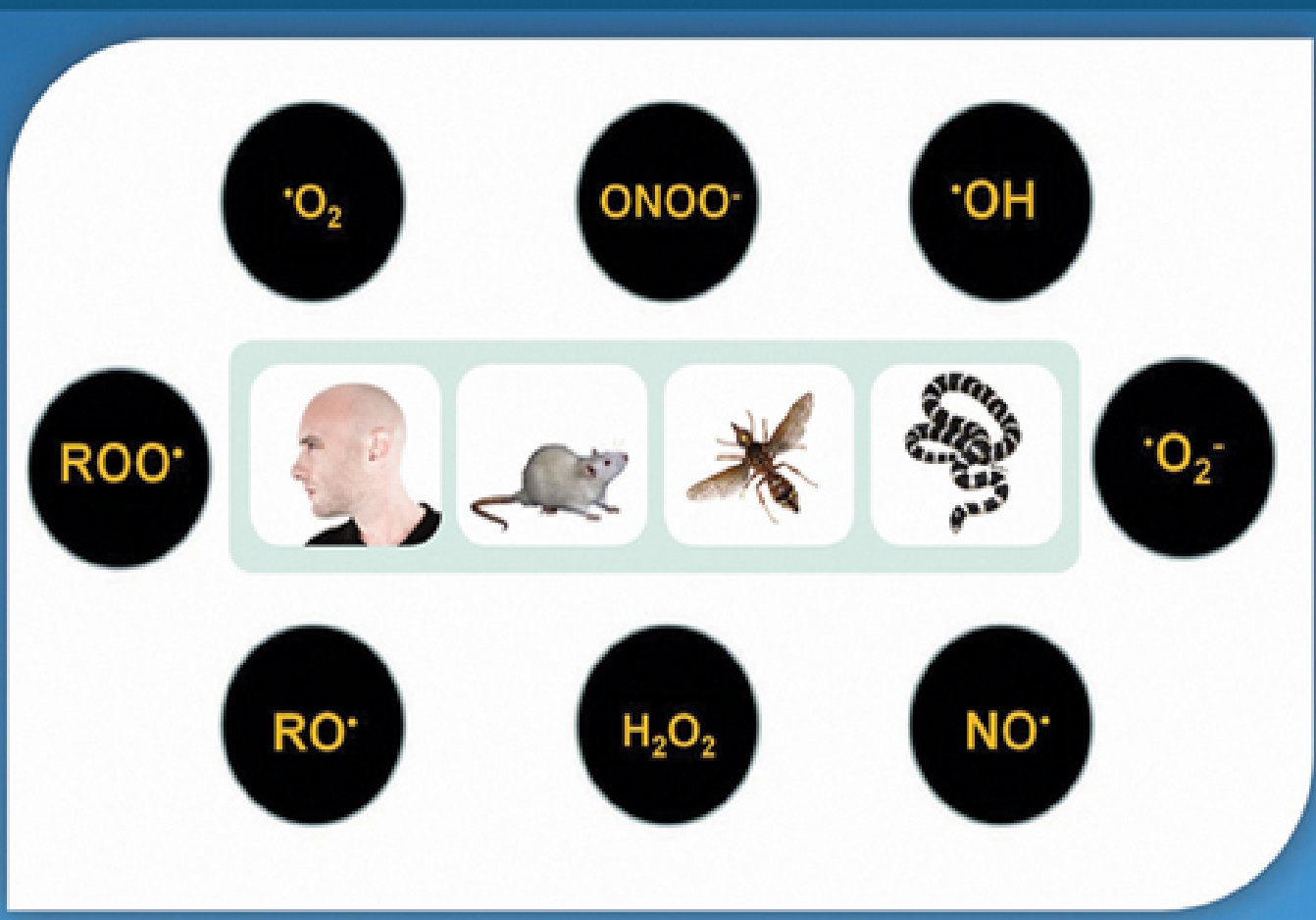
# Oxidative Stress in Vertebrates and Invertebrates

## MOLECULAR ASPECTS OF CELL SIGNALING

*Edited by*
*Tahira Farooqui and Akhlaq A. Farooqui*




WILEY-BLACKWELL

# OXIDATIVE STRESS IN VERTEBRATES AND INVERTEBRATES

# OXIDATIVE STRESS IN VERTEBRATES AND INVERTEBRATES

## Molecular Aspects of Cell Signaling

**Edited by**

**TAHIRA FAROOQUI**
Department of Entomology/Center of Molecular Neurobiology
The Ohio State University
Columbus, Ohio
USA

**AKHLAQ A. FAROOQUI**
Department of Molecular and Cellular Biochemistry
The Ohio State University
Columbus, Ohio
USA

A JOHN WILEY & SONS, INC., PUBLICATION

Published by John Wiley & Sons, Inc., Hoboken, New Jersey
Published simultaneously in Canada



***Library of Congress Cataloging-in-Publication Data***

Oxidative stress in vertebrates and invertebrates : molecular aspects on cell signaling / Tahira Farooqui and Akhlaq A. Farooqui, editors.
p. cm.
Includes index.
ISBN 978-1-118-00194-3 (cloth)
1. Oxidative stress–Molecular aspects. 2. Oxidative stress–Pathophysiology. 3. Vertebrates–Cytology. 4. Invertebrates–Cytology. 5. Vertebrates–Diseases–Molecular aspects. 6. Invertebrates–Diseases–Molecular aspects. 7. Cellular signal transduction. I. Farooqui, Tahira. II. Farooqui, Akhlaq A.
RB170.O955 2012
571.9′453–dc23

2011037215

Printed in the United States of America

oBook ISBN: 978-1-118-14814-3
ePDF ISBN: 978-1-118-14813-6
ePub ISBN: 978-1-118-14811-2
eMobi ISBN: 978-1-118-14812-9

10 9 8 7 6 5 4 3 2 1

*"Live as if you were to die tomorrow. Learn as if you were to live forever."*

Mohandas Karamchand Gandhi

# CONTENTS

# PREFACE

All oxygen-utilizing animals and organisms have to deal with reactive oxygen species (ROS), which include superoxide anions, hydroxyl, alkoxyl, and peroxyl radicals, and hydrogen peroxide. These radicals are common products of life in an aerobic environment, and they are responsible for oxygen toxicity. Proteins, lipids, and nucleic acid are targets for ROS attack, and modification of these molecules can increase the risk of chronic neurodegenerative diseases, visceral diseases, and cancer. "Oxidative Stress in Vertebrates and Invertebrates: Molecular Aspects of Cell Signaling" provides readers with a comprehensive description of the latest research on oxidative stress and antioxidant defenses in vertebrate and invertebrate systems. In biological systems, cells respond to mild oxidative stress by inducing antioxidant defenses and other protective systems. The antioxidant capacities of tissues are well matched to the rates of oxygen consumption and radical production. In vertebrate and invertebrate systems a variety of endogenous antioxidants (reduced glutathione) and antioxidant enzymes (superoxide dismutase, glutathione peroxidase, glutathione reductase, and catalase) act in a concerted manner to protect tissues against oxidative damage. The balance among oxidants and antioxidant enzyme systems, levels of antioxidants, and endogenous antioxidant mechanisms may be of major importance in the protection against oxidative stress-mediated cell injury. Under normal conditions, the rate of production of oxidants is balanced by the rate of oxidant elimination. However, an imbalance between prooxidants and antioxidants results in oxidative stress. Increase in ROS production has a substantial impact cellular metabolism and may lead to either defective cellular function and aging or chronic neurodegenerative and visceral diseases. Therefore, a better understanding of the roles of ROS-mediated signaling in normal cellular function as well as in disease is necessary for the development of therapeutic agents for oxidative stress-related chronic diseases.

Unlike other edited books that focus on oxidative stress in mammals, this unique book provides a comparative account of oxidative stress and antioxidant defenses in vertebrates and invertebrates, dealing not only with basic mechanisms and biomarkers but also with oxidative stress-mediated chronic diseases. This edited book is a valuable source of information for both basic scientists and clinicians who are interested in basic mechanism and oxidative stress-associated diseases. In this book, chapters are organized into two sections: (1) oxidative stress in vertebrates (Chapters 1–17) and (2) oxidative stress in invertebrates (Chapters 18–26), followed by a perspective (Chapter 27).

In Part I, Chapters 1 and 2 deal with the generation of ROS in the brain and their signaling associated with neural cell survival, cell suicide, and diseases. Chapters 3 and 4 discuss mitochondrial DNA mutation-induced oxidative stress underlying biochemical and pathological consequences and redox therapy in mitochondrial diseases and changes in kainic acid-induced neurotoxicity, which can be implicated in the pathogenesis of neurotraumatic and neurodegenerative diseases. Chapter 5 covers the historical aspects of the discovery of NF-E2-related factor 2 (Nrf2), recent advances in molecular aspects of its function, and updates involving Nrf2 association with various pathological conditions. Chapter 6 discusses modulation of oxidative stress by caloric restriction, suggesting that a calorie-restricted diet and the composition of diet may significantly improve ROS homeostasis both in single cells as well as in the whole body. Chapters 7–10

deal with the contribution of oxidative stress and inflammation to the pathogenesis of neurodegenerative diseases (Alzheimer disease, Parkinson disease). Chapter 11 summarizes free radical contribution to the development of cardiovascular diseases and discusses the applicability of antioxidant therapy based on data from clinical trials. Chapter 12 provides a comparison between vertebrates and invertebrates with regard to oxidative stress and aging. Chapter 13 addresses various environmental stressor-induced toxicities in experimental animals like rats and humans to elucidate the molecular mechanisms underlying oxidative stress. Chapter 14 discusses the role of selenoproteins in cellular redox regulation and signaling. Chapter 15 gives a clinical demonstration of the effectiveness of antioxidant administration in different diseases. Chapter 16 demonstrates that grape-derived bioactive polyphenolic components from wine effectively protect against the onset and progression of Alzheimer disease phenotypes, suggesting that moderate wine consumption may have preventive and/or therapeutic value in Alzheimer disease. Finally, Chapter 17 discusses pharmacological and therapeutic properties of propolis, a resinous mixture that honeybees collect from tree buds, sap flow, and other botanical sources, which is very beneficial for human health because of its richness in phenolic compounds.

In Part II, Chapter 18 reviews the endocrine control of oxidative stress in insects. Chapter 19 focuses on oxidative stress and innate immune system in airway epithelial cells of the fruit fly *Drosophila melanogaster*. Chapter 20 explores the molecular mechanisms of antioxidant protective processes in the honeybee *Apis mellifera*. Chapter 21 describes a hypothetical mechanism associated with iron-induced oxidative stress, implicating ROS production in olfactory dysfunction in the honeybee brain. Chapter 22 covers cutting-edge information on the Keap1/Nrf2 system in flies as well as its implications in combating human diseases. Chapter 23 is devoted to orchestration of oxidative stress responses in *Drosophila melanogaster* and promoter analysis study of circadian regulatory motifs. Chapter 24 deals with the protective role of sestrins (a unique family of proteins that is critically involved in cellular defense) against chronic target of rapamycin complex activation and oxidative stress in *Drosophila*. Chapter 25 explores current advances in the studies of oxidative stress and age-related memory impairment in the nematode *C. elegans*. Chapter 26 elegantly reviews oxidative challenge and redox sensing in mollusks by focusing on effects of natural and anthropic stressors. Finally, Chapter 27 provides readers with an in-depth perspective on current progress on our understanding of oxidative stress. It also presents readers and researchers with information that will be important for future research dealing with oxidative stress.

Biochemists, neuropharmacologists, toxicologists, and clinicians will find this book useful for understanding basic mechanisms of oxidative stress in vertebrate and invertebrate systems. It is hoped that "Oxidative Stress in Vertebrates and Invertebrates: Molecular Aspects of Cell Signaling" will further stimulate young and senior scientists to perform research on oxidative stress and oxidative stress-associated diseases.

Tahira Farooqui
Akhlaq A. Farooqui

# FOREWORD

Oxidative stress is a cytotoxic process that occurs in cells when antioxidant mechanisms are overwhelmed by reactive oxygen species (ROS). This imbalance not only causes damage to important biomolecules (lipids, proteins, and nucleic acids) in cells, but also has an impact on functional activities in both vertebrates and invertebrates. This new volume entitled "Oxidative Stress in Vertebrates and Invertebrates: Molecular Aspects of Cell Signaling" brings together important information from expert researchers in the oxidative stress-mediated cell signaling area in both vertebrate and invertebrate systems. Accumulation of high levels of ROS and significant reduction in cellular redox systems are common processes associated with acute and chronic visceral and neurodegenerative diseases, including hypertension, preeclampsia, arteriosclerosis, acute renal failure, diabetes, and Alzheimer and Parkinson diseases. This well-organized book presents up-to-date and comprehensive information on oxidative stress-related signaling events in vertebrates and invertebrates. The text is clear, concise, and easily accessible. Subject matter is divided into a vertebrate section (17 chapters) and an invertebrate section (10 chapters). The editors are known for their work on oxidative stress and neurodegeneration. They have done a commendable job in putting together this volume, and have contributed 5 chapters. These editors have taken great care in selecting the topics and describing progress that has been recently made in this field. The authors of this book also tried to ensure uniformity and mode of presentation in a simple and clear manner.

Topics addressed in the vertebrate section include the generation of ROS and their roles in cell survival and suicide; ROS-induced signal transduction and human diseases; biochemical and pathological consequences and redox therapy; pathogenesis of neurotraumatic and neurodegenerative diseases; oxidative stress mediated by caloric restriction; the role of oxidative stress and neuroinflammation in Alzheimer disease and Parkinson disease; selenoproteins in cellular redox regulation and signaling; antioxidant therapy and its effectiveness in oxidative stress-mediated disorders; pharmacological and therapeutic properties of propolis; comparison of oxidative stress in aging between vertebrates and invertebrates; and finally, oxidative stress-mediated signaling pathways by environmental stressors. The invertebrate section includes oxidative stress-induced signaling in three important phyla, namely, arthropoda, annelida, and mollusca. Topics addressed in this section include effect of oxidative stress on insect endocrine control; the innate immune system in airway epithelial cells of *Drosophila*; age-related memory impairment in *C. elegans*; olfactory learning and memory in *Apis mellifera*; Keap1/Nrf2 signaling in *Drosophila*; circadian rhythm in *Drosophila*; molecular antioxidant protective processes in *Apis mellifera*; protective role of sestrins against chronic TOR; and oxidative challenge and redox sensing in mollusks.

The subject matter in this book develops logically and progresses from one topic to another with an extensive bibliography along with major primary references. These references will help readers in pursuing their areas of interest. In order to facilitate better understanding and easier reading, this book also contains a large number of figures and line diagrams of signal transduction pathways. This book fills the gap between basic science and

clinical studies and provides the reader with the skills to apply basic science to clinical settings of chronic diseases associated with oxidative stress.

This book is essential reading material for a broad range of individuals, including researchers, clinicians, graduate and medical students, as well as the many health-conscious individuals who wish to know more about the emerging field of oxidative stress in vertebrate and invertebrate systems. It can be used as a supplemental text for a range of biology courses. It is anticipated that senior neuroscientists may also find some inspiration from this book to overcome problems encountered in their research on oxidative stress in vertebrate and invertebrate systems.

GRACE Y. SUN

*Department of Biochemistry*
*Department of Pathology and Anatomical Sciences, and*
*Department of Nutritional Sciences*
*University of Missouri*
*Columbia, MO*

# ACKNOWLEDGMENTS

We express our deepest appreciation in acknowledging our teachers for their excellent teaching, guidance, inspiration, and influence on our lives. We also thank all authors for sharing their expertise by contributing chapters of high standard, thus making our editorial task much easier. We are grateful for the cooperation and patience of Dr. Karen E. Chambers and Anna Ehler at John Wiley & Sons for helpful discussions and advice during compilation of this book. We are also thankful to Kris Parrish at Wiley and Shanmuga Priya at Macmillan Publishing Solutions for handling the production process in a most efficient, cooperative, and remarkable manner.

TAHIRA FAROOQUI
AKHLAQ A. FAROOQUI

# CONTRIBUTORS


**Hiromi Akanuma**, *National Institute for Environmental Studies, Tsukuba, Ibaraki, Japan*

**Danielle Anderson**, *Aging-Osteopathic Consortium, Department of Basic Sciences, College of Osteopathic Medicine, Touro University-California, Vallejo, CA, USA*

**Michael E. Andrades**, *Postgraduate Program in Cardiology, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil*

**Kandangath Raghavan Anilakumar**, *Biochemistry & Nutrition Discipline, Defence Food Research Laboratory, Mysore, India*

**Amarinder Singh Bawa**, *Biochemistry & Nutrition Discipline, Defence Food Research Laboratory, Mysore, India*

**Marla J. Berry**, *Department of Cell and Molecular Biology, University of Hawaii, Biomedical Sciences Building, Honolulu, HI, USA*

**Ethan Bier**, *Section of Cell and Developmental Biology, University of California San Diego, La Jolla, California, USA*

**Dirk Bohmann**, *University of Rochester Medical Center, Department of Biomedical Genetics, Rochester, NY, USA*

**Kelly Cabana**, *Aging-Osteopathic Consortium, Department of Basic Sciences, College of Osteopathic Medicine, Touro University-California, Vallejo, CA, USA*

**Fabrice Durand**, *Laboratoire d'Ecotoxicologie—Milieux Aquatiques EA, Université du Havre, Le Havre Cedex, France*

**Anne Eckert**, *Neurobiology Laboratory for Brain Aging and Mental Health, Psychiatric University Clinics, University of Basel, Basel, Switzerland*

**Akhlaq A. Farooqui**, *Department of Molecular and Cellular Biochemistry, The Ohio State University, Columbus, Ohio, USA*

**Tahira Farooqui**, *Department of Entomology/Center of Molecular Neurobiology, The Ohio State University, Columbus, Ohio, USA*

**Louisa R. Gaifullina**, *Institute of Biochemistry and Genetics, Ufa Science Center RAS, Ufa, Russia*

**Doyle Graham**, *Duke-NUS Graduate Medical School, Singapore*

**Amandine Grimm**, *Neurobiology Laboratory for Brain Aging and Mental Health, Psychiatric University Clinics, University of Basel, Basel, Switzerland*

**Tommaso Iannitti**, *Department of Biological and Biomedical Sciences, Glasgow Caledonian University, Glasgow, UK*

**Klaudia Jomova**, *Department of Chemistry, Faculty of Natural Sciences, Constantine The Philosopher University, Nitra, Slovakia*

**Farhath Khanum**, *Biochemistry & Nutrition Discipline, Defence Food Research Laboratory, Mysore, India*

**Dalibor Kodrík**, *Institute of Entomology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic*

**Natraj Krishnan**, *Department of Zoology, Oregon State University, Corvallis, OR, USA and Department of Biochemistry, Molecular Biology, Entomology and*

*Plant Pathology, Mississippi State University, Starkville, MS, USA*

**Jun Hee Lee**, *Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, Michigan, USA*

**François Leboulenger**, *Laboratoire d'Ecotoxicologie—Milieux Aquatiques EA, Université du Havre, Le Havre Cedex, France*

**Julie Letendre**, *Laboratoire d'Ecotoxicologie—Milieux Aquatiques EA, Université du Havre, Le Havre Cedex, France*

**Kah-Leong Lim**, *Department of Physiology, National University of Singapore, Singapore; Duke-NUS Graduate Medical School, Singapore; Neuroscience Research Partnership, A*STAR, Singapore; and National Neuroscience Institute, Singapore*

**Rodrigo Lorenzi**, *Faculté de Médecine, Lille 2 University, Lille, France*

**Yi-Shing Ma**, *Department of Biochemistry and Molecular Biology, School of Life Sciences, National Yang-Ming University, Taipei, Taiwan*

**Ayikoe Guy Mensah-Nyagan**, *Equipe Stéroïdes, Neuromodulateurs et Neuropathologies, Universitéde Strasbourg, Strasbourg, France*

**Masanobu Morita**, *Department of Medical Biochemistry, Tohoku University Graduate School of Medicine, Aoba-ku, Sendai, Japan*

**Hozumi Motohashi**, *Center for Radioisotope Sciences, Tohoku University Graduate School of Medicine, Aoba-ku, Sendai, Japan*

**Shin Murakami**, *Aging-Osteopathic Consortium, Department of Basic Sciences, College of Osteopathic Medicine, Touro University-California, Vallejo, CA, USA*

**Xiao-Hui Ng**, *Department of Physiology, National University of Singapore, Singapore*

**Alexey G. Nikolenko**, *Institute of Biochemistry and Genetics, Ufa Science Center RAS, Ufa, Russia*

**Kanti Bhooshan Pandey**, *Department of Biochemistry, University of Allahabad, Allahabad, India*

**Beniamino Palmieri**, *Department of General Surgery and Surgical Specialties, Medical School and Surgical Clinic, University of Modena and Reggio Emilia, Modena, Italy*

**Giulio Maria Pasinetti**, *Department of Neurology, The Mount Sinai School of Medicine, New York, USA*

**M. Mahidur Rahman**, *University of Rochester Medical Center, Department of Biomedical Genetics, Rochester, NY, USA*

**Kuntol Rakshit**, *Department of Zoology, Oregon State University, Corvallis, OR, USA*

**Arjun V. Raman**, *Department of Cell and Molecular Biology, University of Hawaii, Biomedical Sciences Building, Honolulu, HI, USA*

**Syed Ibrahim Rizvi**, *Department of Biochemistry, University of Allahabad, Allahabad, India*

**Thomas Roeder**, *Christian Albrechts Universitaet zu Kiel, Zoophysiology II, Kiel, Germany*

**Elena S. Saltykova**, *Institute of Biochemistry and Genetics, Ufa Science Center RAS, Ufa, Russia*

**Jan Škrha**, *Laboratory for Endocrinology and Metabolism and 3rd Department of Internal Medicine, 1st Faculty of Medicine, Charles University in Prague, U Nemocnice, Prague, Czech Republic*

**Hideko Sone**, *National Institute for Environmental Studies, Tsukuba, Ibaraki, Japan*

**Gerasimos P. Sykiotis**, *University of Patras Medical School, Department of Internal Medicine, Division of Endocrinology and Metabolism, Patras, Greece*

**Philippe Taupin**, *School of Biotechnology, Dublin City University, Glasnevin, Dublin, Ireland*

**Marian Valko**, *Department of Chemistry, Faculty of Natural Sciences, Constantine The Philosopher University, Nitra, Slovakia and Faculty of Chemical and Food Technology, Slovak Technical University, Bratislava, Slovakia*

**Yau-Huei Wei**, *Department of Biochemistry and Molecular Biology, School of Life Sciences, National Yang-Ming University, Taipei, Taiwan and Department of Medicine, Mackay Medical College, New Taipei City, Taiwan*

**Shi-Bei Wu**, *Department of Biochemistry and Molecular Biology, School of Life Sciences, National Yang-Ming University, Taipei, Taiwan*

**Yu-Ting Wu**, *Department of Biochemistry and Molecular Biology, School of Life Sciences, National Yang-Ming University, Taipei, Taiwan*

# PART I

# OXIDATIVE STRESS IN VERTEBRATES

# 1

# GENERATION OF REACTIVE OXYGEN SPECIES IN THE BRAIN: SIGNALING FOR NEURAL CELL SURVIVAL OR SUICIDE

Akhlaq A. Farooqui

*Department of Molecular and Cellular Biochemistry, The Ohio State University, Columbus, Ohio, USA*

## 1.1 INTRODUCTION

Oxidative stress is a redox-sensitive process that occurs in the cell when antioxidant mechanisms are overwhelmed by the generation of reactive oxygen species (ROS), leading to oxidation of lipids, proteins, and DNA in ways that impair cellular function [1]. Thus oxidative stress is a threshold phenomenon characterized by a major increase in the amount of oxidized cellular components. ROS include superoxide anions, hydroxyl, alkoxyl, and peroxyl radicals, and hydrogen peroxide, which are generated as by-products of oxidative metabolism, in which energy activation and electron reduction are involved. The chemical reactivity of ROS varies from the very toxic hydroxyl ($^{\bullet}OH^{-}$) to the less reactive superoxide radical ($O_2^{\bullet -}$). The initial product, $O_2^{\bullet -}$, results from the addition of a single electron to molecular oxygen. $O_2^{\bullet -}$, is rapidly dismutated by superoxide dismutase (SOD), yielding $H_2O_2$ and $O_2$, which can be reused to generate superoxide radical. In the presence of reduced transition metals, $H_2O_2$, although less reactive than $O_2^{\bullet -}$, and highly diffusible, can be converted into the highly reactive hydroxyl radical $HO^{\bullet}$. The tight regulation of ROS generation and removal makes fluctuations in their levels transient, a feature that is characteristic of second messengers. ROS may also act as an intracellular "rheostat," closely modulating the activity of a discrete set of biochemical reactions, which contribute to cell proliferation, migration, and survival [2]. ROS not only inactivate membrane proteins and DNA but also promote peroxidation of neural membrane polyunsaturated fatty acids associated with glycerophospholipids, enhance levels of ceramide, and facilitate the formation of hydroxyl/ketocholesterol levels (Fig. 1.1). These processes promote neurodegeneration through apoptosis [3–5]. The polyunsaturated fatty acids, which are located at the *sn*-2 position of glycerol moiety in the glycerophospholipid, are most susceptible to free radical attack at the $\alpha$-methylene carbon in the alkyl chain of the fatty acid that is adjacent to the carbon-carbon double bond. Under aerobic conditions a polyunsaturated fatty acid with an unpaired electron undergoes a molecular rearrangement by reaction with $O_2$ to generate a peroxyl radical. The peroxyl radical captures hydrogen atoms from the adjacent fatty acids to form a lipid hydroperoxide. The lipid hydroperoxides thus formed are not completely stable in vivo and, in the presence of iron, can further break down to radicals that can propagate the chain reactions started by an initial free radical attack. The major sources of ROS are the mitochondrial respiratory chain, where $O_2^{\bullet -}$, is generated by electron leakage from complexes I and III of the electron transport chain (Fig. 1.2) [6, 7]. Microsomes and peroxisomes are also sources of ROS, primarily $H_2O_2$, whereas immune cells such as neutrophils and macrophages possess oxygen-dependent mechanisms to fight against invading microorganisms. Enzymes, such as xanthine/xanthine oxidase, myeloperoxidase, cytochrome *P*450 in cell cytoplasm,

*Oxidative Stress in Vertebrates and Invertebrates: Molecular Aspects of Cell Signaling*, First Edition.
Edited by Tahira Farooqui and Akhlaq A. Farooqui.

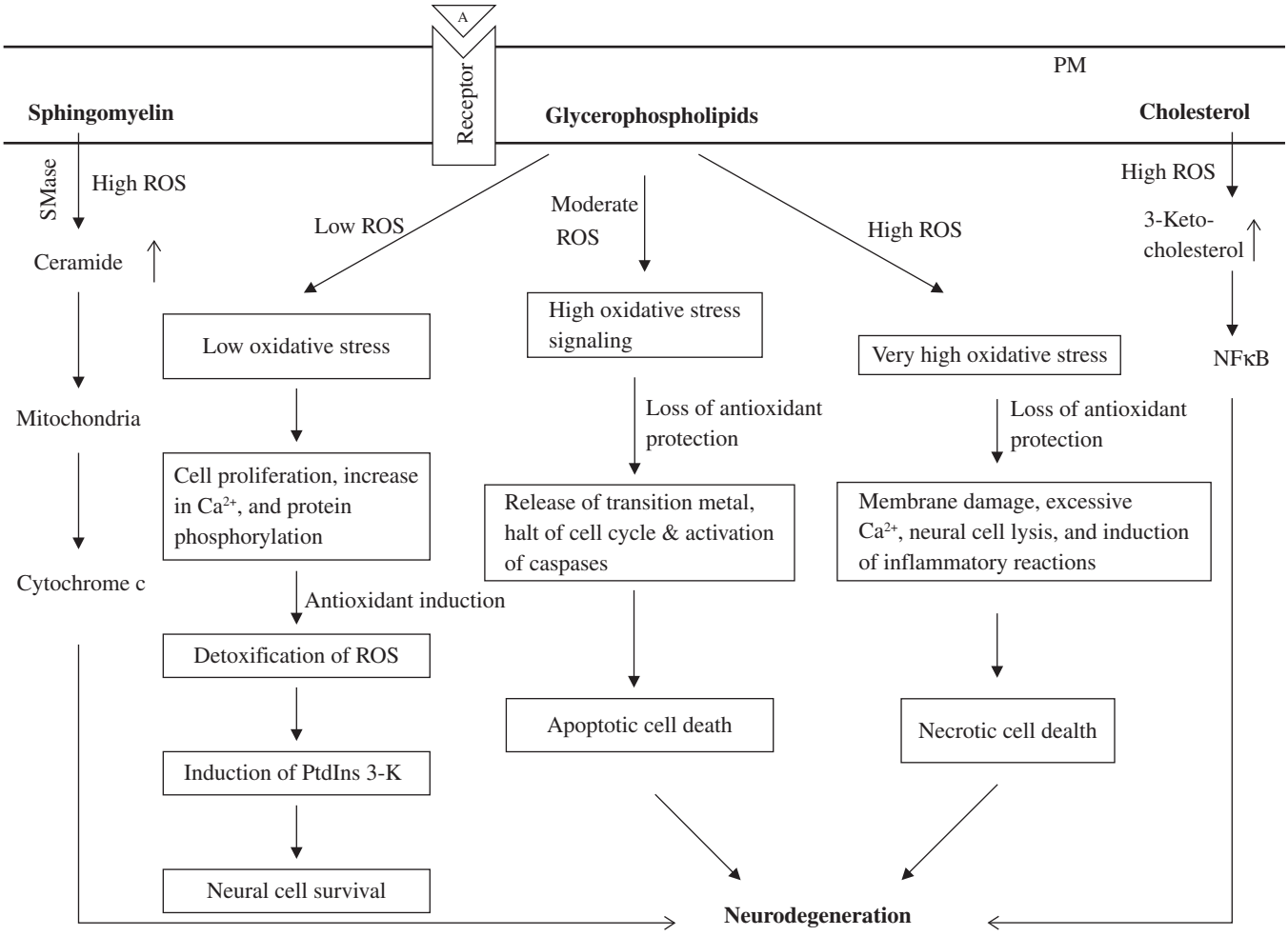



**Fig. 1.1** Effect of reactive oxygen species (ROS) on lipid constituents (glycerophospholipid, sphingolipid, and cholesterol) of neural membranes. Low ROS levels promote neural cell survival, whereas high ROS levels promote neurodegeneration through apoptotic and necrotic cell death. PM, plasma membrane; NF-κB, nuclear factor-κB; SMase, sphingomyelinases.

COX, LOX, nitric oxide synthase, and NADPH oxidase contribute to ROS production in plasma membranes and mitochondria (Fig. 1.2). The presence of redox-active metals, such as iron and copper, also contributes to ROS generation. In the presence of $Fe^{2+}$ and $Fe^{3+}$, $HO^{\bullet}$ can be generated through the Fenton reaction or the Haber–Weiss reaction [7].

## 1.2 ROLE OF REACTIVE OXYGEN SPECIES IN NEURAL CELLS

As stated above, in brain ROS are generated during oxidative metabolism. ROS-mediated damage to neural membranes is accompanied by (a) changes in physicochemical properties of neural membranes (microviscosity and fluidity) not only resulting in exchange of phospholipids between the two halves of the lipid bilayer but also altering the orientation of optimal domains of receptors, enzymes, and ion channels; (b) changes in the number of receptors and their affinity for neurotransmitters; and (c) inhibition of ion pump operation and entry of $K^+$ and $Ca^{2+}$ into neural cells resulting in changes in ion homeostasis. The presence of peroxidized glycerophospholipids in neural membranes may also induce a membrane-packing defect, making the *sn*-2 ester bond more accessible to the action of phospholipase $A_2$ ($PLA_2$) and the release of free arachidonic acid (ARA) or docosahexaenoic acid (DHA). ARA and DHA act as substrates for the synthesis of eicosanoids and docosanoids, respectively [3]. Lyso-phospholipid, the other product of $PLA_2$-catalyzed reaction, not only induces detergent-like effects leading to further disorganization of neural membranes but also acts as substrate for platelet-activating factor (PAF) [8]. Emerging evidence suggests that enzymic and nonenzymic oxidation of polyunsaturated fatty acids

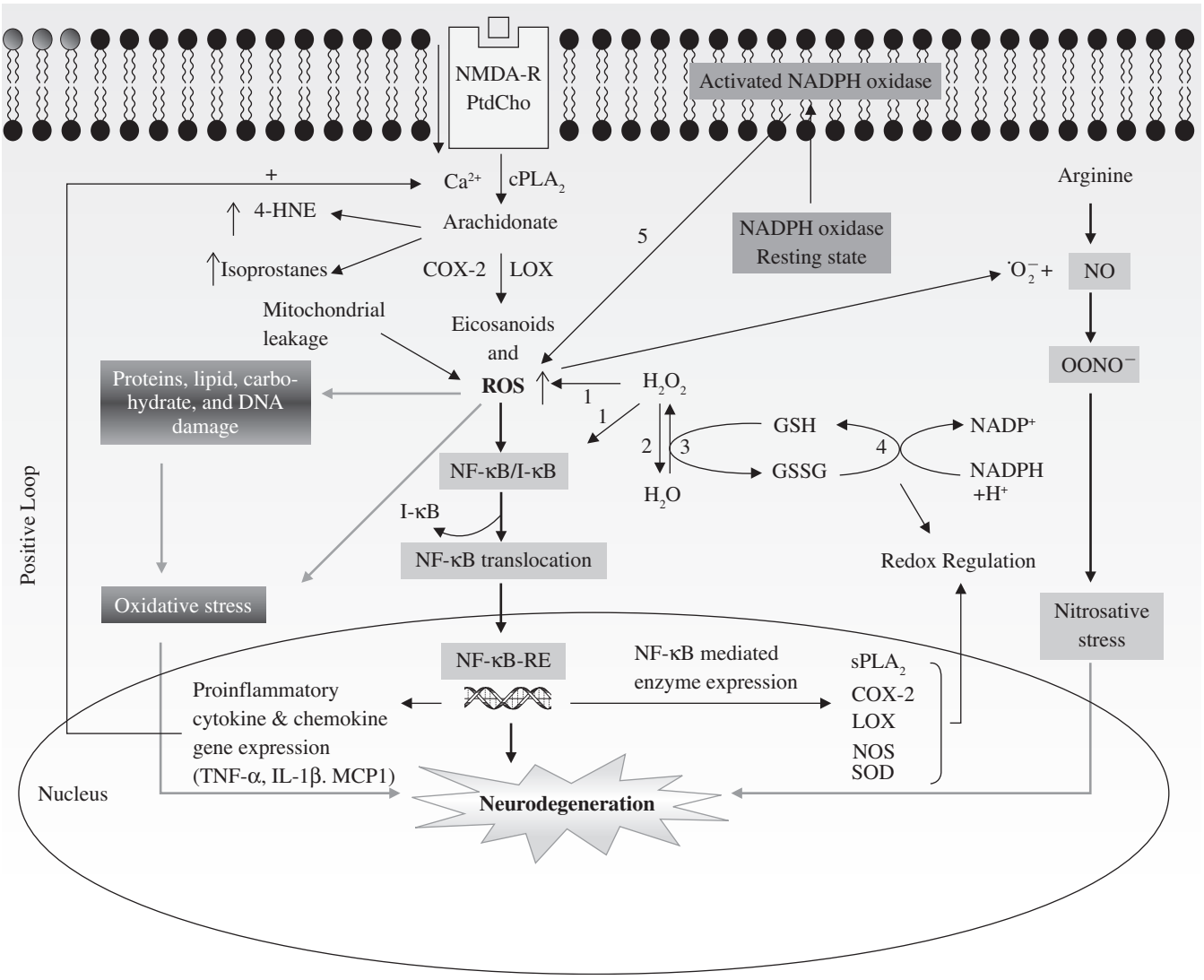



**Fig. 1.2** Generation of reactive oxygen species (ROS) and enzymic and nonenzymic markers for oxidative stress. 1, Superoxide dismutase (SOD); 2, catalase; 3, glutathione peroxidase; 4, glutathione reductase; 5, NADPH oxidase. $cPLA_2$, cytosolic phospholipase $A_2$; $sPLA_2$, secretory phospholipase $A_2$; COX-2, cyclooxygenase-2; LOX, lipoxygenase; NOS, nitric oxide synthase; GSH, reduced glutathione; GSSG, oxidized glutathione; $H_2O_2$, hydrogen peroxide; 4-HNE, 4-hydroxynonenal; NO, nitric oxide; $OONO^-$, peroxynitrite. Activation of NF-κB by ROS leads to its translocation to the nucleus, where it facilitates the transcription of proinflammatory enzymes ($sPLA_2$, COX-2, NOS, and SOD) and proinflammatory cytokines (TNF-α and IL-1β). These cytokines upregulate activities of $cPLA_2$ and $sPLA_2$ through a positive loop mechanism in cytoplasm and neural membranes. Upward arrows indicate increase in levels of metabolites. *(See color insert.)*

leads to the formation and accumulation of ARA-derived eicosanoids, 4-hydroxy-2-nonenal (4-HNE), isoprostanes, isofurans, and isoketals and DHA-derived docosanoids, 4-hydroxyhexanal, neuroprostanes, neurofurans, and neuroketals that induce specific cellular dysfunction [3, 5, 9]. In addition, lipid peroxidation also leads to the generation of lipid hydroperoxides, which inhibit the reacylation of phospholipids in neuronal membranes [10]. The detoxification of glycerophospholipid hydroperoxides is accomplished through the combined enzymic activity of $PLA_2$ and reduction of the released fatty acid hydroperoxides with phospholipid hydroperoxide glutathione peroxidase [11–13]. The latter enzyme not only acts on membranes and reduces glycerophospholipid hydroperoxides to the nontoxic hydroxyl derivatives [14, 15] but reduces $H_2O_2$ to water to limit its harmful effects. Phospholipid hydroperoxide glutathione peroxidase is different from the classic glutathione

peroxidase, which mainly reduces $H_2O_2$. The restoration of neural membrane integrity by the reaction catalyzed by phospholipid hydroperoxide glutathione peroxidase is achieved by the reinsertion of nonoxidized fatty acyl groups through the involvement of the deacylation/reacylation cycle [16]. Nonenzymically, ROS buildup can be prevented by vitamins E and C. These vitamins terminate lipid chain reactions involving peroxyl radicals. In addition to being a cofactor of various antioxidant enzymes, GSH, which is the most abundant peptide in cells, performs many functions. The thioredoxin system is another important thiol antioxidant system consisting of thioredoxin (Trx) and thioredoxin reductase. Trx is a multifunctional selenoprotein containing two redox-active cysteines and a conserved active site (Cys-Gly-Pro-Cys) [17, 18]. Although many ROS are quenched by GSH, other thiol-containing proteins also participate in neutralizing ROS [19].

### 1.2.1 Modulation of Enzyme Activities, Transcription Factors, and Genes by ROS

ROS regulate activities of several enzymes in neural cells. Thus ROS not only modulate activities of protein tyrosine kinases, protein phosphatases, and mitogen-activated protein kinases [extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK)/stress-activated protein kinase, and p38 pathway] (Fig. 1.3) but also play an important role in regulating intracellular $Ca^{2+}$ homeostasis and RhoA/Rho kinase signaling [20]. Low and moderate levels of ROS activate PtdIns 3-kinase signaling and promote cell survival. PtdIns 3-kinase/protein kinase B (Akt) transduces the signal for cell survival mainly through phosphorylation of target molecules by Akt. This results in the inactivation of proapoptotic proteins and activation of transcription factors that target the expression of antiapoptotic proteins. ROS increase vascular $[Ca^{2+}]_i$ by stimulating inositol trisphosphate-mediated $Ca^{2+}$ mobilization, by increasing cytosolic $Ca^{2+}$ accumulation through sarcoplasmic/endoplasmic reticulum $Ca^{2+}$-ATPase inhibition, and by stimulating $Ca^{2+}$ influx through $Ca^{2+}$ channels. Increased ROS production enhances $Ca^{2+}$ signaling and upregulates RhoA/Rho kinase, thereby altering vascular contractility and tone in the vasculature [21]. ROS also inactivate protein tyrosine phosphatases in a dose- and time-dependent manner [22, 23]. ROS activate metalloproteinases, and this stimulation is blocked by *N*-acetyl cysteine [24]. In addition, ROS modulate transcription factors (NF-κB, HIF, CREB, AP-1, ATF2, A-1, CHOP-1, and E2F), modulate the cell cycle, and ion transport (Fig. 1.3). Although the molecular mechanisms underlying ROS-mediated alterations of kinases and transcription factors are not fully understood, it is becoming increasingly evident that the regulation of stress-responsive proteins by ROS may be closely associated with the above alterations. ROS-mediated cellular changes involve (a) the direct effect of ROS on the kinase or transcription factor, which can alter conformation and activity, and (b) the effect of cysteine-rich, redox-sensitive proteins, which are associated with the regulation of stress-responsive proteins. Oxidative stress not only produces conformational changes in redox-responsive proteins but also facilitates the generation of dimers/multimers of these proteins. The redox-responsive proteins include thioredoxin and glutathione *S*-transferase [25]. Emerging evidence suggests that low levels of ROS induce minor changes in levels of $Ca^{2+}$, enzyme activities, transcription factors, cell cycle, and ion transporters, which support and maintain normal cell function through the tight regulation of diverse intracellular signaling networks. However, moderate and high levels of ROS can inflict damage to all subcellular organelles (e.g., mitochondria, endoplasmic reticulum, etc.), eventually leading to cell death.

The brain processes large amounts of $O_2$ in relatively small mass and has a high content of substrates available for oxidation in conjunction with low antioxidant activities making polyunsaturated fatty acids found in glycerophospholipids extremely susceptible to oxidative damage. In addition, neurons of certain regions of the brain, such as the hippocampus, may be particularly vulnerable to oxidative stress because of their low endogenous levels of vitamin E and glutathione relative to other brain regions. Such a depressed defense system may be adequate under normal circumstances. However, generation of high levels of ROS following acute neural trauma (stroke, spinal cord trauma, and traumatic brain injury) and neurodegenerative diseases, such as Alzheimer disease (AD), Parkinson disease (PD), and amyotrophic lateral sclerosis (ALS), and low antioxidant defenses can predispose the brain to high oxidative stress leading to neuronal injury and death [3].

### 1.2.2 Modulation of Genes by ROS

In nonneural cells, ROS regulate many genes, including adhesion molecules and chemotactic factors, antioxidant enzymes, and vasoactive substances [2]. Some of these genes are associated with adaptive responses. This includes the induction of superoxide dismutase (SOD), catalase, and glutathione peroxidase (Gpx) by $H_2O_2$, supporting the view that newly synthesized protective proteins are needed for adaptive responses [26, 27]. Most redox-sensitive genes have been identified on the basis of their responsiveness to externally applied oxidant stress; only a few have been shown to be

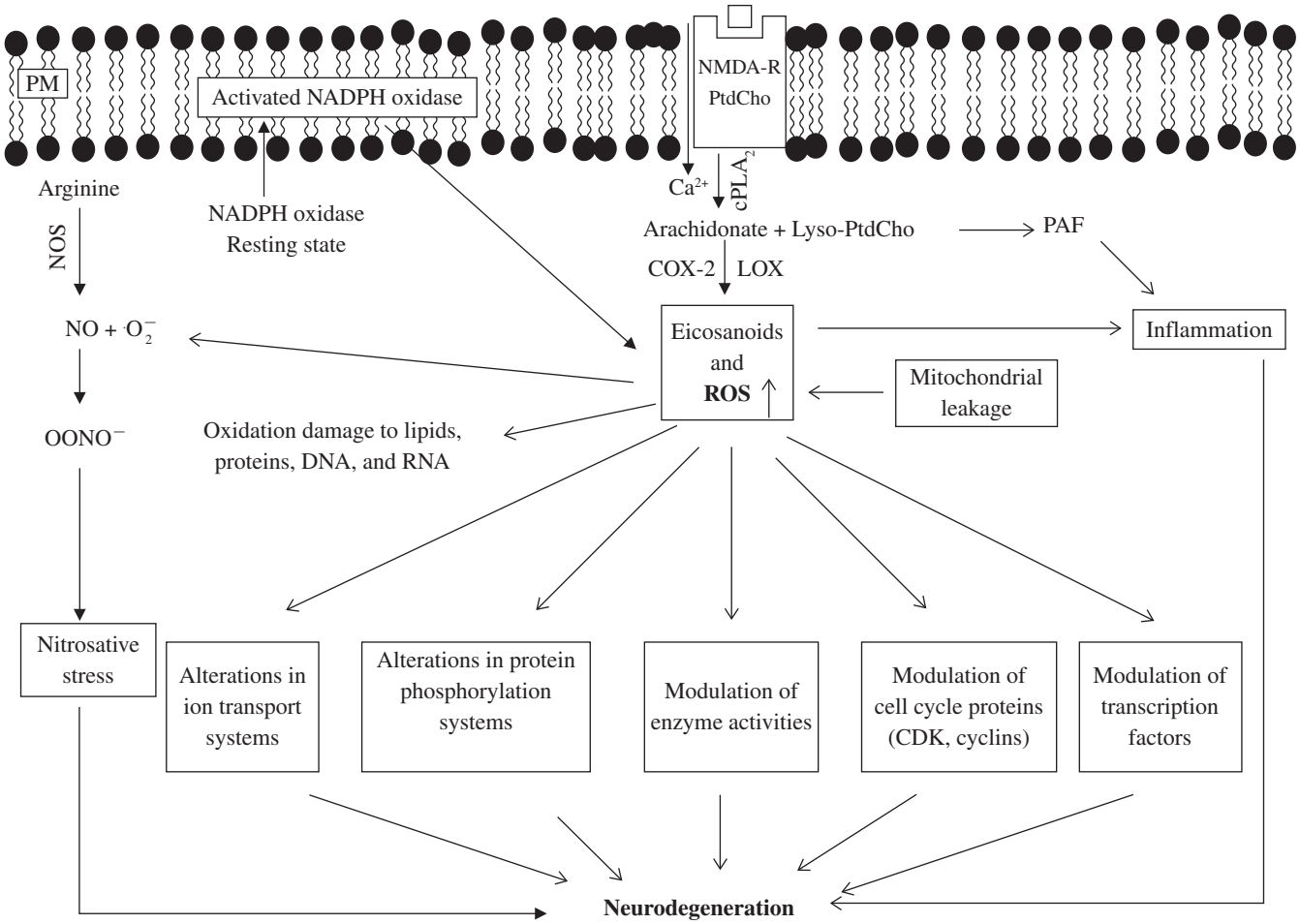



**Fig. 1.3** Roles of reactive oxygen species (ROS) in the brain. $cPLA_2$, cytosolic phospholipase $A_2$; COX-2, cyclooxygenase-2; LOX, lipoxygenase; NOS, nitric oxide synthase; NO, nitric oxide; $OONO^-$, peroxynitrite; PAF, platelet-activated factor. In brain, ROS are generated through mitochondrial dysfunction, ARA oxidation, and NADPH oxidase activation. Low ROS levels modulate ion transport, protein phosphorylation, enzymic activities, cell cycle, and transcription factors, but high ROS levels damage neural membrane lipids, protein, and nucleic acid.

downstream of an endogenous source of ROS, such as the NADPH oxidase. These include TNF-α- and lactosylceramide-mediated induction of intercellular adhesion molecule (ICAM-1) [28] and Ang II, PDGF, and TNF-α stimulation of monocyte chemotactic protein (MCP)-1 [29]. In contrast, stimulation of MCP-1 by IL-1β in vascular smooth muscle cells (VSMCs) is not affected by antioxidants, suggesting that the control of gene expression by ROS is both stimulus- and tissue specific [30]. Neural and nonneural cells possess signaling pathways that can sense oxidative stress and launch adaptive responses that bolster the antioxidant defense networks. Accumulating evidence suggests that modulation of gene expression by ROS occurs at cellular and subcellular levels. Low ROS levels involve modulation of some neuroprotective genes along with redox-sensitive transcription factors (AP-1 and Nrf2). These factors modulate genes for antioxidant response element (ARE), endogenous antioxidants, phase II detoxifying enzymes, and transporters. Nrf2 is a transcription factor that regulates the basal and inducible expression of a wide array of antioxidant genes. After phosphorylation and dissociation from the cytosolic protein Keap1, a scaffolding protein that binds Nrf2 and Cul3 ubiquitin ligase for proteasomal degradation, Nrf2 rapidly translocates to the nucleus, where it activates the ARE in the promoter region of many antioxidant genes [31]. Nrf2 activates transcription primarily through the formation of a dimer with a small musculoaponeurotic fibrosarcoma oncogene family of proteins (Maf) [32, 33]. The binding

of the small Maf-Nrf2 dimers to ARE sequences leads to a coordinated transcriptional activation of a battery of antioxidant enzymes and detoxifying proteins. This regulated adaptive response is called the "phase II detoxification response" [34]. Activation of Nrf2 not only increases the abundance of thioredoxins and glutathione-synthesizing enzymes and glutathione *S*-transferases but also enhances the expression of molecular chaperones, proteasome subunits, and various other cytoprotective proteins [35]. Expression of the Nrf2-dependent proteins is critical to maintaining cellular redox homeostasis through elimination of toxins [2]. Modulation of other genes involves translocation of a specific transcription factor NF-κB, which facilitates expression of proinflammatory enzymes, chemokines, and cytokines. The mechanism by which cytokines (TNF-α) induce neurodegeneration appears to be related not only to the depletion of GSH but also to the redox-dependent generation of ceramide from sphingomyelin, formation of 4-HNE and isoprostane from membrane glycerophospholipids, and generation of hydroxyl- and ketocholesterol from cholesterol [5, 6]. Hydroperoxy fatty acids and $H_2O_2$ promote the expression of c-Fos and Jun 2 proteins that form heterodimers and activate AP-1 [36]. Activation of nitric oxide synthase (NOS) during oxidative stress generates $NO^{\bullet}$, an important signaling molecular and vasodilator. $NO^{\bullet}$ increases the transcription of Iκ-B, the inhibitory factor that binds NF-κB and facilitates its retention in the cytoplasm [37]. The turnover of I-κB protein is also oxidant sensitive, and antioxidants can block agonist-mediated stimulation of I-κB phosphorylation and degradation [2, 37]. Conversely, $H_2O_2$ increases translocation of NF-κB to the nucleus, where it facilitates the transcription of responsive genes [38]. In addition, several other mammalian transcription factors are directly modified by ROS or by reducing proteins that modify cysteine residues involved in DNA binding [2]. These transcription factors include AP-1, NF-κB, and hypoxia-inducible factor-1 (HIF-1) [2, 39, 40]. Both c-Fos and c-Jun contain a conserved cysteine in a basic motif that, when oxidized, interferes with the interaction of these proteins with AP-1 consensus sequences. Conversely, if c-Fos/c-Jun heterodimers are complexed with AP-1, they cannot be oxidized [39]. The oxidation state of these important proteins is modulated by redox factor-1 [1], a protein that, in cooperation with thioredoxin, facilitates the cycling of the critical cysteines between reduced and oxidized forms [2, 39]. Thioredoxin also modulates HIF-1-dependent transcription [40] and modifies the DNA binding and transcriptional activity of NF-κB by reducing cysteine 62 [41]. Collectively, these studies indicate the importance of the nuclear redox state in regulating ROS-mediated gene expression [2].

### 1.2.3 Modulation of Long-Term Potentiation, Cognition, and Memory Formation by ROS

It is well known that hippocampus is involved in synaptic plasticity associated with cognitive function and learning and memory. This region is highly susceptible to oxidative stress [42]. Long-term potentiation (LTP) is defined as a long-lasting increase in synaptic efficacy following high-frequency stimulation of afferent fibers. Treatment of hippocampal slices with $H_2O_2$ at millimolar concentrations produces oxidative stress [43] and inhibition of LTP, whereas micromolar concentrations of $H_2O_2$ enhance LTP [44, 45]. The action of $H_2O_2$ is mediated through the release of calcium ions from internal stores, modulating the activity of specific calcium-dependent protein phosphatases, $PLA_2$, and phospholipase C (PLC). These enzymes modulate synaptic plasticity. The above observations are supported by studies in aged mice overexpressing extracellular SOD. These mice perform better in a water maze memory task than aged control mice [46]. It is also reported that an increase in SOD activity, which impairs LTP, is caused by a secondary increase in $H_2O_2$ levels and catalase reverses the effects of SOD. The molecular mechanism associated with $H_2O_2$-mediated impairment of LTP is not fully understood. However, it is becoming increasingly evident that serine/threonine phosphatases (PP2A) contribute to the impairment of LTP [45, 47]. The ketogenic diet (high-fat and low-carbohydrate with anticonvulsant), which induces ketonemia, not only downregulates PP2A activity and expression of this enzyme but also prevents oxidative stress-mediated impairment of LTP by inhibiting PP2A [48–50]. It is proposed that oxidative stress-mediated impairment of hippocampal LTP is associated with low levels of ROS production, changes in synaptic plasticity, and activation of PP2A, and that ketone bodies prevent this impairment of LTP through the inhibition of PP2A. The regulation of synaptic activity by ROS is not confined to hippocampal synapses. A series of studies have indicated that $H_2O_2$ modulates dopamine release in dorsal striatum through a ROS sensor on potassium channels that control the excitability of the dopamine-releasing neurons [51]. Emerging evidence suggests that the signal transduction network associated with synaptic plasticity involves many players, including protein kinases, phosphatases, phospholipases, transcription factors, and other $Ca^{2+}$-dependent enzymes [52], which contribute to the generation of ROS.

### 1.2.4 Modulation of Cell Death by ROS

As mentioned above, the brain consumes large quantities of oxygen relative to its contribution to total body

mass. This, together with low levels of vitamin E, glutathione, and lipoic acid and low activities of SOD, catalase, and peroxidase, places the brain at the risk for damage mediated by ROS [6]. ROS generation through mitochondrial dysfunction gradually disrupts the intracellular calcium homeostasis, which modulates neuronal excitability and synaptic transmission, making neurons more vulnerable to additional oxidative stress, and leads to neurodegeneration. Among neural membrane components lipids are most susceptible to oxidative modification. Lipid peroxidation produces lipid radicals, which can further attack the subsequent lipid molecules and propagate through a chain reaction. Lipid peroxidation leads to the formation of a number of aldehyde byproducts, including malondialdehyde (MDA), 4-HNE, and acrolein. The most abundant aldehydes are 4-HNE and MDA, while acrolein is the most reactive. In addition, stimulation of $PLA_2$s, sphingomyelinases, and cytochrome *P*450 hydroxylases produces high levels of glycerophospholipid-, sphingolipid-, and cholesterol-derived lipid mediators, which support inflammatory processes leading to neural cell death in the brain [5, 6]. The highly reactive $OH^{\bullet}$, generated through the Fenton reaction, and $ONOO^{-}$ formed from the reaction between $O_2^{\bullet -}$ and nitric oxide ($NO^{\bullet}$), target protein components of neural membranes. Irreversible protein oxidation includes nitrosylation of cysteine sulfhydryl groups, tyrosine, methionine, and tryptophan by $ONOO^{-}$. Nitration of tyrosine residues may inhibit its phosphorylation or adenylation, important for protein function [53]. Severe oxidative stress can induce disulfide bond-mediated protein cross-linkage or secondary oxidative modifications such as adduct formation between oxidized proteins and lipid peroxides or glycation products, leading to accumulation of damaged proteins and cell death [54]. Some protein modifications, such as phosphorylation, are reversible modifications that can be overcome by specific enzymes (protein phosphatases) that cause a protein to "revert" back to its original protein structure, while other protein modifications, such as protein nitration and HNE-mediated modification (4-HNE-histidine and glutathione-4-HNE Michael adducts), are irreversible. Oxidative modification of proteins may induce alterations in the structure of proteins with subsequent loss of normal physiological cell functions leading to cell death.

Compared with lipids and proteins, neural cell DNA is less susceptible to oxidative modifications because of its double-helix structure and the protective shield from histone and other coating proteins. However, under severe oxidative stress nuclear DNA damage is also oxidized with the generation of 8-hydroxy-2-deoxyguanosine (8-OHdG) [55]. Collective evidence suggests that ROS-mediated alterations in neural membrane components, generation of high levels of glycerophospholipid-, sphingolipid-, and cholesterol-derived lipid mediators, modifications of proteins and DNA, loss of $Ca^{2+}$ homeostasis, and induction of mitochondrial dysfunction may result in neural cell death.

## 1.3 ROS-MEDIATED SURVIVAL SIGNALING IN NEURAL CELLS

The cellular response to ROS depends not only on their concentration but also on their chemical nature. Low concentrations of ROS do not cause cell death but instead induce an adaptive and survival response to the oxidative stress through modulation of proliferation, synaptic plasticity, gene transcription, and neuronal excitability (Fig. 1.4). Adaptive and survival responses are modulated by cellular $Ca^{2+}$ gradient. In neural cells, maintenance of $Ca^{2+}$ gradients requires reduction in ATP level, which is associated with generation of ROS through respiratory control mechanisms. The selective oxidation of calmodulin (a $Ca^{2+}$ binding protein) and alteration in $Ca^{2+}$-ATPase (a $Ca^{2+}$-dependent enzyme associated with efflux of $Ca^{2+}$) activity during oxidative stress may represent an adaptive response to oxidative stress that functions to downregulate energy metabolism and the associated generation of ROS. During oxidative stress, enhanced sensitivity of $Ca^{2+}$ binding proteins is closely associated not only with modulation of signal transduction processes but also with intracellular energy metabolism, supporting the view that the selective oxidation of critical signal transduction proteins may represent a regulatory mechanism that functions to minimize the generation of ROS through respiratory control. Thus decrease in the rate of ROS formation, in turn, may promote cellular survival under conditions of low oxidative stress, when ROS overwhelm cellular antioxidant defense systems, by minimizing the nonselective oxidation of a range of lipid, proteins, and nucleic acids [56, 57]. In addition, ROS may function as signaling molecules that fine-tune neural cell metabolism through the selective oxidation of $Ca^{2+}$ binding proteins in order to minimize widespread oxidative damage and protein aggregation. Formation of low ROS levels also minimizes protein oxidation, which promotes intracellular repair mechanisms that function to eliminate damaged and partially unfolded proteins. Since the rates of protein repair or degradation compete with the rate of protein aggregation, the modulation of intracellular $Ca^{2+}$ concentrations and energy metabolism through the selective oxidation of critical signal transduction proteins ($Ca^{2+}$ binding proteins) maintains cellular function by minimizing protein aggregation [58]. Furthermore, ROS, specifically $H_2O_2$, are also essential for

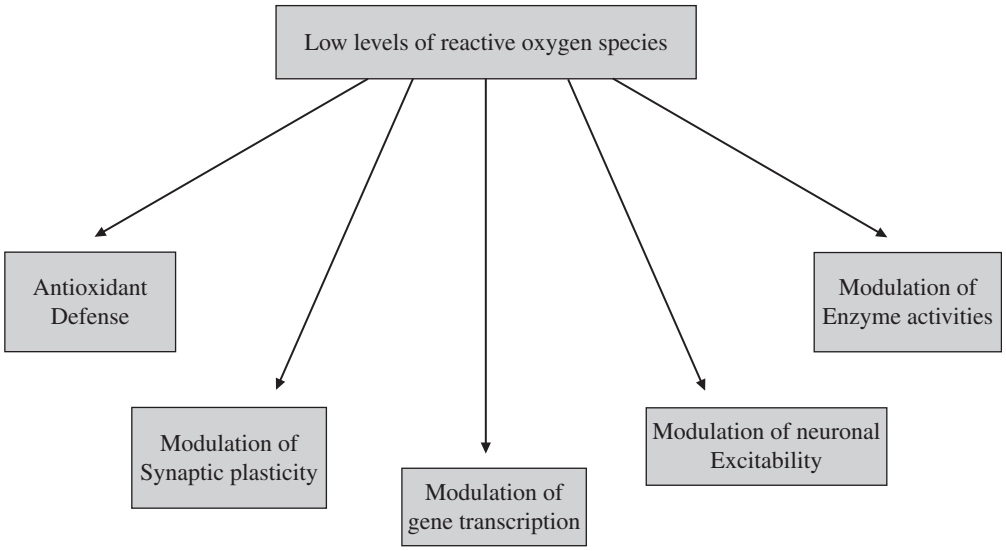



**Fig. 1.4** Modulation of neural cell survival by low levels of ROS generated during normal metabolic conditions.

growth factor-mediated signal transduction, mitochondrial function, and maintenance of normal thiol redox-balance. These processes are closely associated with neural cell survival.

ROS activate both acidic and basic sphingomyelinases (SMases). The activation of SMases results in generation of ceramide. Low levels of this lipid mediator stimulate and modulate signaling pathways involved in the regulation of cell viability, differentiation, growth, and survival [59]. However, excessively high levels of ceramide can trigger apoptosis through the release of cytochrome *c*. Ceramides generated in response to membrane-associated oxidative stress have been implicated in the dysfunction and death of cells in neurotraumatic and neurodegenerative diseases [5].

## 1.4 ROS-MEDIATED INJURY IN NEURAL CELLS

High ROS levels induce neurodegeneration through apoptotic and necrotic cell death. The reaction between high ROS levels and proteins leads to a chemical cross-linking of membrane proteins and phospholipids resulting in alterations in membrane-bound enzymes and reduction in membrane unsaturation [60]. Alterations in activities of membrane-bound enzymes and depletion of unsaturation in membrane lipids are associated not only with decrease in activities of membrane-bound enzymes but also with decreased membrane fluidity and altered activities of ion channels and receptors [6, 61]. Oxidative damage to cellular proteins along with the loss of calcium homeostasis contributes to protein aggregation and deposition, a process that occurs in neurodegenerative diseases [58]. In addition, high levels of 4-HNE generated during severe oxidative stress contribute to neurodegeneration by forming adducts with sulfhydryl groups (thiols) on proteins involved in neurotransmission [4, 62]. Moreover, in the presence of metal ions, such as $Fe^{2+}$ and $Cu^{2+}$, $H_2O_2$ can be further transformed into hydroxyl radical ($^{\bullet}OH$) through the Fenton reaction. Hydroxyl radicals can attack polyunsaturated fatty acids in membrane phospholipids, forming the peroxyl radical ($^{\bullet}ROO$), and then propagate the chain reaction of lipid peroxidation. Furthermore, high levels of ROS modulate the expression of genes responsible for modulating activities of cytokines and chemokines [3, 6]. Neurons are most susceptible to ROS-mediated oxidative injury. ROS also contribute to brain damage by activating a number of cellular pathways resulting in the expression of stress-sensitive genes and proteins associated with oxidative injury [63]. ROS-mediated injury to astrocytes induces apoptosis-like cell death through a caspase-3-independent mechanism [63]. In reactive microglia, activation of NADPH oxidase orchestrates the generation of superoxide, which is converted into $O_2$ and $H_2O_2$ by SOD.

Under severe oxidative stress, high levels of $NO^{\bullet}$ are formed through enzymic oxidation of L-arginine to citrulline. $O_2^{\bullet-}$ reacts with $NO^{\bullet}$ to form peroxynitrite ($ONOO^-$), a strong oxidant that can initiate lipid peroxidation and formation of nitrotyrosine in proteins (Fig. 1.5). This metabolite not only inhibits enzymes of the mitochondrial respiratory chain and inactivates glyceraldehyde-3-phosphate dehydrogenase, but also inhibits membrane $Na^+/K^+$-ATPase and inactivates sodium channels in the membrane [64]. In addition, *S*-nitrosylation or covalent reaction of NO with specific