Heat Shock Proteins 17
Series Editors: Alexzander A. A. Asea · Stuart K. Calderwood

Alexzander A. A. Asea
Punit Kaur *Editors*

Heat Shock Proteins in Signaling Pathways

Springer

Heat Shock Proteins

Volume 17

Series editors
Alexzander A. A. Asea
Professor, Department of Medicine and Director
Precision Therapeutics Proteogenomics Diagnostic Center
Eleanor N. Dana Cancer Center
University of Toledo College of Medicine and Life Sciences
Toledo, USA

Stuart K. Calderwood
Professor and Director, Division of Molecular and Cellular Radiation Oncology
Department of Radiation Oncology
Beth Israel Deaconess Medical Center and Harvard Medical School
Boston, USA

Heat Shock Proteins: key mediators of Health and Disease. Heat shock proteins (HSP) are essential molecules conserved through cellular evolution required for cells to survive the stresses encountered in the environment and in the tissues of the developing and aging organism. These proteins play the essential roles in stress of preventing the initiation of programmed cell death and repairing damage to the proteome permitting resumption of normal metabolism. Loss of the HSP is lethal either in the short-term in cases of acute stress or in the long-term when exposure to stress is chronic. Cells appear to walk a fine line in terms of HSP expression. If expression falls below a certain level, cells become sensitive to oxidative damage that influences aging and protein aggregation disease. If HSP levels rise above the normal range, inflammatory and oncogenic changes occur. It is becoming clear that HSP are emerging as remarkably versatile mediators of health and disease. The aim of this series of volumes is to examine how HSP regulation and expression become altered in pathological states and how this may be remedied by pharmacological and other interventions.

More information about this series at http://www.springer.com/series/7515

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Alexzander A. A. Asea
Professor, Department of Medicine and
Director, Precision Therapeutics
Proteogenomics Diagnostic Center
Eleanor N. Dana Cancer Center
University of Toledo College of Medicine
and Life Sciences
Toledo, OH, USA

Punit Kaur
Department of Experimental Radiation
Oncology
MD Anderson Cancer Center
Houston, TX, USA

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Preface

Signaling pathway is a comprehensive mechanism by which all cellular organisms communicate internally and externally with their microenvironment. This is a highly complex and exact process. Errors in signaling pathways and in the processing of cellular information are known to be responsible for the majority of diseases including cancer and inflammatory and neurological disorders. Knowledge gained from the better understanding of signaling pathways will help in elucidating disease processes and will assist in the development and design of novel targeted treatment therapies to combat human diseases and disorders. Heat shock proteins (HSP) are uniquely involved in a number of critical signaling pathways.

The book ***Heat Shock Proteins in Signaling Pathways*** provides the most comprehensive review on contemporary knowledge on the role of HSP in signaling pathways relevant to a number of diseases. Using an integrative approach, the contributors provide a synopsis of novel mechanisms, signal transduction pathways. To enhance the ease of reading and comprehension, this book has been subdivided into various sections: Section I reviews current progress on our understanding of inflammatory signaling pathways, Section II focuses on oncology signaling pathways, and Section III emphasizes neurological signaling pathways.

Key basic and clinical research laboratories from major universities, academic medical hospitals, and biotechnology and pharmaceutical laboratories around the world have contributed chapters that review present research activity and importantly project the field into the future. The book is a must-read for graduate students, medical students, basic science researchers, and postdoctoral scholars in the fields of Translational Medicine, Clinical Research, Human Physiology, Biotechnology, and Cell and Molecular Medicine and also for pharmaceutical scientists and researchers involved in drug discovery.

Toledo, OH, USA — Alexzander A. A. Asea
Houston, TX, USA — Punit Kaur

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About the Editors

Prof. Dr. Alexzander A. A. Asea is a highly innovative and accomplished world renowned clinical and basic research scientist and visionary executive leader who has exceptional experience spearheading clinical and basic science research, training, education, and commercialization initiatives within top ranked academic biomedical institutes. Prof. Dr. Asea's initial findings studying the effects of Hsp72 on human monocytes lead to the proposal of a novel paradigm that Hsp72, previously known to be an intracellular molecular chaperones, can be found in the extracellular milieu where it has regulatory effects on immuno-competent cells - a term now called chaperokine. Prof. Asea has authored over 255 scientific publications including peer-reviewed articles, reviews, books, book chapters, editorials, and news headliners in a wide range of biomedical-related disciplines. Prof. Asea is the series editor of the widely successful book series *Heat Shock Proteins* (Springer Nature Publishing) and is an editorial board member of numerous scientific peer-reviewed journals. Currently, Prof. Dr. Asea is at the University of Toledo College of Medicine and Life Sciences in Toledo, USA.

Dr. Punit Kaur is an expert in onco-proteogenomics, with extensive training and experience in quantitative mass spectrometry imaging, protein chemistry and biomarker discovery. Her main research focus is on the use of heat-induced nanotechnology in combination with radiotherapy and chemotherapy in the cancer stem cell therapy. She has published more than 40 scientific articles, book chapters, and reviews, and currently serves as editorial board member for the *European Journal of Cancer Prevention* and the *Journal of Proteomics and Bioinformatics*. She is an editor of eight books in the highly successful *Heat Shock Proteins* book series by Springer Nature Publishers. Currently, she is a Visiting Scientist Professor at the University of Texas MD Anderson Cancer Center in Houston, USA.

Part I
Inflammatory Signaling Pathways

Chapter 1
Thiol-Based Redox Signaling: Impacts on Molecular Chaperones and Cellular Proteostasis

Amy E. Ford and Kevin A. Morano

Abstract Signaling through protein cysteine residues to regulate diverse biological processes is widely conserved from bacterial to human cells. Differential cysteine reactivity enables cells to sense and respond to perturbations in the cellular redox environment, which may impact protein structure and activity. This chapter will focus on how redox signaling regulates components of the protein quality control network to mitigate proteotoxic stress caused by redox active compounds. While specifics of redox-based activation of the endoplasmic reticulum unfolded protein response and the cytoplasmic heat shock and oxidative stress responses differ, the presence of regulatory proteins containing reactive cysteines is a common feature. Moreover, several protein chaperones are reversibly regulated via cysteine switches that govern their ability to protect or refold damaged polypeptides. These responses are biologically indispensable, given the propensity of dysregulated cells to produce endogenous reactive oxygen species and the prevalence of thiol-reactive xenobiotics in the external environment.

Keywords Chaperone · Oxidative stress · Proteostasis · Reactive oxygen species · Redox · Signaling

A. E. Ford
Department of Microbiology and Molecular Genetics, University of Texas McGovern Medical School at Houston, Houston, TX, USA

MD Anderson UT Health Graduate School of Biomedical Sciences, Houston, TX, USA

K. A. Morano (✉)
Department of Microbiology and Molecular Genetics, University of Texas McGovern Medical School at Houston, Houston, TX, USA
e-mail: kevin.a.morano@uth.tmc.edu

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Abbreviations

CRD	cysteine-rich domain
Cys	cysteine
ER	endoplasmic reticulum
HMW	high molecular weight
HS	heat shock
HSP	heat shock protein
HSR	heat shock response
LMW	low molecular weight
NBD	nucleotide binding domain
NEF	nucleotide exchange factor
OS	oxidative stress
OSR	oxidative stress response
PDI	protein disulfide isomerase
PQC	protein quality control
Prx	peroxiredoxin
ROS	reactive oxygen species
SOH	sulfenic acid
TF	transcription factor
Ub	ubiquitin
UPR	unfolded protein response

1.1 Introduction

Cysteine (Cys) is one of the least abundant amino acids, but serves critical and unique roles in protein structure and chemistry due to its irreplaceable functionality as the only amino acid with a readily ionizable thiol group (Marino and Gladyshev 2010). Thiol reactivity depends on its accessibility and protonation state (pK_a), the latter of which is influenced by local protein microenvironment properties such as pH, secondary structure, and hydrogen bonding (Kortemme and Creighton 1995; Ferrer-Sueta et al. 2011). Although methionine also contains a sulfur atom, the thioether is in a relatively less reactive form and is typically not involved in biologically relevant reactions. Cys residues are most often buried within the interior of the protein structure; however, they can also be found exposed to the solvent (Poole 2015). Additionally, cysteines are typically clustered into two or more groups, characteristic of metal binding and redox centers. These chemical and functional properties allow for rapid and reversible redox regulation of protein activity, frequently but not exclusively through the formation of intramolecular disulfide bonds, to sense and control diverse cellular states and processes.

Reactive oxygen species (ROS) produced as a byproduct of aerobic metabolism, oxidative protein folding, and exposure to oxidants and highly toxic xenobiotics

have the potential to modify reactive thiols (Marnett et al. 2003; Tu and Weissman 2004; West et al. 2012). Oxidants such as hydrogen peroxide and diamide can react with protein thiols to form both reversible and irreversible thiolations (Winterbourn and Hampton 2008; Paulsen and Carroll 2010). Following initial formation of sulfenic acid (SOH), the modified thiol can either be further oxidized into sulfinic (SOOH) or sulfonic (SOOOH) acid or form a disulfide bond with a nearby free thiol (e.g. intramolecularly with a proximal Cys residue or with glutathione). These modifications play biological roles in sensing and regulation of activity of redox enzymes and transcriptional programs. The highly toxic heavy metal cadmium and metalloid-anion arsenite can target proteins in multiple ways – covalent binding of free thiols, metal ion displacement, and catalyzing oxidation (Tamás et al. 2014). In addition to oxidants and heavy metals, Cys residues are susceptible to modification by organic electrophiles, which form thiol adducts and may induce intermolecular cross-links between proteins (Zhang et al. 1995; Sánchez-Gómez et al. 2010). While xenobiotics are not involved in normal, steady state redox regulation, exposure to these agents can mimic endogenous modifications and induce similar downstream signaling.

Protein homeostasis ("proteostasis") is essential for cellular function, and is defined as the status of the protein complement of a cell as determined by protein synthesis, assembly and degradation/turnover. Molecular chaperones assist proteins in their proper folding and prevent non-native conformations that lead to misfolding and aggregation (reviewed by Verghese et al. 2012). Proteins that cannot be folded properly or any non-native confirmations that arise are shuttled to specific protein aggregation sites and/or degradation pathways. These functions are performed by a variety of different chaperone classes and machines that make up the protein quality control (PQC) network. Members of the highly conserved Hsp70 class of chaperones are located in all major subcellular compartments and function in many aspects of proteostasis including native folding, transport, disaggregation, and degradation. Hsp70 performs these functions with the assistance of co-chaperones such as J-domain-containing Hsp40 proteins and nucleotide exchange factors (NEF), including the Hsp110, HspBP1 and Bag protein families (Bracher and Verghese 2015). Unlike Hsp70, the conserved Hsp90 system of chaperones interacts with specific "client" proteins, including kinases, receptors, and transcription factors, to aid in protein maturation and assembly of macromolecular complexes (Röhl et al. 2013). Cells also utilize small heat shock proteins that form multimers to aid in disaggregation (Verghese et al. 2012).

Cys modification by thiol-reactive compounds (described above) has the potential to alter protein structure and affect protein stability and solubility. Using in vitro folding assays, live cell imaging, and proteomic approaches, thiol stress has been found to induce protein aggregation (Sharma et al. 2008; Jacobson et al. 2012, 2017; Weids et al. 2016). Accumulation of protein aggregates resulting from exposure to these compounds can be toxic to cells as demonstrated by dose-dependent loss of cell viability (West et al. 2011). Protein aggregation is linked to diverse human diseases including diabetes, cancers, and neurodegenerative disorders such as Alzheimer's, Parkinson's and Lou Gehrig's diseases (Valastyan and Lindquist 2014;

Hipp et al. 2014). In addition to protein aggregation, oxidative stress (OS) and metal dyshomeostasis have been implicated, suggesting that disruption of redox balance and, therefore, redox regulation, as a contributing factor to these diseases.

Multiple studies have investigated the in vivo redox state of thiol-containing proteins during steady state conditions, peroxide stress, and changes to redox status due to aging or genetic mutations (Le Moan et al. 2006; Brandes et al. 2011, 2016). A common theme amongst these studies is the diversity of cellular processes that depend on redox-active thiol-containing proteins – redox systems, energy metabolism, translation, and, notably, protein folding. In this chapter, we will discuss the interplay between the PQC network and redox signaling with respect to changes in the protein folding environment.

1.1.1 Regulation of Stress Responses

The ability to respond and adapt to environmental changes through transcriptional reprogramming is essential for survival and proliferation. Bacterial responses to stress are numerous due to the diversity of niches and are regulated by specific or over-lapping stresses (Chalancon and Madan Babu 2011; Helmann 2011). Transcriptional activators, repressors and alternative sigma factors block or recruit RNA polymerase and additional co-regulators to regulate gene expression. Activity of these proteins is often controlled through anti-sigma factors that act as stress sensors and interact with the transcriptional regulator to sequester or facilitate its degradation (Hughes et al. 1998; Zhou et al. 2001; Arsène and Tomoyasu 2000). The major chaperone Hsp70 system composed of DnaK/DnaJ/GrpE (*E. coli*) and the Hsp60 chaperonin (GroEL/ES in *E. coli*) machines protect nascent polypeptides from insults to the folding environment and assist in refolding or degradation of damaged proteins. The two chaperone systems have been implicated in stress response sensing and regulation, most notably the Hsp70 system that regulates stability of the bacterial stress factor σ^{32} (Arsène and Tomoyasu 2000).

A distinguishing feature of eukaryotes is the presence of membrane-bound organelles that allow for the compartmentalization of distinct protein folding environments that differ in redox status: a reducing environment predominates in the cytosol and nucleus, and an oxidizing one is characteristic of the ER and mitochondrial inner membrane space, as well as the extracellular milieu. Changes in the redox balance within these compartments are sensed via protein thiol modifications which lead to activation of transcriptional responses (see Fig. 1.1). Within the ER, the response to redox imbalance is well characterized and is known as the unfolded protein response (UPR). In the cytosol, cells activate a specific transcriptional program to oxidative stress called the oxidative stress response (OSR). On the other hand, the response to misfolded proteins, classically termed the heat shock response (HSR), is primarily modulated by the transcription factor (TF) Hsf1; however, the mechanism of Hsf1 activation by oxidation of the reducing environment is unclear. Within the last 10 years, studies have investigated the connection between OS and