Akira Nakai *Editor*

Heat Shock Factor

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Preface

All organisms sense varying conditions in the environment that they live in, and respond and ultimately adapt to them through sophisticated mechanisms. These adaptive mechanisms play pivotal roles for homeostasis and biological defense; therefore, the elucidation of the adaptive mechanisms has grown to be major research field in molecular biology. A prominent system among them is a primitive and evolutionally conserved adaptive mechanism called heat shock response, which induces the heat shock proteins against proteotoxic stress including high temperature.

It has been 53 years since the discovery of the heat shock response (in 1962), and 27 years after the molecular cloning of the key regulator, heat shock factor (HSF) (isolated in 1988). This adaptive response to high temperature or protein misfolding is a fundamental mechanism to maintain the capacity of protein homeostasis, or proteostasis, and is evolutionally conserved among all living organisms, including bacteria and humans, on the earth. Furthermore, physiological and pathological roles of HSF have been extensively studied in fruit fly, worm, and mouse models for the last 18 years (starting in 1997). It has been revealed that HSF plays roles in development of the brain, reproductive and sensory organs, and in ageing, inflammation, and circadian rhythm. Analysis of the mechanisms have uncovered that HSF exerts a wide range of effects on gene expression and epigenetic status on the whole genome. Moreover, loss or gain of HSF function is also closely related to protein-misfolding diseases including neurodegenerative diseases, psychiatric diseases, heart diseases, and cancers. Therefore, HSF is now thought to be a promising therapeutic target for treatment of these refractory diseases.

At this point (2015), we should bring a large amount of HSF-related information, describe core observations about molecular mechanisms and pathophysiological roles, and provide fundamental concepts on the basis of information from diverse aspects in one book. The aim of this publication is to provide a resource on the heat shock response and HSF for undergraduate students. This book will not only be a guide of the heat shock response and HSF to be understandable to students and

young researchers in other fields, but will also be a cornerstone for future works in the field related to the heat shock response and HSF. I would like to take this opportunity to thank all the authors for their contributions and the staff members at Springer Japan for their enthusiasm and effort.

Yamaguchi, Japan Akira Nakai

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Part I
The Basis of HSF Biology

Chapter 1
Proteostasis and Adaptation to High Temperature Stress

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Abstract All living organisms respond to elevated temperatures by rapidly producing a set of highly conserved proteins, known as heat shock proteins (HSPs) or chaperones, which facilitate proper protein folding. Simultaneously, they produce non-HSP proteins with diverse functions including protein degradation. This adaptive response to elevated temperature or proteotoxic stress is called as the heat shock response and is mainly regulated at the transcription level by heat shock factor (HSF). Thus, HSF regulates the capacity of protein homeostasis (proteostasis) or buffering capacity against protein misfolding. This review describes history of the discovery of the heat shock response, chaperones, and HSF and explains mechanisms by which proteostasis capacity is regulated in cells. Furthermore, it discusses the fundamental function of the heat shock response, which is required for adaptation in all organisms, including those living in extreme high-temperature environments.

Keywords Chaperone • Energy landscape • Hyperthermia • Protein folding • Thermotolerance • Transcription

1.1 Introduction

It is assumed that all living organisms on the earth come from a common ancestor. This is because organisms possess common properties, e.g., they transcribe RNA from DNA and synthesize proteins based on the code written in the RNA (Barton et al. 2007). In particular, the nucleotide sequences of the genes encoding ribosomal proteins and RNAs have been highly conserved during evolution, and those of genes encoding a group of heat shock proteins (HSPs), which facilitate protein folding, are equally well conserved (Craig 1985). Organisms each live at an optimal temperature for them and dramatically induce HSPs when they are exposed to elevated temperatures (Lindquist 1986). This adaptive response to heat stress or

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proteotoxic stress is called the heat shock response and is present in all organisms including those living in extreme environments from cold areas in Antarctica to hot hydrothermal vents at the bottom of the deep sea (Conway de Macario and Macario 1994). Thus, the heat shock response is a primitive adaptive mechanism to respond to proteotoxic stresses including heat shock, which had been acquired by a common ancient ancestor of all organisms that exist today.

The focus of this review is the fundamental function of the heat shock response and transcription factors that regulate this adaptive response. After reprising the history of the discovery of the heat shock response and functions of HSPs, I describe the processes of protein folding and mechanisms that maintain protein homeostasis. Furthermore, I review heat shock transcription factors and their requirement for cell growth and survival of various organisms in normal and proteotoxic stress conditions.

1.2 Discovery of the Heat Shock Response

In the early 1960s, gene expression in the polytene chromosomes of *Drosophila* was monitored as chromosome puffing under the light microscope. Different puffing patterns were observed in various tissues and at particular developmental stages. Ferruccio Ritossa was studying the nucleic acid produced in puffs of the salivary gland of *Drosophila busckii*, as these were thought to represent gene activation. One day, he noticed a different puffing pattern in larvae that were accidentally incubated at a high temperature (Ritossa 1996). He found that a unique puffing pattern was observed when larvae grown at 25 °C were subjected to a temperature shock at 30 °C for 30 min and the induced puffs receded during recovery at 25 °C (Ritossa 1962). It is worth noticing that the regression of some normal puffs was also observed during the temperature shock. Thereafter, several laboratories showed that a similar pattern of puffing appeared in different ages and strains of *Drosophila* during temperature shock. In *D. melanogaster*, there were nine heat-inducible puffs (33B, 63C, 64F, 67B, 70A, 87A, 87C, 93D, and 95D) (Ashburner 1970).

Meanwhile, it was established that the puffs were the sites of RNA synthesis, and the RNA synthesized in a particular puff was transported to the cytoplasm and translated into proteins (Daneholt and Hosick 1973). In 1974, Tissières and Mitchell examined proteins synthesized after heat shock (Tissières et al. 1974). Larvae of *D. melanogaster* were exposed to 37.5 °C for 20 min, and the isolated salivary glands were cultured for 20 min in medium containing [^{35}S]methionine. The labeled proteins were separated on a polyacrylamide gel and detected as bands on the autoradiograph. They found the rapid appearance of six strong bands after heat shock and suggested that the heat-induced puffs were associated with the synthesis of these proteins. Parallel changes in puffing activity and the induced synthesis of new proteins after heat shock were further confirmed by using an inhibitor of puffing (Lewis et al. 1975). The synthesis of the small set of proteins

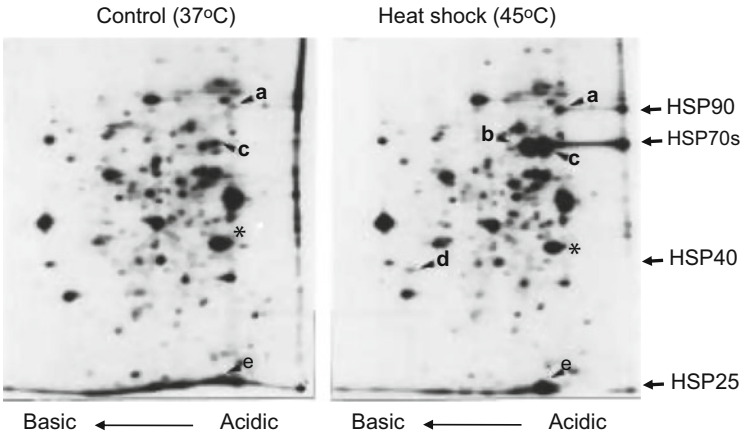

Fig. 1.1 Synthesis of major heat shock proteins (*HSPs*) was induced in avian cells upon upshift of growth temperature. Chicken B lymphocyte DT40 cells were maintained at 37 °C. Cells were metabolically labeled with [^{35}S]methionine for 60 min at 37 °C (control), or heat shocked at 45 °C for 30 min, allowed to recover at 37 °C for 3 h, and metabolically labeled with [^{35}S]methionine during the last 60 min of the recovery period (heat shock). Cell extracts were subjected to two-dimensional gel electrophoresis consisting of 10 % SDS-PAGE and nonequilibrium pH gradient gel electrophoresis (NEPHGE). *Arrowheads a* to *e* indicate the bands for HSP90, HSP70, HSC70, HSP40, and HSP25, respectively, which are also marked on the *right*. *Asterisks* indicate β-actin bands. Acidic and basic sides are indicated at the *bottom* (Tanabe et al. 1998)

was also induced in different tissues and various *Drosophila* species (Daneholt and Hosick 1973; Lewis et al. 1975). The apparent molecular weights of these proteins in *D. melanogaster* were 82,000; 70,000; 68,000; 36,000; 27,000; 26,000; 23,000; and 22,000 daltons. These heat shock polypeptides or proteins (HSPs) were called as HSP82, HSP70, and so on (Ashburner and Bonner 1979).

The selective induction of HSPs by heat shock was thought to be unique to flies for about 15 years after the discovery of induced puffing after heat shock. In 1978, Kelly and Schlesinger found that heat shock induced the synthesis of similar proteins in cultured avian and mammalian cells, suggesting that this response was a conserved system of gene regulation between species (Kelly and Schlesinger 1978) (Fig. 1.1). It was also reported at almost the same time that the synthesis rate of four to five proteins was induced in bacteria and yeast upon upshift of the growth temperature (Lemaux et al. 1978; Yamamori et al. 1978; Miller et al. 1979). Therefore, it was assumed that this response, termed the heat shock response, was a general phenomenon of cellular adaptation to high temperature.

The potential benefit of hyperthermia in the treatment of human cancers had been recognized (Bronk 1976). The exposure of mammalian cells maintained at 37 °C to temperatures above 40 °C led to reproductive death, which was progressive with increasing time at the elevated temperature (Hahn 1974). The plating

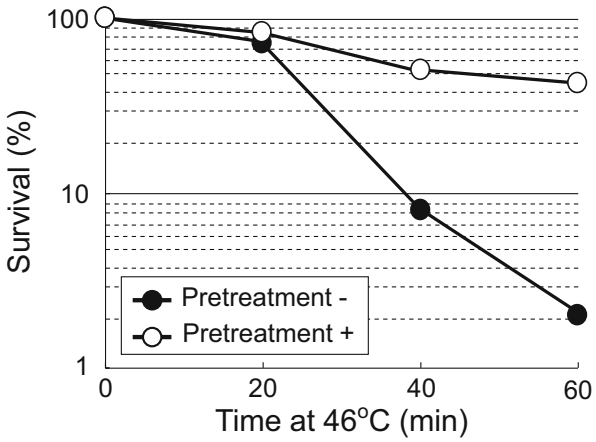

Fig. 1.2 Avian cells pretreated with sublethal heat shock acquire thermotolerance. Chicken B lymphocyte DT40 cells, maintained at 37 °C, were then incubated at 46 °C for the indicated periods (*closed circles*; pretreatment −). Some cells were pretreated with sublethal heat shock at 45 °C for 20 min and allowed to recover at 37 °C for 2 h (open circles; pretreatment +). The numbers of surviving cells were counted using a colony formation assay. Survival percentages are shown (Tanabe et al. 1998)

efficiency or proliferative capacity, which was monitored as colony-forming ability in vitro, was used as a measure of cell survival. It was revealed that a single hyperthermic pretreatment to human carcinoma HeLa cells induced a transient resistance to heat, and this induced heat resistance required the return of the culture temperature at 37 °C for 2–3 h (Gerner and Schneider 1975; Gerner et al. 1976). This phenomenon is universal and has been termed thermotolerance (Henle and Dethlefsen 1978) (Fig. 1.2). As was expected, the acquisition of a transient thermotolerance was tightly correlated with the induced synthesis of a set of HSPs (Li and Werb 1982). Agents that elevated the synthesis of HSPs also induced thermotolerance.

1.3 Heat Shock Proteins as Molecular Chaperones

The heat shock response was thus characterized originally by the induction of the synthesis of a set of HSPs upon stress conditions. This response was induced by many kinds of treatments, which had the common property of causing the accumulation of denatured or damaged proteins within the cells. First, the synthesis of abnormal proteins in avian and mammalian cells treated with amino acid analogs led to this response (Kelly and Schlesinger 1978; Hightower 1980). Second, HSPs

were strongly induced in mouse ts85 cells, which exhibited temperature sensitivity in ubiquitin-protein conjugation, at the nonpermissive temperature (Finley et al. 1984). In these cells, abnormal proteins would be accumulated. Third, the heat shock response was induced by the production of abnormal proteins in *Escherichia coli* (Goff and Goldberg 1985) or the microinjection of a denatured protein into *Xenopus* oocytes (Ananthan et al. 1986). These observations suggested that HSPs might prevent denaturation of cellular proteins in stressed conditions.

Numerous studies examining the function of HSPs have focused on the major heat shock protein, HSP70. DnaK, an *E. coli* homologue of mammalian HSP70, had ATPase activity (Zylicz et al. 1983) and mammalian HSP70 bound to ATP (Welch and Feramisco 1985). Upon heat shock, HSP70 was immobilized in the nuclei and nucleoli (Lewis and Pelham 1985), probably owing to its binding to hydrophobic protein precipitates (Evan and Hancock 1985). HSP70 was released from heat-shocked nuclei and nucleoli by treatment with ATP, but not with non-hydrolysable ATP analogues (Lewis and Pelham 1985). Pelham speculated that HSP70 had a general affinity for denatured or abnormal proteins and proposed in 1986 that HSP70 directly prevented aggregate formation of denatured proteins which expose hydrophobic regions during heat shock and promoted disaggregation by using energy from ATP hydrolysis (Pelham 1986). The latter idea was supported by the function of another isoform of HSP70, which was initially known as uncoating ATPase. Rothman and colleagues purified uncoating ATPase, which bound to and released clathrin trimers from clathrin cages in coated vesicles (Schlossman et al. 1984). This protein hydrolyzed ATP in this uncoating process. Uncoating ATPase was subsequently renamed HSC70 (Ungewickell 1985; Chappell et al. 1986). Therefore, it was speculated that HSP70 family proteins catalyzed the assembly and disassembly of a variety of proteins by coupling to the energy of ATP hydrolysis (Rothman and Schmid 1986). The mechanism of substrate binding and the release cycle of HSP70 have more recently been elucidated in much greater detail (Mayer and Bukau 2005) (Fig. 1.3). Because HSC70 was abundantly expressed in unstressed cells, Pelham and colleagues further proposed that it recognized newly synthesized proteins, which by definition were denatured, and was involved in protein folding (Pelham 1986).

In 1987, Ellis coined the term “molecular chaperone” to describe the new class of proteins whose function was to ensure that the folding of other proteins and their assembly into oligomeric structures occurred correctly (Ellis 1987). The term molecular chaperone was used first by Lasky et al. to describe nucleoplasmin, an acidic nuclear protein required for the assembly of nucleosomes from DNA and histones (Laskey et al. 1978). It was later extended by Ellis to include an abundant chloroplast protein (now known as chloroplast chaperonin) that functions to keen nascent Rubisco large subunits from forming insoluble aggregates and was further refined after the discoveries of the roles of HSPs in protein folding and assembly as described above (Ellis 1987). Molecular chaperones were originally defined as proteins whose role was to mediate the folding of certain polypeptides and, in