Heat Shock Proteins 15
Series Editors: Alexzander A. A. Asea · Stuart K. Calderwood

Alexzander A. A. Asea · Punit Kaur
Editors

Heat Shock Proteins and Stress

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Heat Shock Proteins

Volume 15

Series editors

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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.

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Preface

The clinical definition of stress is a physical, mental, or emotional factor that causes bodily or mental tension. On a whole-body level, stresses can be induced by environmental, psychological, or social situations or by illness, or from a medical procedure. At the cellular level, stress can be induced by a wide variety of conditions including heat shock, cold shock, pH shift, hypoxia, UV light, and during wound healing or tissue remodeling. Increased expression of heat shock proteins (HSP) protects the cell by stabilizing unfolded proteins, giving the cell time to repair or resynthesize damaged proteins.

The book *Heat Shock Proteins and Stress* provides the most comprehensive review on contemporary knowledge on the role of HSP in stress. Using an integrative approach to understanding the regulation of HSP responses, the contributors provide a synopsis of novel mechanisms by which HSP responses are regulated under normal physiological and pathophysiological conditions.

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 HSP in cellular stress; Section II evaluates the role of HSP in oxidative stress; Section III focuses the reader on the role of HSP in stress response pathway in invertebrates, vertebrate, plants, and aquatic organisms.

Key basic and clinical research laboratories from major universities and academic medical hospitals around the world contribute chapters that review present research activity and importantly project the field into the future. The book is a must read for researchers, postdoctoral scholars, and graduate students in the fields of Translational Medicine, Clinical Psychology, Human Physiology, Zoology, Botany, Biotechnology, Molecular Medicine, Infectious Diseases, and Pathology.

Toledo, OH, USA — Alexzander A. A. Asea
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About the Editors

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. Asea's initial findings studying the effects of Hsp72 on human monocytes led to the proposal of a novel paradigm that Hsp72, previously known to be as intracellular molecular chaperones, can be found in the extracellular milieu where it has regulatory effects on immunocompetent 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 Publications) and is an editorial board member of 13 other scientific peer-reviewed journals. Currently, Prof. Asea is at the University of Toledo College of Medicine and Life Sciences in Toledo, USA.

Punit Kaur is an expert in onco-proteogenomics, with extensive training and experience in quantitative mass spectrometry imaging, protein chemistry, and biomarker discovery. Dr. Kaur's main research focus is on the use of heat-induced nanotechnology in combination with radiotherapy and chemotherapy in the cancer stem cell therapy. Dr. Kaur 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*. Dr. Kaur is an editor of five books in the highly successful *Heat Shock Proteins* book series by Springer Nature Publishers. Currently, Dr. Kaur is a Visiting Scientist Professor at the University of Texas MD Anderson Cancer Center in Houston, USA.

Part I
Cellular Stress

Chapter 1
Molecular Chaperones and the Nuclear Response to Stress

Lynn Boyd and Katherine M. Sampuda

Abstract Chaperones are a well conserved class of proteins that reside in many different cellular compartments. The nucleus is a compartment of special interest because it houses the genetic material and allows for the expression and maintenance of genes. Many chaperones localize to the nucleus under stress conditions. The current body of evidence indicates that the nuclear function of chaperones is similar to chaperone function in the cytoplasm. Emerging evidence on the nuclear import pathway for chaperones suggests that novel pathways exist that allow chaperones to enter the nucleus under conditions of environmental stress. One such pathway, the Hikeshi pathway, is responsible for the transport of HSP70 and possibly other molecular chaperones.

Keywords Chaperone · HSP · HSP70 · Nuclear import · Protein aggregation · Stress

Abbreviations

CHIP	c-terminus of Hsc70-interacting protein
HOP	HSP70/HSP90 organizing protein
HSF1	Heat shock factor 1
HSP	Heat shock protein
PQC	Protein quality control
sHSP	Small heat shock protein
SING	Stress induced nuclear granule
TPR	Tetratricopeptide repeat
UPS	Ubiquitin protein system
UV	Ultraviolet

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1.1 Introduction

The different compartments of the eukaryotic cell each have their own characteristic proteome. Each compartment possesses a protein quality control (PQC) system that functions to monitor and repair damage to the proteome. The nucleus is a compartment of special interest since it houses the genetic material which must be maintained, replicated, and protected from damage. Several human diseases, such as Huntington's disease, are associated with protein misfolding in the nucleus. It has been reported that the nucleus is the most susceptible compartment in regards to protein damage following a heat insult and this damage can be mitigated by overexpression of a nuclear targeted molecular chaperone, HSP70 (Hageman et al. 2007). This suggests that chaperones can work similarly in the nucleus and cytosol. The nucleus differs from other membrane-bound compartments in that trafficking between the nucleus and cytosol is more dynamic than that of the other organelles such as the ER or mitochondria. Thus, it has been more difficult to discern which PQC pathways might be specific to the nucleus versus cytosol. The nuclear pore provides a channel through which small proteins can travel freely and in both directions between the nucleus and cytosol. Larger proteins must use a transport pathway to travel through the nuclear pore. Many molecular chaperones are localized to both the nucleus and cytosol. Molecular chaperones are known mediators of protein folding and thus may be required during times of proteotoxic stress. Chaperones recognize exposed hydrophobic patches on a protein's surface and help the protein to refold into its proper conformation. Several chaperones are known to relocate to the nucleus under stress conditions (discussed below). Although, most studies have looked at the stress of elevated temperature, some recent studies have shown that other types of stress can also induce this nuclear response.

Several neurodegenerative diseases are associated with nuclear protein aggregates. A link between these aggregation events and chaperones is well established. First, chaperones have been shown to localize to these aggregates (see Table 1.1). Second, overexpression of chaperones is known to reduce the level of nuclear aggregates (reviewed in Nath and Lieberman 2017). Third, reduced expression or mutations in chaperones makes cells more susceptible to nuclear aggregation events (Nath and Lieberman 2017). In addition to nuclear protein aggregates, several other nuclear bodies have been described. In several cases, chaperones have been shown to localize to these nuclear bodies. Table 1.1 shows known nuclear bodies and nuclear aggregates that are associated with localization of chaperones.

There are a large number of individual chaperones and co-chaperones that have been identified. Generally, these can be divided up into several different classes: the HSP60 family, the HSP70 family, the HSP90 family, the HSP100 family, the HSP110 family, and the small heat shock proteins (sHSP). Additionally, there are several co-chaperone families including HSP40 (DNAJ) proteins and the TPR domain containing proteins (such as HOP/Sti1). First, we discuss the entry of these proteins into the nucleus. Next, we look at the evidence surrounding the actual function of these chaperones inside the nucleus.

Table 1.1 Nuclear bodies and aggregates with chaperone localization

Nuclear bodies with chaperone localization		
Nuclear body	**Chaperone protein**	**Reference**
Clastome	Hsc70, Hsp70	(Lafarga et al. 2002)
Cajal body	NOPP140, Telomere Cajal body protein 1 (TCAB1), Survival Motor Neuron (SMN)	(Isaac et al. 1998; Raimer et al. 2016)
Nuclear speckles	HSPB1, alpha B-crystallin (HSPB5), HSPB7, Hsp27 (Drosophila), HSPA6 (HSP70B'), HSPA1A (HSP70–1)	(Bao et al. 2002; Van den IJssel et al. 2003; Michaud et al. 2008; Vos et al. 2009)
Nuclear stress body	HSF1, HSF2	(Morimoto and Boerkoel 2013)
Nuclear stress granules	HSF1, HSF2, and Hsp70	(Alastalo et al. 2003; Sarge et al. 1993)
PML body	DEK proto-oncogene, death domain-associated protein 6 (DAXX), ATP-dependent helicase ATRX (ARTX), and histone cell cycle regulator (HIRA)	(Ivanauskiene et al. 2014)
Nuclear aggregates with chaperone localization		
Disease	**Chaperone protein**	**Reference**
Huntington's disease (Poly (Q) aggregate)	Hsp70, Hsc70, Hsp26 and Hsp104	(Jana et al. 2000; Walter et al. 2011)
Spinobulbar muscular atrophy (Poly(Q) aggregate)	Hsp70, Hsp90, HDJ-2/HSDJ	(Cummings et al. 1998; Stenoien et al. 1999)
Dentatorubral Pallidoluysian atrophy (Poly(Q) aggregate)	HDJ-2/HSDJ, HSP70	(Cummings et al. 1998)
Spinocerebellar Ataxia (Poly(Q) aggregates)	HDJ-2/HSDJ, Hsc70 (Hsp73), Hsp70 (Hsp72)	(Chai et al. 1999; Cummings et al. 1998)

1.1.1 Nuclear Protein Quality Control

The presence of chaperones and ubiquitin proteasome pathway components in the nucleus suggests that PQC in the nucleus may work similarly to the cytosolic system. Although it has been suggested that misfolded nuclear proteins might be exported from the nucleus for degradation (Chen and Madura 2014), other reports have suggested just the opposite, that some proteins are imported into the nucleus specifically for degradation. The most detailed description of nuclear protein quality control comes from studies in the budding yeast, *Saccharomyces cerevisiae*. The ubiquitin pathway enzymes Cdc34p, Ubc1p, and San1p target four temperature sensitive nuclear proteins for degradation by the 26S proteasome (Gardner et al. 2005). Further studies in *S. cerevisiae* identified a chaperone-assisted degradation pathway where Hul5, an E3 ligase associated with the 26S proteasome, HSP70, and Bag102, a co-chaperone, mediate the tagging and the removal of the nuclear kinetochore component Spc7–23 (Kriegenburg et al. 2014). In mammalian COS cell culture,

modified β-galactosidase from *Escherichia coli* was shown to be degraded rapidly in the nucleus whereas its unmodified form remained stable (Tsuneoka and Mekada 1992). However, the molecular pathways of nuclear PQC in mammalian cell culture have yet to be discovered. In the nematode, *C. elegans*, expression of chaperones can repress the formation of stress induced nuclear granules (SINGs) (Sampuda et al. 2017). These nuclear granules contain high concentrations of ubiquitin and proteasome and occur after exposure to proteotoxic stress such as high salt or oxidative stress, but not in response to heat shock. The SINGs may be sites of localized protein degradation in the nucleus. That the expression of chaperones can repress SING formation suggests that SINGs are a nuclear response to protein misfolding.

Many chaperones like heat shock protein 70 (HSP70), heat shock protein 90 (HSP90) and small heat shock proteins (sHSP) are induced by heat shock and other stress conditions. Heat shock induced expression of chaperones is mediated by the heat shock factor 1 (HSF1) transcription factor (Brunquell et al. 2016; Shibata and Morimoto 2014). Since many key chaperones are above the size of free diffusion through the nuclear pore transport into the nucleus would require some sort of transport mechanism such as that involving the Ran and importin proteins (Weis 2003). The topic of yeast nuclear chaperones and their role in nuclear PQC has recently been reviewed (Jones and Gardner 2016). In this chapter, we will first discuss the circumstances and mechanisms for delivery of chaperones into the nucleus. In the following section, we discuss the known functions of those chaperones in the nucleus.

1.1.2 Nuclear Import of Chaperones

In addition to responding to heat stress, chaperones are constitutively expressed under normal physiological conditions. They help to fold newly synthesized proteins as they leave the ribosome and aid in protein translocation across the membrane. However, during stress conditions such as heat shock, chaperones shift to refolding thermally damaged proteins to prevent them from aggregating. Chaperone expression is upregulated by HSF1. In unstressed states, cytosolic HSF1 monomers are suppressed by a chaperone complex consisting of molecular chaperones HSP70 and HSP90. During stressed conditions like heat shock, cadmium sulfate, or azetidine, HSP70 and HSP90 release HSF1 and interact with misfolded proteins. Unbound HSF1 is then translocated into the nucleus to form a transcriptionally active HSF1 trimer that binds to heat shock elements found upstream of HSP genes. Upon recovery from heat shock, HSP70 binds to HSF1 and translocates to the cytosol where HSP90 binds to form the HSP70/HSP90 complex to inhibit HSF1 (Trinklein et al. 2004).

After transcription, heat shock proteins are translated in the cytosol where many of them function. As previously mentioned, a portion of these chaperones translocate to the nucleus. Nuclear import of many of these chaperones requires active

transport. In some cases, chaperones contain a nuclear localization sequence that promotes transport through a classical nuclear import pathway. In other cases, chaperones have been known to piggyback into the nucleus as they transport proteins from the cytosol into the nucleus (Melchior and Gerace 1995).

The classical nuclear import pathway revolves around the Importin α/β family. In this pathway, importins bind to cargo proteins and help transport them across the membrane and into the nucleus. Once the cargo protein is in the nucleus, Ran GTPase binds to the importin prompting the release of the cargo protein. Importin is then shuttled back into the cytosol by Ran (Melchior and Gerace 1995). This pathway is reliant on the Ran gradient, which is higher in the nucleus and lower in the cytosol. Heat shock, oxidative stress, and UV irradiation downregulate the classical importin α/β-mediated pathway by altering the distribution of Ran (Czubryt et al. 2000; Kodiha et al. 2004; Kose et al. 2012; Yasuda et al. 2006).

The yeast chaperone HSP104 has a nuclear localization signal and is imported into the nucleus after heat shock (Tkach and Glover 2008). Interestingly, nuclear import under heat stress does not depend upon the nuclear localization signal and must depend upon some non-canonical import pathway. In the yeast, *Saccharomyces cerevisiae,* both starvation and ethanol stress induced nuclear accumulation of the HSP70 family member Ssa4p (Chughtai et al. 2001; Quan et al. 2004). Nuclear localization following starvation is dependent upon a short hydrophobic region near the N-terminus on the protein and requires the activity of β importin. Nuclear localization following ethanol stress also depends upon β importin and the N-terminal domain.

CHIP (C-terminus of Hsc70-interacting protein) is a TPR family co-chaperone and E3 ligase that ubiquitinates proteins that HSP70 and HSP90 are unable to fold. After heat stress, CHIP localizes predominantly to the nucleus (Dai et al. 2003). This localization coincides with the translocation of HSF1 into the nucleus and it has been proposed that CHIP functions as a regulator of HSF1 activity in the nucleus (Dai et al. 2003).

The co-chaperone HOP (HSP70/HSP90 organizing protein) serves as a linker for the HSP70 and HSP90 chaperones. Thus, it plays a crucial role in linking these two major chaperone activities. HOP contains a bipartite nuclear localization signal required for its nuclear localization under non-stress conditions. However, its translocation into the nucleus following stress does not depend upon this NLS (Daniel et al. 2008).

The classical import pathway is reduced during stress conditions (Kodiha et al. 2004). However, chaperones still localize to the nucleus. This implied that a non-canonical import pathway was utilized by molecular chaperones. Kose et al. (2012) showed that a carrier protein, Hikeshi, does not bind to Ran and functions under heat shock to transport HSP70 into the nucleus. This pathway was termed the Hikeshi-mediated nuclear import pathway. It is currently unclear if chaperones other than HSP70 are transported by the Hikeshi pathway.