Preface

Recent research had demonstrated a critical role of the poly-ADP-ribose (pADPr) pathway in genotoxic stress response as well as in developmentally regulated processes, including chromatin reorganization, control of gene expression, ribosomal biogenesis, nuclear traffic, and stem cell regulation. Poly(ADP-ribose) Polymerase (PARP) enzymatic activity is required for normal assembly of higher-order chromatin structures and for the transcriptional activation of genes. The role of PARP in the biology of eukaryotes and its relevance to human health are impossible to overstate. The PubMed database annotates hundreds of publication on the pathway per month. Nevertheless, the poly(ADP-ribosylating) pathway remains a terra incognita. This important pathway is hardly ever mentioned in biology textbooks and literature for general biology readers. This book aims to cover this gap in literature by presenting multiple new and classical methods for studying pADPr pathway.

The first edition of the *Methods in Molecular Biology* series on Poly(ADP-ribose) Polymerase (PARP) was published in 2011. Despite being called the second edition, this present volume introduces multiple methods that were not covered in the first edition of the book and hence represents a second volume that complements rather than supersedes the first edition. Chapters of this volume do not replace or update chapters published in 2011. The overwhelming majority of the chapters are being published here for the first time.

The collection of methods published in the first edition covered the classical biochemical, genetics, molecular biology, and medical biology approaches that are focused on the study of PARP-1 functions in the cells, as well as its structure and enzymology. The second edition presents new methods that were developed during recent efforts to detect and quantify the product of PARP enzymatic activity, i.e., pADPr in different tissues, as well as to detect variation in pADPr production during different parts of cell cycle and cell compartment specificity (Part I). pADPr serves as a biomarker for phases of normal development and pathogenesis. Thus, the ability to detect and visualize pADPr production is of vital interest for developmental biologists and clinicians.

Chapters in Part II are focused on the identification of pADPr protein acceptors, based on advanced biochemical approaches and mass spectrometry. New revolutionary approaches using "clickable NAD+ analogs" have made it possible to identify PARP protein targets at a new level of specificity and sensitivity. Combining the clickable NAD+ analogs methods with the classical genetics approaches allows us not only to target the identification of PARP targets with high precision but also link each target with a specific member of the PARP protein superfamily.

Chapters in Part III cover a rapidly growing field of methods focusing on studying molecular mechanisms of PARP functions in eukaryotic cells, particularly those involved in control of DNA repair and oxidative stress, as well as in expression regulation.

Nucleosomal histones, nucleosomes, and nucleosomal arrays control multiple aspects of the PARP-1 protein localization in chromatin, dynamics of PARP-1 in nuclei, and regulation of PARP-1 enzymatic activation during PARP-1 dependent transcription and in genotoxic stress response. The two chapters of Part IV describe approaches to the in vitro reconstitution of PARP-1 interaction with chromatin.

Part V presents methods that allowed us to develop and test small molecule PARP inhibitors. The clinical potential of PARP-1 inhibitors has been recognized over the past two decades, prompting intensive research on their therapeutic application, albeit not without some setbacks. Therefore, identification of new inhibitors is an important step forward for clinicians involved in the developments of PARP-1 therapies targeting PARP functions.

The inclusion of Part VI, which focuses on methods oriented towards studying the functions of understudied members of PARP family, reflects the recent research shift in the field of poly(ADP-ribosyl)ation from studies focused on the most abundant PARP-1 to other members of the PARP superfamily and a group of antagonistic proteins controlling pADPr catabolism. Methods presented in this section are oriented towards unstudied components of the PARP pathway.

And the last but not least: I would like to acknowledge the cooperation of 29 outstanding groups of authors who collectively contributed to this volume, as well as the valuable support provided by the series editor, Mr. John Walker. I would also like to express my gratitude to Kate Pechenkina for her help and advice during this volume assembly and preparation. Finally, I thank my sons, Denis and Roman, for their help and emotional support.

Philadelphia, PA, USA *Alexei V. Tulin*

http://www.springer.com/978-1-4939-6992-0

Poly(ADP-Ribose) Polymerase
Methods and Protocols
Tulin, A.V. (Ed.)
2017, XV, 528 p. 82 illus., 47 illus. in color. With online files/update., Hardcover
ISBN: 978-1-4939-6992-0
A product of Humana Press