conjugation to mitochondriotropic triphenylphosphonium cations. Utilizing this strategy, Richard Hartley describes the preparation of MitoB and MitoP as exomarkers of mitochondrial hydrogen peroxide. Gerard D' Souza and his group describe the use of triphenylphosphonium cations for the preparation of phospholipid conjugates which in turn are the basis for preparing mitochondria-targeted liposomes. Triphenylphosphonium cations are used by Jung-Joon Min and Dong-Yeon Kim for the synthesis of 18F-labeled fluoroalkyl triphenylphosphonium conjugates as mitochondrial voltage sensors for PET myocardial imaging. Fernanda Borges and her group describe the utilization of triphenylphosphonium cations for the development and application of a new antioxidant based on dietary cinnamic acid. Tamer Elbayoumi and colleagues have utilized the intrinsic mitochondriotropism of Genistein to design mitochondria-targeted cationic lipid-based nanocarrier systems including micelles and nanoemulsions. Since Genistein, a major soy isoflavone, exhibits extensive proapoptotic anticancer effects which are mediated predominantly via induction of mitochondrial damage, this delivery system is potentially suited to enhance anticancer efficacy of different coformulated chemotherapeutic agents. Shanta Dhara and her group outline in one chapter the synthesis and characterization of a functionalized polymer for building mitochondria-targeted nanoparticles (NPs), and in a second chapter she describes the application of such mitochondria-specific nanoparticles for the delivery of a photosensitizer to mitochondria for photodynamic therapy. Hideyoshi Harashima and Yuma Yamada describe the construction and application of a mitochondria-targeted dual function liposome-based nanocarrier termed DF-MITO-Porter.

The utilization of α -aminophosphonates as external probes in combination with ^{31}P -NMR allows the simultaneous pH measurements of cytosolic and acidic compartments in normal and stressed cultured cells. Sylvia Pietria's group has developed this strategy further by using triphenylphosphonium derivatives of aminophosphonates as mitochondria-targeted pH probes. The authors describe the synthesis and ^{31}P -NMR pH titrating properties

of such mitochondria-targeted pH probes as well as their application in green alga cultures. The formation of reactive oxygen species (ROS) in the inner mitochondrial membrane can cause mitochondrial dysfunction eventually followed by induction of apoptosis. Antioxidants conjugated with mitochondria-targeted, membrane-penetrating cations can be used to scavenge ROS inside mitochondria. Vladimir Skulachev and coworkers describe some essential methodological aspects of the application of mitochondria-targeted cations belonging to the MitoQ and SkQ groups which have shown promise for treating oxidative stress-related pathologies. Lucia Biasutto and colleagues describe a step-by-step procedure for synthesizing mitochondria-targeted derivatives of resveratrol and quercetin, two plant polyphenols exhibiting potential health-promoting properties, as well as a method for assessing their mitochondrial accumulation.

The mitochondrial respiratory chain is stress-responsive and responds to mitochondrially targeted anticancer agent by destabilization and induction of massive ROS production eventually leading to apoptosis. Jiri Neuzil's group has developed mitochondrially targeted anticancer agents epitomized by the mitochondrially targeted analogue of the redox-silent compound vitamin E succinate, which belongs to the group of agents that kill cancer cells via their mitochondria-destabilizing activity. The authors describe the use of native blue gel electrophoresis and clear native electrophoresis coupled with in-gel activity assays as methods of choice for trying to understand the molecular mechanism of the effect of such mitochondria-destabilizing agents. Many low-molecular-weight agents that may be of potential clinical relevance act by targeting mitochondria, where they may suppress mitochondrial respiration. Jiri Neuzil and coworkers describe the methodology for assessing respiration in cultured cells as well as in tumor tissue exposed to mitochondria-targeted anticancer agents.

Nina Entelis, Ivan Tarasov, and colleagues have developed mitochondria-targeting RNA vectors for the delivery of therapeutic oligoribonucleotides into human mitochondria. Their group provides a detailed protocol for the transfection of cultured human cells with small recombinant RNA molecules as well as methods for characterizing the mitochondrial transfection efficiency. Genetic transformation of mitochondria in multicellular eukaryotes is of fundamental importance for basic investigations and for applications to gene therapy or biotechnology. Andre Dietrich's group has developed a strategy to target nuclear transgene-encoded RNAs into mitochondria in plants. In their chapter they give a detailed protocol for mitochondrial targeting of trans-cleaving ribozymes destined to knockdown organelle RNAs for regulation studies, inverse genetics, and biotechnological purposes.

Allotopic expression (AE) of mitochondrial proteins, i.e., nuclear localization and transcription of mtDNA genes followed by cytoplasmic translation and transport into mitochondria, has been suggested as a strategy for gene replacement therapy in patients harboring mitochondrial DNA mutations. Carl Pinkert and David Dunn describe the use of AE for transgenic mouse modeling of the pathogenic human T8993G mutation in mtATP6 as a case study for designing AE animal models.

There is increasing evidence that exposure to air pollutants is associated with human disease and may act through epigenetic modification of the nuclear genome, but there have been few publications describing their impact upon the mitochondrial genome. Hyang-Min Byun and Timothy M. Barrow describe a protocol for the isolation of mitochondrial DNA from peripheral blood samples and the analysis of 5-methylcytosine content by bisulfite-pyrosequencing. Stanislaw Pshenichnyuk and Alberto Modelli describe the application of two complementary experimental techniques, Electron Transmission Spectroscopy

(ETS) and Dissociative Electron Attachment Spectroscopy (DEAS), for studying the transfer of electrons unto xenobiotics in the intermembrane space of mitochondria. Additional support of experimental procedures by suitable quantum-chemical calculations is described in detail and illustrated by an example of ETS/DEAS study of rhodamine which shows rich fragmentation under gas-phase resonance electron attachment.

The link between mitochondrial dynamics and human pathologies has spawned significant interest in developing methods for screening proteins involved in mitochondrial dynamics as well as small molecules that modulate mitochondrial dynamics. Antonio Zorzano and Juan Pablo Munoz describe in their chapter functional screening protocols for the in vitro examination of mitochondrial parameters such as mitochondrial morphology, reactive oxygen species (ROS) levels, mitochondrial calcium, and oxygen consumption rate. Dysfunctional mitochondria communicate via retrograde signaling with the nucleus leading to cell stress adaptation by changes in nuclear gene expression. Mitochondria to nucleus signaling pathways have been widely studied in *Saccharomyces cerevisiae*, where retrograde-target gene expression is regulated by RTG genes. Sergio Giannattasio and coworkers describe a method for the assessment of the mitochondrial retrograde pathway activation in yeast cells based on monitoring the mRNA levels of a variety of RTG-target genes. Adaptations to energy stress or altered physiological condition can be assessed by measuring changes of multiple bioenergetic parameters. Dmitri Papkovsky and Alexander Zhdanov describe a simple methodology for high-throughput multiparametric assessment of cell bioenergetics, called Cell Energy Budget (CEB) platform, and demonstrate its practical use with cell models.

Viable disease models for mitochondrial DNA diseases are much needed for elucidating genotype/phenotype relationships and for improving disease management. Alessandro Prigione discusses the potential advantages and critical challenges for the utilization of induced pluripotent stem cells (iPSCs) from patients affected by mtDNA disorders for modeling debilitating mtDNA diseases.

Heteroplasmic mice can be used for studying the segregation of different mtDNA haplotypes in vivo against a defined nuclear background. Thomas Kolbe and colleagues describe two methods involving either the transfer of ooplasm or the fusion of two blastomeres for the creation of such mice models.

H. van der Spek and coworkers describe a robust and efficient method for visualizing and quantifying mitochondrial morphology in *Caenorhabditis elegans*, which is a preferred model for studying mitochondrial deficiencies caused by disease or drug toxicity. Their method allows for a comprehensive analysis of mitochondrial morphology. Mitochondrial DNA (mtDNA) is a useful and reliable biomarker of UV-induced genetic damage in both animal and human skin. Mark Birch-Machin and Amy Bowman describe in their protocol chapter the assessment of UV-induced mtDNA damage, including the extraction of cellular DNA, qPCR to determine the relative amount of mtDNA, and qPCR to determine UV-induced damage within a long strand of mtDNA.

Mitochondrial dysfunction is associated with the pathogenesis of septic disorders, eventually leading to a decline in energy supply. Matthias Hecker and colleagues give a protocol for assessing the influence of short- and medium-chain fatty acids on mitochondrial respiration using high-resolution respirometry under inflammatory and baseline conditions. Hun-Kuk Park and Gi-Ja Lee describe the application of Atomic Force Microscopy (AFM)-based shape analysis for the characterization of nanostructural changes of mitochondria. The authors use AFM to study mitochondrial swelling in heart mitochondria during myocardial ischemia-reperfusion injury employing a rat model. In some tissues such as the heart, abnormal

mitochondrial fusion and fission can go along with mitochondrial apoptosis, but its contribution as cause vs. a consequence remains to be defined. Catherine Brenner and her group give a protocol for the isolation of fresh mitochondria from rat heart by a procedure adapted to the myofibrillar structure of the tissue, and they describe several miniaturized enzymatic assays for probing mitochondria-mediated apoptosis. The pathogenesis of Parkinson's disease (PD) is poorly understood and under intensive investigation. Mitochondrial dysfunction has been linked to the sporadic form of PD. Daniella Arduino's group describes a method for the generation of cytoplasmic hybrid cells as a cellular model of sporadic PD which is based upon the fusion of platelets harboring mtDNA from PD patients with cells in which the endogenous mtDNA has been depleted. JC-1, a commercially available fluorescence dye, is widely used for measuring changes in the mitochondrial membrane potential. Dorit Ben-Shachar and coworkers show that JC-1 can also be used to follow alterations in mitochondrial distribution and mitochondrial network connectivity. The authors describe various applications of JC-1 staining to study mitochondrial abnormalities in different cell types derived from schizophrenia patients and healthy subjects.

We are extremely grateful to all authors for having spent significant parts of their valuable time to contribute to this book. It is our hope that together we have succeeded in providing an essential source of know-how and a source of inspiration to all researchers who are as fascinated as we are about this tiny organelle which so much seems to control life and death of a single cell and the whole organism alike. Last but not least we would like to thank John Walker, the series editor of *Methods in Molecular Biology*, for having accepted our book proposal, which originated from our efforts in organizing a series of annual conferences on Targeting Mitochondria, the fifth one of which has taken place in October 2014 in Berlin, Germany (www.targeting-mitochondria.com). We are also grateful to John Walker for his unlimited guidance and help throughout the whole process.

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