METHODS IN MOLECULAR BIOLOGY

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Nitric Oxide

Methods and Protocols

Edited by

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Humana Press

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ISSN 1064-3745 ISSN 1940-6029 (electronic)
Methods in Molecular Biology
ISBN 978-1-4939-7694-2 ISBN 978-1-4939-7695-9 (eBook)
https://doi.org/10.1007/978-1-4939-7695-9

Library of Congress Control Number: 2018935390

Printed on acid-free paper

This Humana Press imprint is published by the registered company Springer Science+Business Media, LLC part of Springer Nature.
The registered company address is: 233 Spring Street, New York, NY 10013, U.S.A.

Preface

Nitric oxide (NO) is an important signaling molecule in humans, animals, plants, fungi, and bacteria, which is involved in the regulation of growth and development as well as stress response reactions. On the cellular level, NO is sensed through redox modifications of proteins. The most important mode of action of NO is protein S-nitrosation, the covalent attachment of NO to the thiol group of protein cysteine residues. Other major types of NO-dependent modifications are metal nitrosation and tyrosine nitration. Together, these protein modifications can lead to alteration of gene expression and/or direct metabolic changes and finally result in defined physiological responses. The past decade has seen an explosion in the number of articles relating to both the physiological and pathological responses evoked by NO.

Investigation of NO signaling is challenging because many available methods suffer from the lack of specificity for the parameter to be tested. Additionally, S-nitrosation is a thermodynamically unstable modification, impeding its direct detection. Therefore, NO research requires a broad set of complementary methods, which together allow the accurate identification of the functions of NO. In this volume, we tried to describe new methods for NO research with improved specificity and sensitivity compared to traditional methods.

This volume considers three main aspects of NO research and we hope this book is a useful resource for anyone with interest in NO research. Part I includes a protocol for NO treatment using chemical NO donors and several methods for NO detection using a NO-sensitive electrode, electron spin resonance, quantum cascade laser-based spectroscopy, membrane inlet mass spectrometry, and fluorescence-based NO sensor proteins. Part II focuses primarily on techniques to detect and identify NO-dependent modifications. This includes modified protocols of the biotin switch assay for detection, identification, and quantification of S-nitrosated proteins. Moreover, mass spectrometry, surface plasmon resonance, and antibody-based techniques are described to analyze low and high molecular weight S-nitrosothiols or tyrosine nitrated proteins. Additionally, the detection of nitro-fatty acids is described. In Part III of this volume protocols that are designed to measure S-nitrosothiol homeostasis and denitrosation activities are presented.

A special feature of this book is that it includes methods used in human/animal and plant NO research bringing together the experience from both fields. In many cases a transfer of the protocol from one system to the other system is possible with minor modifications. For example, the biotin switch technology is successfully used in human/animal and plant NO research using almost identical protocols. Techniques, which are based on analysis of protein extracts or recombinant proteins, might also be adapted easily. Others such as the detection of NO via NO sensor proteins, which was developed for the human/animal system, are very promising techniques for the plant system, but the transfer might be more challenging.

Although visible progress has been made in developing methods for NO research, future efforts should aim to further increase the sensitivity and specificity of those methods. In addition, many of the described techniques require sophisticated equipment not afford-

able to all NO labs. Better networking and a more open discussion on the problems associated with NO research will potentially lead to more robust and reproducible results in this important research area in the future.

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