Molecular Cell
Review

The Heat Shock Response: Life on the Verge of Death

Klaus Richter,[1,2] Martin Haslbeck,[1,2] and Johannes Buchner[1,*]
[1]Munich Center for Integrated Protein Science (CIPS[M]) and Department Chemie Technische Universität München, 85747 Garching, Germany
[2]These authors contributed equally to this work
*Correspondence: johannes.buchner@ch.tum.de
DOI 10.1016/j.molcel.2010.10.006

Organisms must survive a variety of stressful conditions, including sudden temperature increases that damage important cellular structures and interfere with essential functions. In response to heat stress, cells activate an ancient signaling pathway leading to the transient expression of heat shock or heat stress proteins (Hsps). Hsps exhibit sophisticated protection mechanisms, and the most conserved Hsps are molecular chaperones that prevent the formation of nonspecific protein aggregates and assist proteins in the acquisition of their native structures. In this Review, we summarize the concepts of the protective Hsp network.

Introduction

Organisms have adapted to growth temperatures from the freezing point of water to 113°C (Stetter, 2006). Nevertheless, as a major stressor, heat represents a significant barrier to life. For all living organisms, temperatures only moderately above the respective optimum growth temperature represent a challenging problem for survival.

Experiments on the effects of heat on the fruitfly *Drosophila* were already performed in the 1930s (Goldschmidt, 1935; cf. Morimoto et al., 1990). The appearance of expanded chromosomal puffs after heat shock indicated the onset of locally enhanced transcription (Jamrich et al., 1977; Ritossa, 1962). In the following years, the identification of the transcribed genes and the corresponding proteins were the focus of stress research (Ashburner and Bonner, 1979; Peterson et al., 1979), and the term heat shock proteins (Hsps) was coined. Similar phenomena were also observed in prokaryotes and other eukaryotes (Kelley and Schlesinger, 1978; Lemaux et al., 1978; McAlister and Finkelstein, 1980), suggesting that the heat shock response is a universal and ancient mechanism. From that time, the field began to expand in different directions. Two major areas of investigation were the analysis of the function of Hsps (Lindquist and Craig, 1988) and the regulation of the stress response (Morimoto et al., 1990).

The Damaging Effects of Heat

One of the most amazing aspects of the heat shock response is that it is triggered by a temperature increase of just a few degrees. This is even true for organisms living at extreme temperatures (Brown and Lupas, 1998; D'Amico et al., 2006; Takai et al., 1998). The solution to this conundrum lies in the dynamic character of proteins: they need to be conformationally flexible to perform their functions in the cell. Indeed, they are evolutionarily optimized to be only marginally stable at the respective growth temperatures. A small increase in temperature can cause protein unfolding, entanglement, and unspecific aggregation. Many of the morphological and phenotypic effects of heat stress can be explained by the aggregation of proteins and an imbalance of protein homeostasis in general. It is therefore reasonable to assume that the deleterious accumulation of unfolding proteins is the signal to start counter measures. Interestingly, this scenario implies that the cell does not recognize temperature per se. Rather, it suggests that the heat shock response is triggered by unfolded proteins that are a result of a variety of stresses, including oxidative stress, heavy metals, ethanol, or other toxic substances (Courgeon et al., 1984; Heikkila et al., 1982; Michel and Starka, 1986; Yura et al., 1984).

Heat shock has deleterious effects on the internal organization of the cell (Figure 1) beyond the unfolding of individual proteins (Szalay et al., 2007; Toivola et al., 2010; Welch and Suhan, 1985). Especially in eukaryotes, one of the major damages observed in response to stress conditions are defects of the cytoskeleton. Mild heat stress leads to the reorganization of actin filaments into stress fibers, while severe heat stress results in the aggregation of vimentin or other filament-forming proteins, leading to the collapse of intermediary, actin, and tubulin networks (Toivola et al., 2010; Welch and Suhan, 1985; Welch and Suhan, 1986). Along with the disruption of the cytoskeleton, the loss of the correct localization of organelles and a breakdown of intracellular transport processes are observed. The Golgi system and the endoplasmic reticulum (ER) become fragmented under stress conditions and the number of mitochondria and lysosomes decreases (Welch and Suhan, 1985). The uncoupling of oxidative phosphorylation and the loss of mitochondria are connected to a dramatic drop in ATP levels during heat stress (Lambowitz et al., 1983; Patriarca and Maresca, 1990).

Heat shock also hits nuclear processes. In general, RNA splicing is strongly affected by heat shock (Vogel et al., 1995). Nucleoli, the sites of ribosome assembly, swell, and large granular depositions composed of incorrectly processed ribosomal RNAs and aggregating ribosomal proteins become visible (Welch and Suhan, 1985; see related review in this issue by Boulon et al., 2010). In the cytosol, stress granules are formed. These are large RNA-protein structures, containing nontranslating

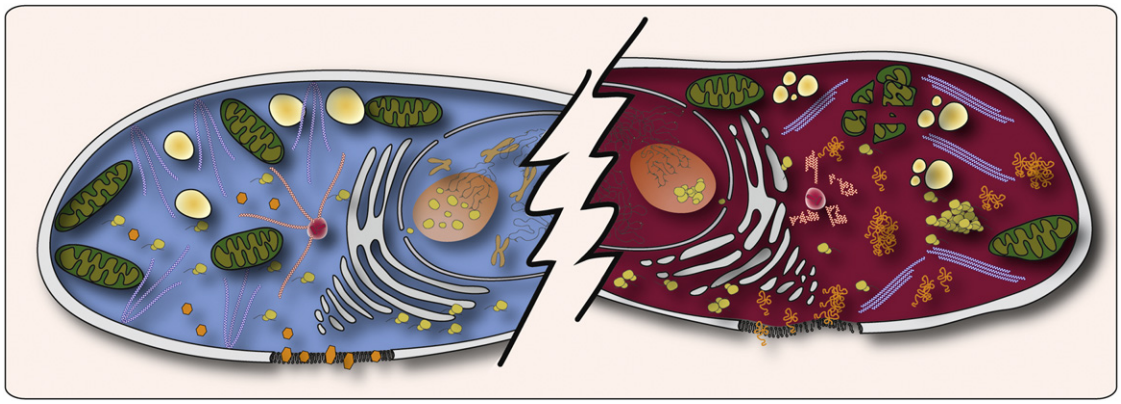

Figure 1. Effects of Heat Shock on the Organization of the Eukaryotic Cell
An unstressed eukaryotic cell (left) is compared to a cell under heat stress (right). Heat stress leads to damage to the cytoskeleton, including the reorganization of actin filaments (blue) into stress fibers and the aggregation of other filaments (microtubuli, red). Organelles like the Golgi and the endoplasmic reticulum (white) become fragmented and disassemble. The number and integrity of mitochondria (green) and lysosomes (yellow-white gradient) decreases. The nucleoli, sites of ribosome (yellow) assembly, swell, and large granular depositions consisting of ribosomal proteins become visible. Large depositions, the stress granula (yellow), resulting from assemblies of proteins and RNA, are found in the cytosol in addition to protein aggregates (hexagonal versus spaghetti style, orange). Finally, there are changes in the membrane morphology, aggregation of membrane proteins, and an increase in membrane fluidity. Together, all these effects stop growth and lead to cell-cycle arrest as indicated by the noncondensed chromosomes in the nucleus.

mRNAs, translation initiation components, and other proteins affecting mRNA function (Nover et al., 1989; Buchan and Parker, 2009). Their formation correlates with a global decrease in translation, one of the early hallmarks of the heat shock response (Ashburner and Bonner, 1979). Finally, in addition to proteins and RNAs, cellular membranes can be severely affected by heat shock. Changes in membrane morphology were observed together with changes in the ratio of protein to lipids and a higher fluidity of the membranes (Kruuv et al., 1983; Vigh et al., 2007). The resulting enhanced membrane permeability leads to a drop in cytosolic pH and changes in ion homeostasis (Coote et al., 1991; Piper et al., 2003).

Together, these effects lead to an arrest of the cell cycle and a stagnation of growth and proliferation (Lindquist, 1980; Yost and Lindquist, 1986; Zeuthen, 1971). Depending on the duration and severity of the heat stress, the accumulation of defects can result in the death of the cell. Importantly, if heat stress is not lethal, it may lead to the tolerance of more severe and otherwise fatal stresses. The increased levels of Hsps synthesized in response to moderate stress conditions are the basis for this resistance (Lindquist, 1986). Interestingly, "crossprotection" is possible: Hsps induced by one type of stress provide protection against other stresses (Lindquist, 1986).

Seven Classes of Heat Shock Proteins

Recently, many studies have addressed the heat shock response on a genome-wide level using differential display, transcriptional profiling, or proteomic approaches in a variety of cells and organisms (Eisen et al., 1998; Gasch et al., 2000; GuhaThakurta et al., 2002; Matsuura et al., 2010; Larkindale and Vierling, 2008; Richmond et al., 1999; Rohlin et al., 2005; Tabuchi et al., 2008). These studies showed that roughly 50–200 genes are significantly induced in different model organisms, from archaea to human cell lines (Table S1). Functionally, stress-inducible proteins can be grouped into seven classes (Figure 2)(Table S1). The predominant class across species, in terms of expression level, are the initially discovered Hsps, which are now commonly referred to as "molecular chaperones" (Ellis et al., 1989). The second class is represented by components of the proteolytic system, which are needed to clear misfolded and irreversibly aggregated proteins from the cell. The third class is involved in curing nonphysiological covalent modifications of nucleic acids. This class includes RNA- and DNA-modifying enzymes, which are necessary to repair DNA damage and processing failures that occur during stress (Jantschitsch and Trautinger, 2003), such as the heat-induced methylation of ribosomal RNAs (Bügl et al., 2000). The fourth Hsp class is composed of metabolic enzymes. Here, the variation among species is most substantial. Although systems approaches to model metabolic pathways during and after stress are largely lacking, evidence indicates that the changes in pathways may be needed to reorganize and stabilize the energy supply of the cell (Malmendal et al., 2006; Voit and Radivoyevitch, 2000). The fifth class includes regulatory proteins like transcription factors or kinases, some of which are needed to further initiate stress response pathways or to inhibit expression cascades, including ribosome assembly pathways (Al Refaii and Alix, 2009). The sixth class comprises proteins involved in sustaining cellular structures such as the cytoskeleton. Finally, the seventh class of upregulated proteins contains transport, detoxifying, and membrane-modulating proteins. These seem to be needed to maintain or restore membrane stability and function. For example, yeast expresses Hsp12, which stabilizes membranes by binding them and decreasing membrane fluidity (Welker et al., 2010). Interestingly, Hsp12 is an intrinsically unstructured protein in its soluble form and becomes α-helical when it interacts with lipids. Homologs outside fungi have not been discovered yet; however, it seems reasonable to assume that protection from membrane damage should be an important aspect of the cellular damage control system in general. In this context, it had been shown that several other chaperones are

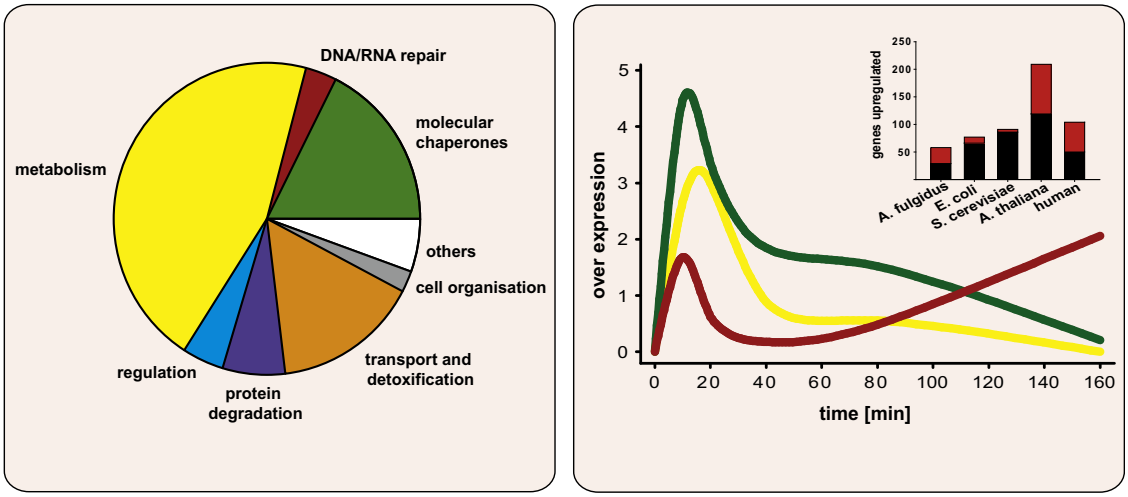

Figure 2. The Heat Shock Proteins

(Left) Summary of the functional classes of proteins upregulated during the heat shock response in *S. cerevisiae* after a shift from 25°C to 35°C for 10 min. Ninety-one genes that are upregulated by a factor of more than 2.8 were grouped into functional classes (in %) (Eisen et al., 1998; Gasch et al., 2000).
(Right) Kinetic pattern of gene expression in response to heat stress. The data are for yeast (Eisen et al., 1998). The change is given in $\log_2$ numbers. Green, a molecular chaperone (Hsp70 [SSA4]); yellow, a metabolic enzyme (citrate synthase [CIT1]); dark red, a DNA/RNA repair enzyme (photolyase [PHR1]). Inset: Analysis of the total number of genes upregulated significantly in organisms of different domains of life. Black, gens grouped into the functional classes shown on the left; red, genes with currently unknown functions or unclear functional annotation (see also Table S1).

targeted to membranes upon stress (Kirkegaard et al., 2010; Nagy et al., 2007; Vigh et al., 2007). It may be that their main task is to take care of structurally compromised membrane proteins rather than interacting with lipids directly.

While genes from these seven protein classes are upregulated upon heat stress in all domains of life (Table S1), the composition of the classes and the number of genes found in different classes vary (Figure 2) (Table S1). Particularly in archaea and yeast, the number of genes involved in the reorganization of metabolism is striking. Nevertheless, as mentioned above, a more detailed, systems-level approach is needed for understanding the metabolic consequences of this reprogramming. Furthermore, it must be taken into account that the quality (i.e., microarray data versus proteome data; date of publication) of the functional annotation of genes is different in the data sets available and summarized here. For example, while yeast genes are well characterized and annotated, a large number of genes in the archaeon *A. fulgidus* are still completely unknown (Figure 2). Moreover, it remains to be seen if and how gene duplications and functional redundancies influence the stress-response patterns in higher eukaryotes and plants, where high-quality expression analysis data are still widely missing. Currently, it might be hypothesized that higher eukaryotes express larger numbers of different chaperones and regulatory proteins while metabolic, nucleic acid repair, and detoxifying gene products are decreased in comparison to archaea and prokaryotes (Table S1). Furthermore, it might be speculated that this difference correlates with the transition from unicellular to multicellular organisms.

The increased need for chaperones and proteases is intuitive for a system that needs to restore its protein homeostasis network to the original balance; the expression of members of the other classes can be seen as measures to deal with specific damages. The comparison of the responses of different organisms indicates that the conservation of specific genes is rather low outside the class of molecular chaperones. This may reflect the fact that the importance of individual proteins within the proteome can vary significantly from species to species due to the respective biochemical lifestyle. Therefore, it may not be surprising that, beyond the conserved Hsps, a "personalized," species-specific set of damage-control proteins has evolved.

The heat shock response is a rapid and transient gene-expression program. However, when analyzed in detail, the expression kinetics for individual heat-inducible genes are quite diverse. In this respect, baker's yeast has been especially well characterized (Eisen et al., 1998; Gasch et al., 2000). When yeast cells are shifted from physiological growth (25°C) to heat shock conditions (37°C), the expression of most Hsps is increased rapidly, reaching maximum levels after 10–15 min (Figure 2). Nevertheless, some genes peak slightly later (after ~20 min) and others do not increase until after 2 hr (Eisen et al., 1998). In particular, the expression of genes involved in cell organization, DNA/RNA repair, and some metabolic processes lags behind. While the first, fast-expression phases correspond to processes that rapidly counteract the consequences of heat shock, the later phases may represent adaptation or recovery processes after stress damage. In terms of expression levels, it is obvious that the chaperone genes are among the most substantially upregulated Hsps not only in yeast but also in other species (Figure 2). Additionally, most of the chaperones are already present at high levels under physiological conditions. The massive need for chaperones reflects the fact that they are required in stoichiometric ratios relative to the unfolded client proteins. Thus, upon stress they become a major constituent of total cytosolic protein levels. A particularly impressive example is the thermosome of the archaeon *Pyrodictium occultum* (Phipps et al.,

1991). This protein, a member of the chaperonins (see below), represents ~13% of total cytosolic protein under physiological conditions and upon stress (i.e., a shift from 102°C to 108°C), it increases to ~35%.

Molecular Chaperones

The predominant class of Hsps, the molecular chaperones, comprises five major and broadly conserved families—Hsp100s, Hsp90s, Hsp70s, Hsp60s, and small heat shock proteins (sHsps). Several other heat-inducible molecular chaperones, like Hsp33 (Jakob et al., 1999; Kumsta and Jakob, 2009), are known. Since these are not ubiquitous, they are not included in a separate class. Interestingly, there is a constant need for chaperone assistance during de novo protein folding and refolding of nonnative polypeptide chains, as the stability of cellular proteins is low and aggregation competes with productive folding even at physiological temperatures (Gragerov et al., 1991; Kerner et al., 2005; Mayer, 2010). In contrast to traditional enzymes, chaperones in general have to work at stoichiometric ratios to decrease the concentration of nonnative proteins and thus the aggregation potential of folding proteins, as protein aggregation is a higher-order reaction and therefore highly dependent on protein concentration (Kiefhaber et al., 1991).

All molecular chaperones interact promiscuously with a broad range of unfolded proteins (Bukau et al., 1996; Sharma et al., 2008; Viitanen et al., 1992; Walter and Buchner, 2002). What discriminates a native protein from its nonnative, partially or globally unfolded counterpart is an increased exposure of hydrophobic amino acids, a feature that is recognized by molecular chaperones. Binding may occur to hydrophobic patches, specific peptide sequences, or structural elements of the nonnative protein (Figure 3). Generally, molecular chaperones do not contribute structural information for folding, but prevent unwanted intermolecular interactions. They do this through controlled binding and release of nonnative proteins, which is usually accomplished by a change of the affinity of the chaperone for its substrate. This change between at least two affinity states is controlled by the binding and hydrolysis of ATP in most chaperone families, with the exception of sHsps. The latter seem optimized for efficient binding of nonnative proteins, thus presenting an efficient first line of defense. These ''holdases'' are often only expressed upon stress, while the ''foldases,'' like Hsp70 and Hsp90, come in stress-induced and constitutively expressed versions (e.g., Hsp70 versus Hsc70).

Chaperonins

Chaperonins are ring-shaped chaperones that encapsulate nonnative proteins in an ATP-dependent manner. In bacteria, the most prominent chaperonin is the GroE machinery (Figure 3). It consists of 14 GroEL subunits, arranged in a cylinder of two heptameric rings, to which the cochaperone GroES, also a heptameric ring, binds (Grallert and Buchner, 2001; Horwich et al., 2006). The closely related proteins in mitochondria are called Hsp60 and Hsp10. Nonnative protein chains in a molecular size range up to 60 kDa can be bound in the central cavity of the GroEL cylinder (Horwich et al., 2006). GroEL encapsulates one nonnative protein per cavity, and the cavity is then closed by the binding of the GroES cofactor in the presence of ATP (Hartl and Hayer-Hartl, 2002; Todd et al., 1994). How GroEL contributes to stress resistance is easy to rationalize. First, it binds a large variety of nonnative proteins; it was shown that about 50% of the *E. coli* proteins can bind to GroEL (Viitanen et al., 1992). Second, it internalizes and sequesters individual polypeptide chains for the length of the ATP hydrolysis cycle. During this time they may fold, or they may gain their native structure after release from GroE, depending on the folding properties of the respective protein. Thus, a GroE-bound protein can start folding in splendid isolation unaffected by other nonnative polypeptide chains. The downside of this mechanism is that a substantial amount of GroE is required to trap a significant fraction of the proteins that unfold under stress conditions. This implies that there is a limit to the protective effect of GroE, given that the level of upregulation of GroE expression is limited. This was also observed during the overexpression of aggregation-prone enzymes in *E. coli*. In these cases, the parallel overexpression of GroE resulted in a boost of the enzymatically active fraction of the recombinantly expressed target protein (Goloubinoff et al., 1989). Furthermore, GroE seems to be the decisive factor limiting the maximum growth temperature of *E. coli*. When an *E. coli* strain was evolved for growth at normally lethal temperature, the most significant change in the proteome was an enormous upregulation of the GroE proteins (Rudolph et al., 2010). This upregulation was much stronger than that observed under stress conditions. The mechanism of this massive GroE expression remains enigmatic. It is easy to imagine that such a potent protein-folding machine, which is essential in bacteria, should also have enormous importance in the stress management of eukaryotic cells. It is therefore surprising that there is no GroE in the eukaryotic or archaeal cytosol. However, it has been replaced by a distant relative, the CCT or TRiC machinery (also called the thermosome in thermophilic archaea), whose architecture and function resembles that of GroE (Dunn et al., 2001). Interestingly, the substrate spectrum of the eukaryotic chaperonin may be more limited (Yam et al., 2008), and, more importantly and stunningly, it is not a heat shock protein in these organisms. TRiC is actually downregulated under stress conditions in yeast (Eisen et al., 1998). This conundrum waits to be resolved: Why remove a protein-folding machine under conditions where it seems to be needed most? There is certainly more to the picture than meets the eye.

Hsp70

One of the most highly conserved chaperones is the Hsp70 protein. The prokaryotic version, called DnaK, shares about 60% sequence identity with eukaryotic Hsp70 proteins, which are found in the cytosol and in organelles, such as the ER, mitochondria, and chloroplasts. Under physiological conditions, Hsp70s are involved in the de novo folding of proteins, and under stress they prevent the aggregation of unfolding proteins and can even refold aggregated proteins (Mayer and Bukau, 2005). Hsp70 consists of two domains, an ATPase domain and a protein binding domain. The binding site accommodates a stretch of seven, mainly hydrophobic, amino acids in an extended conformation (Zhu et al., 1996). The interaction of Hsp70 with unstructured segments of polypeptides is ATP dependent in that it binds substrates with high affinity in the posthydrolysis ADP state (Figure 3). The activity of Hsp70s is regulated by cofactors. The largest class of Hsp70 cofactors is the group of Hsp40/

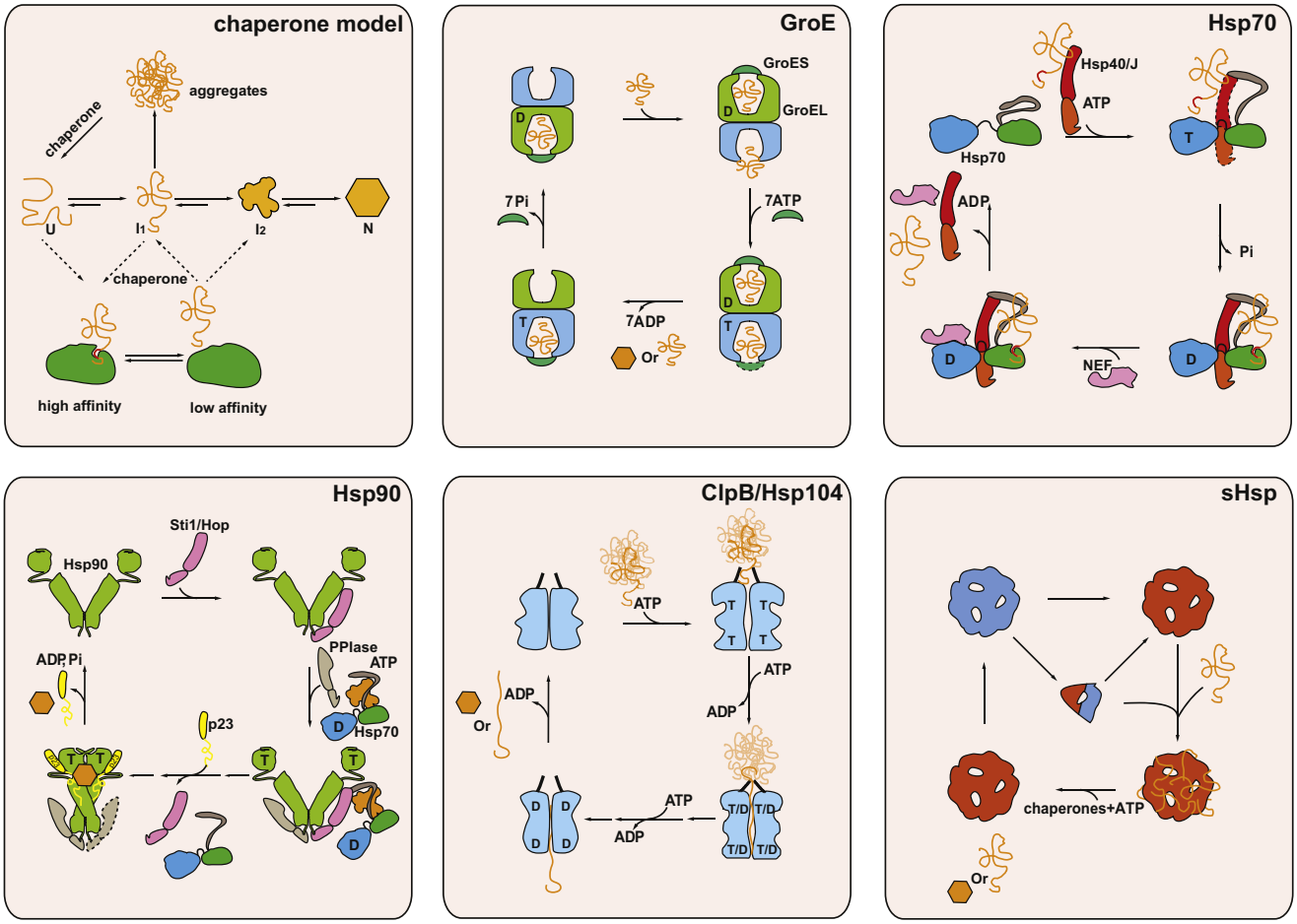

Figure 3. Molecular Chaperone Mechanisms

Chaperone model: In general, proteins fold via increasingly structured intermediates (I_1, I_2) from the unfolded state (U) to the folded state (N). Under heat shock conditions, this process is assumed to be reversed. Molecular chaperones bind proteins in nonnative conformations. The shift from the high-affinity binding state to the low-affinity release state is often triggered by ATP binding and hydrolysis.GroE: The GroE machinery in bacteria, mitochondria, and chloroplasts consists of two identical rings that enclose a central cavity each. Nonnative protein is bound by the apical domains of the rings, and upon binding of ATP and the cochaperone GroES, the protein is encapsulated and released into the cavity. Whether a complex consisting of GroEL and two GroES cochaperones or just one GroES bound is controversial; therefore the second GroES is shown with dashed lines. ATP hydrolysis in one ring results in the release of GroES and substrate protein from the opposite ring. During encapsulation the protein may fold partially or completely, depending on the characteristics of the respective substrate protein.

Hsp70: The Hsp70 system comprises two cochaperones, an activating protein (Hsp40/J-protein) and a nucleotide exchange factor (NEF). The activating protein can bind the nonnative protein and deliver it to Hsp70. It forms a complex with Hsp70 and stimulates its ATPase. The dashed line in the Hsp70-ATP complex indicates a transient interaction. It may also modulate the conformation of Hsp70 to stabilize a substrate protein-accepting state. Hsp70 binds a stretch of seven amino acids in the substrate protein. The NEF will induce the exchange of nucleotide. This further accelerates the ATPase cycle. The substrate protein is released presumably in a nonnative form.

Hsp90: In this chaperone system a large number of proteins work together. First, for a number of substrate proteins, Hsp70 delivers the substrates to Hsp90. It is not clear whether this is true for all substrate proteins and whether this occurs also under stress conditions. More than a dozen cochaperones of Hsp90 exist in eukaryotes, which seem to modulate the system. One of them, Sti1/Hop, binds both Hsp70 and Hsp90 and at the same time inhibits Hsp90s ATPase (in yeast). In this complex, which also contains an additional PPIase cochaperone, the substrate protein is transferred from Hsp70 to Hsp90. Sti1/Hop is released once Hsp90 binds nucleotide and a further cochaperone (p23). In contrast to other chaperones, the protein in complex with Hsp90 is assumed to be bound and released as a structured intermediate (such as I_2, see general chaperone scheme).

ClpB/Hsp104: In bacteria and yeast, this chaperone is able to dissolve aggregates by actively pulling proteins through a central channel of the hexameric structure. Each protomer contains two ATPase sites, which have quite distinct characteristics concerning turnover and function. During passage through the chaperone complex, the substrate protein is unfolded. Refolding can occur upon release, and, to some extent, it can also occur in cooperation with other chaperones.

sHsps: sHps are oligomeric complexes that are often activated, e.g., by heat or modifications. Many are believed to dissociate into smaller oligomers to become active. sHsps can bind many nonnative proteins per complex. Release requires cooperation with other ATP-dependent chaperones such as Hsp70.

J-domain-containing proteins (Kampinga and Craig, 2010). They bind the nonnative protein and deliver it to Hsp70. The J domains of these proteins interact with the ATPase domain of Hsp70 and stimulate the hydrolysis of bound ATP. The release of nucleotide and substrate is further accelerated by nucleotide-exchange factors (Figure 3). While many features of this hydrolysis cycle have been determined, the contribution of Hsp70 to the folding process of a protein or to dissolving aggregates (Goloubinoff

and De Los Rios, 2007) remains an important issue to be addressed.

Hsp90

Hsp90 is present at very high concentrations in the cytosol of bacteria and eukaryotic cells under physiological conditions, and it is further upregulated under stress (Welch and Feramisco, 1982). This chaperone is peculiar in several ways. First, it does not seem as promiscuous in its substrate spectrum as GroE or Hsp70 (Picard, 2002; Pratt and Toft, 2003; Smith, 1998). Second, it does not bind unfolded proteins, but rather native-like proteins (Jakob et al., 1995). Third, it appears to have evolved from a single component system in prokaryotes to the most sophisticated chaperone machinery known in eukaryotes, working together with a large cohort of cochaperones that associate in a defined order during the chaperone cycle (Figure 3) (Pearl and Prodromou, 2006; Taipale et al., 2010; Wandinger et al., 2008; Li et al., 2010). Interestingly, in the context of stress management, only two of these cochaperones are upregulated upon stress in yeast: Sti1 and the prolyl isomerase Cpr6 (Eisen et al., 1998) (Table S1). Sti1 is a noncompetitive inhibitor of the Hsp90 ATPase (Richter et al., 2003) that keeps Hsp90 in a conformation that may facilitate Hsp90s interaction with substrate proteins but prevents further conformational changes required for substrate processing (Hessling et al., 2009). Thus, under heat shock conditions, Sti1 might allow Hsp90 to perform a more basic holding function that prevents aggregation of unfolded proteins. Whether the substrate spectrum of Hsp90 changes under stress conditions is an important open issue. What happens to the Hsp90-bound substrates upon restoration of physiological conditions also remains to be determined.

Hsp100

The Hsp100 family comprises a conserved group of AAA ATPases, which in bacteria includes the proteins ClpA, ClpB, ClpC, ClpE, ClpX, ClpY, and others. Similar proteins are found in mitochondria as well as in plants, yeasts, and mammals (Barends et al., 2010). Hsp100 proteins are subdivided into two classes based on the number of AAA domains. Hsp100, class 1 proteins are dynamic hexameric structures containing two different nucleotide-binding sites in each monomer (Martin et al., 2005), which is true for the bacterial proteins ClpA, ClpB, ClpC, and ClpE as well as their homologs in plants (including ClpD) and yeast Hsp104. Similar proteins, which contain only one nucleotide binding module (Hsp100, class 2), include the proteins ClpX and ClpY (Schirmer et al., 1996). AAA ATPases with homology to Hsp100 proteins are also described in mammals, including p97/Cdc48 (which participates in protein quality control pathways) and the ATPase associated with the proteasome (Schrader et al., 2009). Hsp100 proteins are thought to pull misfolded proteins through the central pore of the hexameric ring in an unfolded state, enabling the proteins to become refolded (Figure 3) (Doyle et al., 2007; Schaupp et al., 2007; Weber-Ban et al., 1999). The exact mechanism of folding is still under debate. Importantly, Hsp100, class 1 proteins are able to support protein disaggregation. The disaggregation system composed of ClpB, Hsp70, and Hsp40-like proteins can extract substrates efficiently from aggregates and fold them in a mechanistically still to be defined way to the native state (Goloubinoff et al., 1999). It is strange that some higher eukaryotes (e.g., nematodes, arthropods, and mammals) lack cytosolic Hsp100, class 1 proteins. In contrast to the situation with CCT, there seems to be no related protein complex with comparable disaggregation properties in the genomes of these organisms.

Small HSPs

The family of sHsps displays a further variation on the theme of protecting proteins from irreversible aggregation by reversible interactions with a chaperone. They comprise the most widespread but also the most poorly conserved family of molecular chaperones. They show high heterogeneity both in sequence and size (Kriehuber et al., 2010). Their common trait is the conserved α-crystallin domain, which refers to the most prominent family member, the eye-lens protein α-crystallin (Horwitz, 2003). sHsps commonly form large dynamic oligomers, often composed of 24 subunits (Van Montfort et al., 2001). Functionally, sHsps are ATP-independent chaperones that interact with large numbers of partially folded target proteins to prevent their aggregation upon stress-induced unfolding (Haslbeck et al., 2005a; McHaourab et al., 2009). In the current view, sHsps serve as a storage depot for unfolded proteins, which can be refolded in the presence of other chaperones such as the Hsp70 and Hsp100 proteins (Mogk et al., 2003; Lee et al., 1997). It seems that sHsps are not only able to form soluble complexes with their unfolding clients but sometimes, especially when protein unfolding is massive in the cell, they are sequestered into the aggregates. This seems to be a special trait related to their passive holdase function which affects the structure of the aggregates and their remodelling by ATP-dependent chaperones (Haslbeck et al., 2005b; Cashikar et al., 2005; Liberek et al., 2008).

Protein Degradation as Tool for Homeostasis Control

Refolding and repairing damaged proteins requires less energy than destroying and synthesizing them anew. Nevertheless, protein degradation machineries are expressed as part of the stress response, especially in bacteria, showing the need for clearing irreversibly damaged proteins.

Proteases, particularly the HslU ATPase and its partner the HslV protease subunit, are among the highly inducible genes in the *E. coli* genome (Richmond et al., 1999). HslUV is a broad specificity protease (Kwon et al., 2004) composed of a hexameric AAA-ATPase subunit (HslU), which is thought to unfold the substrate and present the polypeptide to the catalytically active protease HslV. Other proteases, including Lon, HchA, PrlC, ClpXP, and ClpAP are also upregulated under stress conditions (Table S1) (Richmond et al., 1999). ClpXP and ClpAP are similar in their overall architecture to HslU/HslV (Thibault et al., 2006), and they have partially overlapping substrate spectra (Kwon et al., 2004). In addition to recognizing misfolded proteins, ClpXP and ClpAP recognize SsrA-tagged proteins and the N-end rule proteins (Dougan et al., 2010). Additionally, the adaptor protein ClpS alters the substrate spectrum of these proteases (Sundar et al., 2010; Lee et al., 2010; De Donatis et al., 2010).

In the bacterial periplasm the expression and activity of a protease called DegP is coupled to elevated temperatures. Interestingly, DegP exhibits either chaperone or protease function,

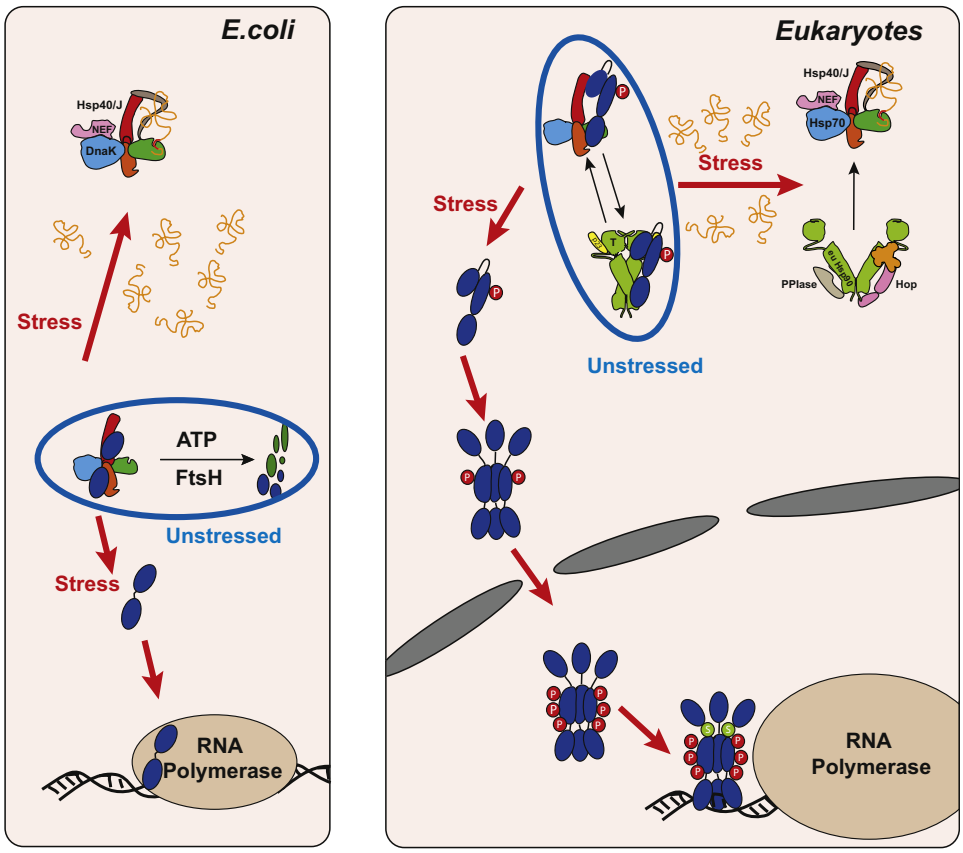

Figure 4. Regulation of the Heat Shock Response
(Left) In eukaryotes and in bacteria, the heat shock specific transcription factor is regulated by molecular chaperones. In Gram-negative bacteria, the Hsp70 and Hsp40 homologs, DnaK and DnaJ, bind to the transcription factor σ32, inactivate it and initiate its rapid degradation via the AAA-protease FtsH. Upon heat stress, chaperones engage in complexes with unfolded proteins and are therefore no longer available to form complexes with σ32. As a consequence, σ32 is active, binds to RNA polymerase, and stimulates the transcription of heat shock genes. (Right) In eukaryotes, a storage form of the heat shock specific transcription factor Hsf1 is maintained in an inactive monomeric form in complexes with the chaperones Hsp70 and Hsp90. As in the case of the prokaryotic system, the titration of chaperones by massive protein unfolding upon proteotoxic stress will result in the release of Hsf1 from chaperone inactivation. Monomeric Hsf1 then trimerizes, is transported into the nucleus, hyperphosphorylated, sumoylated, and activates heat shock gene transcription.

depending on the temperature. It assists the refolding of proteins, if they are not severely damaged, and degrades proteins under conditions where refolding is not feasible (Spiess et al., 1999). The substrate-controlled oligomerization of DegP into active 12-mers or 24-mers plays an important role in activating its proteolytic activity (Krojer et al., 2008; Merdanovic et al., 2010).

In yeast, only four components of its proteolytic system can be found among the most highly induced gene products. Two are vacuolar proteases, suggesting that much of the proteolytic work of the eukaryotic cell is performed in this specialized compartment. The expression of vacuolar proteases also hints to the possible involvement of the autophagic system in protein clearance. In addition to the two vacuolar proteases, the unspecific protease pep4 and ubiquitin are also induced after heat shock (Table S1), indicating that enhanced proteolysis involving proteasomal degradation seems to be necessary for protein degradation after heat shock.

Interestingly, multicellular systems (such as *Caenorhabditis elegans, Arabidopsis thaliana*, and human cell lines) overexpress seemingly fewer components of the proteolytic system compared to bacteria and yeast (Table S1). Few components of the proteasome machinery are upregulated (Parag et al., 1987). It seems that higher eukaryotes rely more on refolding and repair mechanisms, while in bacteria degradation plays a more prominent role (Wong and Houry, 2004).

Regulation of the Heat Shock Response

After the discovery of the heat shock response, it was puzzling how the upregulation of Hsps is achieved. It was immediately clear that the heat shock response requires a specific transcription factor (Wu et al., 1986; Wu, 1984), and it turns out that, in eukaryotes, the critical regulator is heat shock factor, Hsf1. Its binding to the heat shock element (HSE) on DNA initiates the assembly of the transcription machinery. In *E. coli*, the regulatory protein σ32 is responsible for Hsp overexpression (Grossman et al., 1984). σ32 is an alternative subunit of the bacterial RNA polymerase, which replaces the normal regulatory σ70 protein under heat stress. HSF1 and σ32 are not related in terms of structure or sequence, but they share basic mechanistic properties (Figure 4). It is believed that the disturbance of protein

homeostasis leads to the activation of Hsf1 and σ32. A model for the regulation of Hsf1 by chaperones has been proposed and subsequently refined (Voellmy and Boellmann, 2007). The chaperones Hsc70, Hsp90 (in eukaryotes), and Hsp40 have the potential to inhibit Hsf1 and σ32 (Figure 4). Under permissive conditions, σ32 is present in a complex with the Hsp70 protein DnaK and its cofactor DnaJ. It is primed for degradation by these chaperones, thereby reducing its cellular level and keeping the heat shock genes untranscribed (Rodriguez et al., 2008). According to the widely accepted chaperone titration model, the presence of increasing numbers of unfolded proteins upon heat shock releases σ32 from these chaperone complexes, as chaperones are required to bind unfolded proteins. A similar model is suggested for the eukaryotic transcription factor Hsf1. Hsf1 is kept in an inactive complex together with components of the Hsp90 chaperone system. The Hsp70/Hsp40 system also binds Hsf1 or acts as loading helper for the Hsp90 system (Figure 4). Hsf1 regulation is more complex than that of σ32, as phosphorylation, other posttranslational modifications, and oligomerization regulate Hsf1 activity (Akerfelt et al., 2010; Prahlad and Morimoto, 2009). In complex with chaperones, Hsf1 is a monomer. Its release leads to homotrimerization and transport into the nucleus. Here Hsf1 is hyperphosphorylated by several kinases (Holmberg et al., 2001). Further modification events, like sumoylation, regulate the activity of the final transcription factor complex (Hietakangas et al., 2003). Hsf1 acetylation has recently been reported to downregulate it (Westerheide et al., 2009).

The chaperone titration model elegantly explains the inactivation of heat shock transcription factors in the presence of unemployed chaperones, and their dramatic activation if chaperones are busy due to the presence of unfolded proteins. Once the cell returns to normal function, the excess of free chaperones leads again to the downregulation of the transcriptional regulator. It should be noted that additional regulatory processes participate in the transcriptional control, such as the alternate sigma factors σE, of mostly periplasmic genes (Rhodius and Mutalik, 2010). The activation signal seems to be the unfolding of outer membrane porins (Kim et al., 2010; Walsh et al., 2003; Hasenbein et al., 2010). Once σE is activated, it not only increases the expression of its target heat shock genes, but also that of σ32, thus supporting the massive synthesis of heat shock proteins in general (Erickson and Gross, 1989).

Evolutionary Conservation of Chaperone Networks

Generally, the main effectors of the heat shock response are highly conserved in all three domains of life. Interestingly, reduced sets of chaperones exist in a variety of species (Figure 5). While bacteria and lower eukaryotes usually encode all classical families of chaperones, higher eukaryotes (like nematodes, arthropods, and vertebrates) do not encode cytosolic Hsp100 proteins. In archaea, Hsp100 and Hsp90 proteins are usually not found, and many hyperthermophilic species lack Hsp70 in addition. In the archaeal species where members of these three families of molecular chaperones have been found, they are thought to have been acquired by gene-transfer events (Large et al., 2009; Macario et al., 2006). For reduced chaperone sets found in some bacterial and eukaryotic species, however, a secondary reduction from an ancestral complete set might be more likely.

Considerable cooperation between the chaperone systems is to be assumed. This has been shown for the chaperonins and the Hsp70 system and for Hsp70 and Hsp90 during the processing of steroid hormone receptors (Pratt and Toft, 2003). Hsp100 proteins generally are assumed to cooperate with Hsp70s during the processing of aggregated proteins by the chaperone system (Haslberger et al., 2007; Winkler et al., 2010). Furthermore, by virtue of their mode of action, sHps are team players that rely on the Hsp100 and Hsp70 systems. Interestingly, the loss of chaperonins, Hsp70, Hsp90, or Hsp100 in different species implies that each of these foldases can in principle be replaced. Thus, these systems may represent different evolutionary solutions for the same problem, to hold and fold nonnative proteins. Interestingly, sHsps are always present together with at least one of the foldase machineries. It will be fascinating to see, whether the refolding of sHsp-trapped nonnative proteins can be executed by either of the foldases equally well. Certainly, it is important to bear in mind that chaperones are just one (albeit important) segment of the heat shock response. Contributions of Hsps involved in protein degradation or metabolic modulation have to be taken into consideration to understand how these organisms deal with heat stress (Tachdjian and Kelly, 2006; Wong and Houry, 2004).

In the context of these chaperone networks, it should also be pointed out that besides the network involved in protein refolding after stress, there are other chaperone networks that work primarily in de novo protein folding (Albanèse et al., 2006; Deuerling and Bukau, 2004; Rospert and Chacinska, 2006). Certainly, these networks overlap in single components (e.g., Hsp70) but they are also strikingly different in some components, as highlighted by the downregulation of CCT in yeast upon heat stress (Figure 5). Additionally, ribosome-associated chaperones are mainly involved in de novo protein folding and in the cold shock response but not in refolding after heat shock (Rospert and Chacinska, 2006; Albanèse et al., 2006).

With respect to metabolic modulation, it is interesting to note that in plants and in a wide variety of other organisms, like yeast and insects, the enhanced synthesis of the sugar trehalose upon heat stress enhances thermotolerance and protects against desiccation (Singer and Lindquist, 1998b; Crowe et al., 2001). Interestingly, trehalose levels rise very rapidly, correlating with the expression of its major metabolic enzyme Tps1 (Table S1), and rapidly decrease during the recovery from heat stress (Hottiger et al., 1987). Trehalose seems to stabilize proteins and suppresses their aggregation and thus contributes as a "chemical chaperone" under severe stress conditions (Hottiger et al., 1994; Singer and Lindquist, 1998a). In eukaryotes, reversible sumoylation of proteins generally increases in response to heat stress and was shown to increase the solubility of modified proteins (Meulmeester and Melchior, 2008), suggesting that sumoylation might represent another protein stabilization system.

Conclusions

Many questions on the function of molecular chaperones and the heat shock response in general have been answered in the last decades. Still, the precise regulation of the response is not

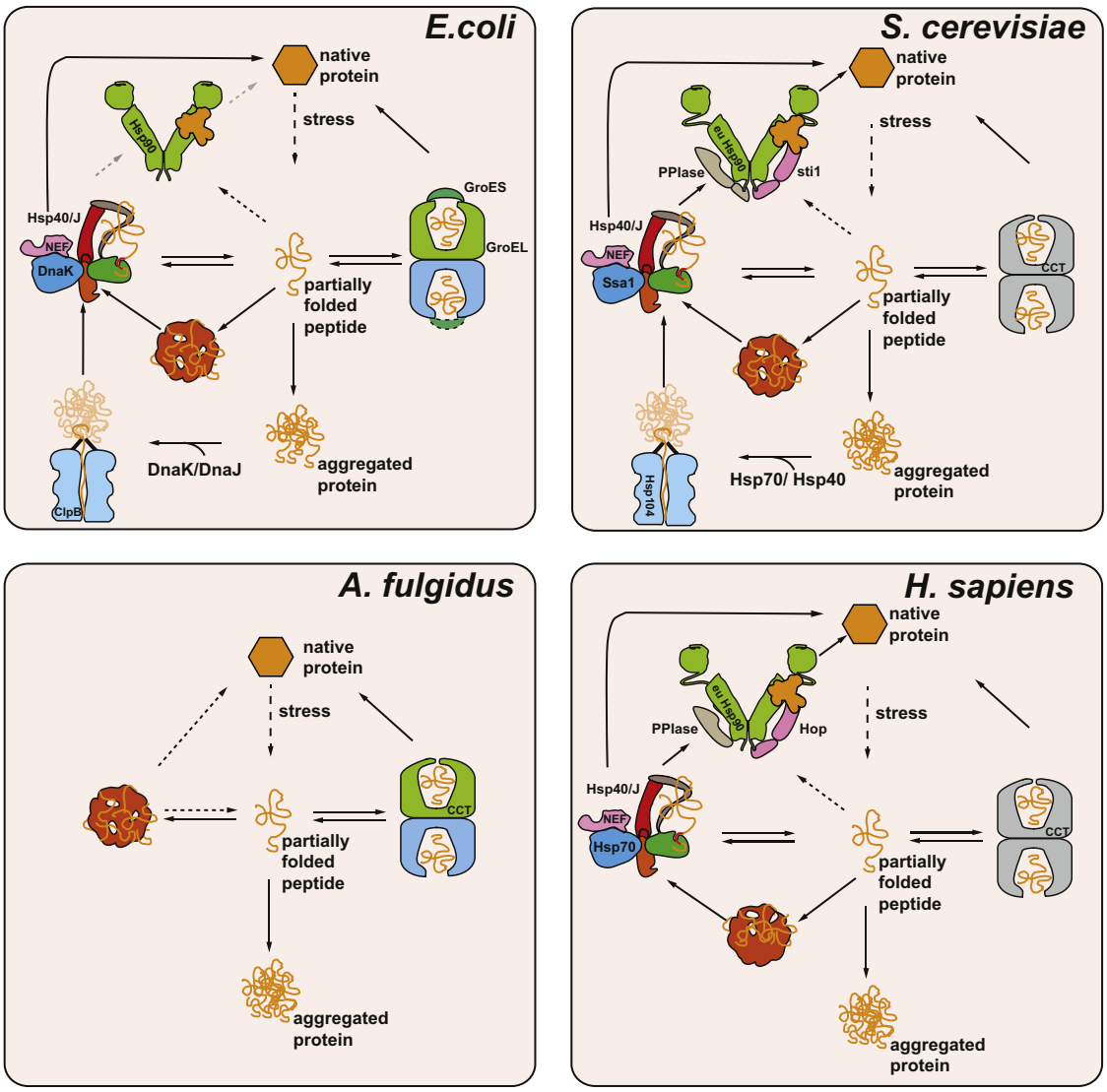

Figure 5. Chaperone Networks

This cartoon summarizes information on the chaperone networks present under heat shock conditions in different organisms. *E. coli:* In bacteria, all the classical molecular chaperones are upregulated, including GroE, Hsp70, sHsps, and Hsp90. In addition ClpB as an aggregate remodeler is present. *S. cerevisiae:* Under stress conditions, yeast seems to rely on Hsp70, sHsps, Hsp104, and a subset of the Hsp90 system, as only two of the many cochaperones of Hsp90 are upregulated upon stress. The GroE homolog CCT/TriC is shown in gray as it is abundant under physiological conditions but downregulated upon heat stress. *A. fulgidus*: In this archaeon, the chaperone system upregulated upon heat shock consists only of two components, sHps and CCT/thermosome. Both are present at high concentration. It is unclear how the protein is released in this case. *H. sapiens*: In human cells, Hsp70, Hsp90, and sHsps are upregulated, while CCT/TriC is not. Note that there is no ClpB/Hsp104 homolog.

understood comprehensively. Certainly, temperature is one of the key parameters for organisms to monitor. In principle, two main ways of monitoring nonphysiological temperatures can be distinguished. First, there are direct, primary thermosensory structures, which "measure" temperature such as DNA, RNA, specific proteins, or lipids (Klinkert and Narberhaus, 2009). Examples for changes in DNA and RNA topology in response to heat and cold are the secondary structure of the 5′UTR for heat shock in the *E. coli* gene *rpoH*, and cold shock in *cspA* (Fang et al., 1997; Morita et al., 1999). Such temperature-sensing characteristics have also been attributed to proteins such as the yeast sHsp Hsp26 (Haslbeck et al., 1999) and the *E. coli* nucleotide-exchange factor GrpE, which changes the DnaK function from foldase to holdase at elevated temperatures (Groemping and Reinstein, 2001). Additionally, the rigidity or fluidity of membranes seems to be monitored by the cell, with a more rigid membrane being a signal to orchestrate the cold shock response (Shivaji and Prakash, 2010; Vigh et al., 1993). In this context, plants seem to sense temperature changes at the membranes where the heat signal transiently activates calcium-permeable channels (Saidi et al., 2009). Second, there are indirect signals such as the accumulation of denatured proteins in the case of heat or the

accumulation of stalled ribosomes under cold shock conditions (Klinkert and Narberhaus, 2009). As detailed above, the regulatory feedback system of the heat shock response is a protein based, very sensitive and integrative thermometer itself, as it "measures" all deviations from physiological conditions based on their cumulative effects on cellular protein homeostasis.

So far we have described the survival program of an individual cell or single-cell organisms, such as archaea, bacteria, and yeast. Certainly, each cell can induce the heat shock response autonomously. However, it is important to understand the additional levels of regulation that might exist in multicellular organisms. Until recently, this has been largely uncharted territory. The established view is that each cell is an independent sensory unit with its own stress management system. This view is certainly supported by experiments with cultured mammalian cells (Welch and Suhan, 1986) and by the conservation of HSF signaling from yeast to man. However, this scenario lacks one of the key traits of multicellular organisms, the coordinated and hierarchical response, which involves integration of the signal in a tissue, an organ, or even the whole organism. Such a model requires specific stress-sensing cells and a way to pass the signal to surrounding tissue or in a systemic, hormone-like manner. Recent experiments on the heat shock response in the nematode *C. elegans* revealed that such a system exists. There are master cells that are specialized in sensing heat shock and other toxic stresses (Prahlad et al., 2008). These cells are specific neurons that use well-known neurotransmitters for stress signaling. How this allows the recipient cells to differentiate between a stress signal and a neurostimulatory effect remains to be seen. It should be noted that the two mechanisms, autonomous decisions on the cell level and an integrative specialized sensing function of certain cells, are not mutually exclusive. On the contrary, only together they allow efficient stress management by combining a local, focused, cell-by-cell response to specific needs or shortcomings due to acute stress situations with a more general, integrative response signal that potentially considers the overall stress status of the organism. Certainly, we are just at the beginning of this area of research. It is of utmost importance to unveil the secrets behind the interplay between cellular, tissue, and organismal stress responses. It will also be important to understand the function of Hsp networks not only in the context of heat shock and other stresses, but also, and maybe more importantly, in the context of disease and aging.

SUPPLEMENTAL INFORMATION

Supplemental Information includes one table and can be found with this article online at doi:10.1016/j.molcel.2010.10.006.

ACKNOWLEDGMENTS

This work was funded by grants from the DFG to K.R., M.H. and J.B. The authors thank Jing Li, Eva Kriehuber, and Thomas Kriehuber for help in creating the figures and the table.

REFERENCES

Akerfelt, M., Morimoto, R.I., and Sistonen, L. (2010). Heat shock factors: integrators of cell stress, development and lifespan. Nat. Rev. Mol. Cell Biol. *11*, 545–555.

Al Refaii, A., and Alix, J.H. (2009). Ribosome biogenesis is temperature-dependent and delayed in Escherichia coli lacking the chaperones DnaK or DnaJ. Mol. Microbiol. *71*, 748–762.

Albanèse, V., Yam, A.Y., Baughman, J., Parnot, C., and Frydman, J. (2006). Systems analyses reveal two chaperone networks with distinct functions in eukaryotic cells. Cell *124*, 75–88.

Ashburner, M., and Bonner, J.J. (1979). The induction of gene activity in drosophilia by heat shock. Cell *17*, 241–254.

Barends, T.R., Werbeck, N.D., and Reinstein, J. (2010). Disaggregases in 4 dimensions. Curr. Opin. Struct. Biol. *20*, 46–53.

Boulon, S., Westman, B.J., Hutten, S., Boisvert, F.M., and Lamond, A.I. (2010). The nucleolus under stress. Mol. Cell *40*, this issue, 216–227.

Brown, J.R., and Lupas, A.N. (1998). What makes a thermophile? Trends Microbiol. *6*, 349–350.

Buchan, J.R., and Parker, R. (2009). Eukaryotic stress granules: the ins and outs of translation. Mol. Cell *36*, 932–941.

Bügl, B., Staker, B.L., Zheng, F., Kushner, S.R., Saper, M.A., Bardwell, J.C., and Jakob, U. (2000). RNA methylation under heat shock control. Mol. Cell *6*, 349–360.

Bukau, B., Hesterkamp, T., and Luirink, J. (1996). Growing up in a dangerous environment: a network of multiple targeting and folding pathways for nascent polypeptides in the cytosol. Trends Cell Biol. *6*, 480–486.

Cashikar, A.G., Duennwald, M., and Lindquist, S.L. (2005). A chaperone pathway in protein disaggregation. Hsp26 alters the nature of protein aggregates to facilitate reactivation by Hsp104. J. Biol. Chem. *280*, 23869–23875.

Coote, P.J., Cole, M.B., and Jones, M.V. (1991). Induction of increased thermotolerance in Saccharomyces cerevisiae may be triggered by a mechanism involving intracellular pH. J. Gen. Microbiol. *137*, 1701–1708.

Courgeon, A.M., Maisonhaute, C., and Best-Belpomme, M. (1984). Heat shock proteins are induced by cadmium in Drosophila cells. Exp. Cell Res. *153*, 515–521.

Crowe, J.H., Crowe, L.M., Oliver, A.E., Tsvetkova, N., Wolkers, W., and Tablin, F. (2001). The trehalose myth revisited: introduction to a symposium on stabilization of cells in the dry state. Cryobiology *43*, 89–105.

D'Amico, S., Collins, T., Marx, J.C., Feller, G., and Gerday, C. (2006). Psychrophilic microorganisms: challenges for life. EMBO Rep. *7*, 385–389.

De Donatis, G.M., Singh, S.K., Viswanathan, S., and Maurizi, M.R. (2010). A single ClpS monomer is sufficient to direct the activity of the ClpA hexamer. J. Biol. Chem. *285*, 8771–8781.

Deuerling, E., and Bukau, B. (2004). Chaperone-assisted folding of newly synthesized proteins in the cytosol. Crit. Rev. Biochem. Mol. Biol. *39*, 261–277.

Dougan, D.A., Truscott, K.N., and Zeth, K. (2010). The bacterial N-end rule pathway: expect the unexpected. Mol. Microbiol. *76*, 545–558.

Doyle, S.M., Shorter, J., Zolkiewski, M., Hoskins, J.R., Lindquist, S., and Wickner, S. (2007). Asymmetric deceleration of ClpB or Hsp104 ATPase activity unleashes protein-remodeling activity. Nat. Struct. Mol. Biol. *14*, 114–122.

Dunn, A.Y., Melville, M.W., and Frydman, J. (2001). Review: cellular substrates of the eukaryotic chaperonin TRiC/CCT. J. Struct. Biol. *135*, 176–184.

Eisen, M.B., Spellman, P.T., Brown, P.O., and Botstein, D. (1998). Cluster analysis and display of genome-wide expression patterns. Proc. Natl. Acad. Sci. USA *95*, 14863–14868.

Ellis, R.J., van der Vies, S.M., and Hemmingsen, S.M. (1989). The molecular chaperone concept. Biochem. Soc. Symp. *55*, 145–153.

Erickson, J.W., and Gross, C.A. (1989). Identification of the sigma E subunit of Escherichia coli RNA polymerase: a second alternate sigma factor involved in high-temperature gene expression. Genes Dev. *3*, 1462–1471.

Fang, L., Jiang, W., Bae, W., and Inouye, M. (1997). Promoter-independent cold-shock induction of cspA and its derepression at 37 degrees C by mRNA stabilization. Mol. Microbiol. *23*, 355–364.

Gasch, A.P., Spellman, P.T., Kao, C.M., Carmel-Harel, O., Eisen, M.B., Storz, G., Botstein, D., and Brown, P.O. (2000). Genomic expression programs in the response of yeast cells to environmental changes. Mol. Biol. Cell *11*, 4241–4257.

Goldschmidt, R. (1935). Gen und Ausseneigenschaft. Indukt. Abstammungs Vererbungsl. *69*, 38–131.

Goloubinoff, P., Gatenby, A.A., and Lorimer, G.H. (1989). GroE heat-shock proteins promote assembly of foreign prokaryotic ribulose bisphosphate carboxylase oligomers in Escherichia coli. Nature *337*, 44–47.

Goloubinoff, P., Mogk, A., Zvi, A.P., Tomoyasu, T., and Bukau, B. (1999). Sequential mechanism of solubilization and refolding of stable protein aggregates by a bichaperone network. Proc. Natl. Acad. Sci. USA *96*, 13732–13737.

Goloubinoff, P., and De Los Rios, P. (2007). The mechanism of Hsp70 chaperones: (entropic) pulling the models together. Trends Biochem. Sci. *32*, 372–380.

Gragerov, A.I., Martin, E.S., Krupenko, M.A., Kashlev, M.V., and Nikiforov, V.G. (1991). Protein aggregation and inclusion body formation in Escherichia coli rpoH mutant defective in heat shock protein induction. FEBS Lett. *291*, 222–224.

Grallert, H., and Buchner, J. (2001). Review: a structural view of the GroE chaperone cycle. J. Struct. Biol. *135*, 95–103.

Groemping, Y., and Reinstein, J. (2001). Folding properties of the nucleotide exchange factor GrpE from Thermus thermophilus: GrpE is a thermosensor that mediates heat shock response. J. Mol. Biol. *314*, 167–178.

Grossman, A.D., Erickson, J.W., and Gross, C.A. (1984). The htpR gene product of E. coli is a sigma factor for heat-shock promoters. Cell *38*, 383–390.

GuhaThakurta, D., Palomar, L., Stormo, G.D., Tedesco, P., Johnson, T.E., Walker, D.W., Lithgow, G., Kim, S., and Link, C.D. (2002). Identification of a novel cis-regulatory element involved in the heat shock response in Caenorhabditis elegans using microarray gene expression and computational methods. Genome Res. *12*, 701–712.

Hartl, F.U., and Hayer-Hartl, M. (2002). Molecular chaperones in the cytosol: from nascent chain to folded protein. Science *295*, 1852–1858.

Hasenbein, S., Meltzer, M., Hauske, P., Kaiser, M., Huber, R., Clausen, T., and Ehrmann, M. (2010). Conversion of a regulatory into a degradative protease. J. Mol. Biol. *397*, 957–966.

Haslbeck, M., Franzmann, T., Weinfurtner, D., and Buchner, J. (2005a). Some like it hot: the structure and function of small heat-shock proteins. Nat. Struct. Mol. Biol. *12*, 842–846.

Haslbeck, M., Miess, A., Stromer, T., Walter, S., and Buchner, J. (2005b). Disassembling protein aggregates in the yeast cytosol. The cooperation of Hsp26 with Ssa1 and Hsp104. J. Biol. Chem. *280*, 23861–23868.

Haslbeck, M., Walke, S., Stromer, T., Ehrnsperger, M., White, H.E., Chen, S., Saibil, H.R., and Buchner, J. (1999). Hsp26: a temperature-regulated chaperone. EMBO J. *18*, 6744–6751.

Haslberger, T., Weibezahn, J., Zahn, R., Lee, S., Tsai, F.T., Bukau, B., and Mogk, A. (2007). M domains couple the ClpB threading motor with the DnaK chaperone activity. Mol. Cell *25*, 247–260.

Heikkila, J.J., Schultz, G.A., Iatrou, K., and Gedamu, L. (1982). Expression of a set of fish genes following heat or metal ion exposure. J. Biol. Chem. *257*, 12000–12005.

Hessling, M., Richter, K., and Buchner, J. (2009). Dissection of the ATP-induced conformational cycle of the molecular chaperone Hsp90. Nat. Struct. Mol. Biol. *16*, 287–293.

Hietakangas, V., Ahlskog, J.K., Jakobsson, A.M., Hellesuo, M., Sahlberg, N.M., Holmberg, C.I., Mikhailov, A., Palvimo, J.J., Pirkkala, L., and Sistonen, L. (2003). Phosphorylation of serine 303 is a prerequisite for the stress-inducible SUMO modification of heat shock factor 1. Mol. Cell. Biol. *23*, 2953–2968.

Holmberg, C.I., Hietakangas, V., Mikhailov, A., Rantanen, J.O., Kallio, M., Meinander, A., Hellman, J., Morrice, N., MacKintosh, C., Morimoto, R.I., et al. (2001). Phosphorylation of serine 230 promotes inducible transcriptional activity of heat shock factor 1. EMBO J. *20*, 3800–3810.

Horwich, A.L., Farr, G.W., and Fenton, W.A. (2006). GroEL-GroES-mediated protein folding. Chem. Rev. *106*, 1917–1930.

Horwitz, J. (2003). Alpha-crystallin. Exp. Eye Res. *76*, 145–153.

Hottiger, T., De Virgilio, C., Hall, M.N., Boller, T., and Wiemken, A. (1994). The role of trehalose synthesis for the acquisition of thermotolerance in yeast. II. Physiological concentrations of trehalose increase the thermal stability of proteins in vitro. Eur. J. Biochem. *219*, 187–193.

Hottiger, T., Schmutz, P., and Wiemken, A. (1987). Heat-induced accumulation and futile cycling of trehalose in Saccharomyces cerevisiae. J. Bacteriol. *169*, 5518–5522.

Jakob, U., Lilie, H., Meyer, I., and Buchner, J. (1995). Transient interaction of Hsp90 with early unfolding intermediates of citrate synthase. Implications for heat shock in vivo. J. Biol. Chem. *270*, 7288–7294.

Jakob, U., Muse, W., Eser, M., and Bardwell, J.C. (1999). Chaperone activity with a redox switch. Cell *96*, 341–352.

Jamrich, M., Greenleaf, A.L., and Bautz, E.K. (1977). Localization of RNA polymerase in polytene chromosomes of Drosophila melanogaster. Proc. Natl. Acad. Sci. USA *74*, 2079–2083.

Jantschitsch, C., and Trautinger, F. (2003). Heat shock and UV-B-induced DNA damage and mutagenesis in skin. Photochem. Photobiol. Sci. *2*, 899–903.

Kampinga, H.H., and Craig, E.A. (2010). The HSP70 chaperone machinery: J proteins as drivers of functional specificity. Nat. Rev. Mol. Cell Biol. *11*, 579–592.

Kelley, P.M., and Schlesinger, M.J. (1978). The effect of amino acid analogues and heat shock on gene expression in chicken embryo fibroblasts. Cell *15*, 1277–1286.

Kerner, M.J., Naylor, D.J., Ishihama, Y., Maier, T., Chang, H.C., Stines, A.P., Georgopoulos, C., Frishman, D., Hayer-Hartl, M., Mann, M., and Hartl, F.U. (2005). Proteome-wide analysis of chaperonin-dependent protein folding in Escherichia coli. Cell *122*, 209–220.

Kiefhaber, T., Rudolph, R., Kohler, H.H., and Buchner, J. (1991). Protein aggregation in vitro and in vivo: a quantitative model of the kinetic competition between folding and aggregation. Biotechnology (N. Y.) *9*, 825–829.

Kim, D.Y., Kwon, E., Choi, J., Hwang, H.Y., and Kim, K.K. (2010). Structural basis for the negative regulation of bacterial stress response by RseB. Protein Sci. *19*, 1258–1263.

Kirkegaard, T., Roth, A.G., Petersen, N.H., Mahalka, A.K., Olsen, O.D., Moilanen, I., Zylicz, A., Knudsen, J., Sandhoff, K., Arenz, C., et al. (2010). Hsp70 stabilizes lysosomes and reverts Niemann-Pick disease-associated lysosomal pathology. Nature *463*, 549–553.

Klinkert, B., and Narberhaus, F. (2009). Microbial thermosensors. Cell. Mol. Life Sci. *66*, 2661–2676.

Kriehuber, T., Rattei, T., Weinmaier, T., Bepperling, A., Haslbeck, M., and Buchner, J. (2010). Independent evolution of the core domain and its flanking sequences in small heat shock proteins. FASEB J. *24*, 3633–3642.

Krojer, T., Sawa, J., Schäfer, E., Saibil, H.R., Ehrmann, M., and Clausen, T. (2008). Structural basis for the regulated protease and chaperone function of DegP. Nature *453*, 885–890.

Kruuv, J., Glofcheski, D., Cheng, K.H., Campbell, S.D., Al-Qysi, H.M., Nolan, W.T., and Lepock, J.R. (1983). Factors influencing survival and growth of mammalian cells exposed to hypothermia. I. Effects of temperature and membrane lipid perturbers. J. Cell. Physiol. *115*, 179–185.

Kumsta, C., and Jakob, U. (2009). Redox-regulated chaperones. Biochemistry *48*, 4666–4676.

Kwon, A.R., Trame, C.B., and McKay, D.B. (2004). Kinetics of protein substrate degradation by HslUV. J. Struct. Biol. *146*, 141–147.

Lambowitz, A.M., Kobayashi, G.S., Painter, A., and Medoff, G. (1983). Possible relationship of morphogenesis in pathogenic fungus, Histoplasma capsulatum, to heat shock response. Nature *303*, 806–808.

Large, A.T., Goldberg, M.D., and Lund, P.A. (2009). Chaperones and protein folding in the archaea. Biochem. Soc. Trans. *37*, 46–51.

Larkindale, J., and Vierling, E. (2008). Core genome responses involved in acclimation to high temperature. Plant Physiol. *146*, 748–761.

Lee, G.J., Roseman, A.M., Saibil, H.R., and Vierling, E. (1997). A small heat shock protein stably binds heat-denatured model substrates and can maintain a substrate in a folding-competent state. EMBO J. *16*, 659–671.

Lee, M.E., Baker, T.A., and Sauer, R.T. (2010). Control of substrate gating and translocation into ClpP by channel residues and ClpX binding. J. Mol. Biol. *399*, 707–718.

Lemaux, P.G., Herendeen, S.L., Bloch, P.L., and Neidhardt, F.C. (1978). Transient rates of synthesis of individual polypeptides in E. coli following temperature shifts. Cell *13*, 427–434.

Li, J., Richter, K., and Buchner, J. (2010). Mixed Hsp90-cochaperone complexes are important for the progression of the reaction cycle. Nat. Struct. Mol. Biol., in press.

Liberek, K., Lewandowska, A., and Zietkiewicz, S. (2008). Chaperones in control of protein disaggregation. EMBO J. *27*, 328–335.

Lindquist, S. (1980). Varying patterns of protein synthesis in Drosophila during heat shock: implications for regulation. Dev. Biol. *77*, 463–479.

Lindquist, S. (1986). The heat-shock response. Annu. Rev. Biochem. *55*, 1151–1191.

Lindquist, S., and Craig, E.A. (1988). The heat-shock proteins. Annu. Rev. Genet. *22*, 631–677.

Macario, A.J., Brocchieri, L., Shenoy, A.R., and Conway de Macario, E. (2006). Evolution of a protein-folding machine: genomic and evolutionary analyses reveal three lineages of the archaeal hsp70(dnaK) gene. J. Mol. Evol. *63*, 74–86.

Malmendal, A., Overgaard, J., Bundy, J.G., Sørensen, J.G., Nielsen, N.C., Loeschcke, V., and Holmstrup, M. (2006). Metabolomic profiling of heat stress: hardening and recovery of homeostasis in Drosophila. Am. J. Physiol. Regul. Integr. Comp. Physiol. *291*, R205–R212.

Martin, A., Baker, T.A., and Sauer, R.T. (2005). Rebuilt AAA + motors reveal operating principles for ATP-fuelled machines. Nature *437*, 1115–1120.

Matsuura, H., Ishibashi, Y., Shinmyo, A., Kanaya, S., and Kato, K. (2010). Genome-wide analyses of early translational responses to elevated temperature and high salinity in Arabidopsis thaliana. Plant Cell Physiol. *51*, 448–462.

Mayer, M.P., and Bukau, B. (2005). Hsp70 chaperones: cellular functions and molecular mechanism. Cell. Mol. Life Sci. *62*, 670–684.

Mayer, M.P. (2010). Gymnastics of molecular chaperones. Mol. Cell *39*, 321–331.

McAlister, L., and Finkelstein, D.B. (1980). Heat shock proteins and thermal resistance in yeast. Biochem. Biophys. Res. Commun. *93*, 819–824.

McHaourab, H.S., Godar, J.A., and Stewart, P.L. (2009). Structure and mechanism of protein stability sensors: chaperone activity of small heat shock proteins. Biochemistry *48*, 3828–3837.

Merdanovic, M., Mamant, N., Meltzer, M., Poepsel, S., Auckenthaler, A., Melgaard, R., Hauske, P., Nagel-Steger, L., Clarke, A.R., Kaiser, M., et al. (2010). Determinants of structural and functional plasticity of a widely conserved protease chaperone complex. Nat. Struct. Mol. Biol. *17*, 837–843.

Meulmeester, E., and Melchior, F. (2008). Cell biology: SUMO. Nature *452*, 709–711.

Michel, G.P., and Starka, J. (1986). Effect of ethanol and heat stresses on the protein pattern of Zymomonas mobilis. J. Bacteriol. *165*, 1040–1042.

Mogk, A., Schlieker, C., Friedrich, K.L., Schönfeld, H.J., Vierling, E., and Bukau, B. (2003). Refolding of substrates bound to small Hsps relies on a disaggregation reaction mediated most efficiently by ClpB/DnaK. J. Biol. Chem. *278*, 31033–31042.

Morimoto, R., Tissieres, A., and Georgopoulos, C.P. (1990). Stress Proteins. In Biology and Medicine. R.I., Morimoto, A. Tissieres and C. Georgopoulos, eds. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory), p. 450.

Morita, M.T., Tanaka, Y., Kodama, T.S., Kyogoku, Y., Yanagi, H., and Yura, T. (1999). Translational induction of heat shock transcription factor sigma32: evidence for a built-in RNA thermosensor. Genes Dev. *13*, 655–665.

Nagy, E., Balogi, Z., Gombos, I., Akerfelt, M., Björkbom, A., Balogh, G., Török, Z., Maslyanko, A., Fiszer-Kierzkowska, A., Lisowska, K., et al. (2007). Hyperfluidization-coupled membrane microdomain reorganization is linked to activation of the heat shock response in a murine melanoma cell line. Proc. Natl. Acad. Sci. USA *104*, 7945–7950.

Nover, L., Scharf, K.D., and Neumann, D. (1989). Cytoplasmic heat shock granules are formed from precursor particles and are associated with a specific set of mRNAs. Mol. Cell. Biol. *9*, 1298–1308.

Parag, H.A., Raboy, B., and Kulka, R.G. (1987). Effect of heat shock on protein degradation in mammalian cells: involvement of the ubiquitin system. EMBO J. *6*, 55–61.

Patriarca, E.J., and Maresca, B. (1990). Acquired thermotolerance following heat shock protein synthesis prevents impairment of mitochondrial ATPase activity at elevated temperatures in Saccharomyces cerevisiae. Exp. Cell Res. *190*, 57–64.

Pearl, L.H., and Prodromou, C. (2006). Structure and mechanism of the Hsp90 molecular chaperone machinery. Annu. Rev. Biochem. *75*, 271–294.

Peterson, N.S., Moller, G., and Mitchell, H.K. (1979). Genetic mapping of the coding regions for three heat-shock proteins in Drosophila melanogaster. Genetics *92*, 891–902.

Phipps, B.M., Hoffmann, A., Stetter, K.O., and Baumeister, W. (1991). A novel ATPase complex selectively accumulated upon heat shock is a major cellular component of thermophilic archaebacteria. EMBO J. *10*, 1711–1722.

Picard, D. (2002). Heat-shock protein 90, a chaperone for folding and regulation. Cell. Mol. Life Sci. *59*, 1640–1648.

Piper, P.W., Millson, S.H., Mollapour, M., Panaretou, B., Siligardi, G., Pearl, L.H., and Prodromou, C. (2003). Sensitivity to Hsp90-targeting drugs can arise with mutation to the Hsp90 chaperone, cochaperones and plasma membrane ATP binding cassette transporters of yeast. Eur. J. Biochem. *270*, 4689–4695.

Prahlad, V., Cornelius, T., and Morimoto, R.I. (2008). Regulation of the cellular heat shock response in Caenorhabditis elegans by thermosensory neurons. Science *320*, 811–814.

Prahlad, V., and Morimoto, R.I. (2009). Integrating the stress response: lesson for neurodegenerative diseases from C. elegans. Trends Cell Biol. *2*, 52–61.

Pratt, W.B., and Toft, D.O. (2003). Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery. Exp. Biol. Med. (Maywood) *228*, 111–133.

Rhodius, V.A., and Mutalik, V.K. (2010). Predicting strength and function for promoters of the Escherichia coli alternate sigma factor, σ. E. Proc. Natl. Acad. Sci. USA *107*, 2854–2859.

Richmond, C.S., Glasner, J.D., Mau, R., Jin, H., and Blattner, F.R. (1999). Genome-wide expression profiling in Escherichia coli K-12. Nucleic Acids Res. *27*, 3821–3835.

Richter, K., Muschler, P., Hainzl, O., Reinstein, J., and Buchner, J. (2003). Sti1 is a non-competitive inhibitor of the Hsp90 ATPase. Binding prevents the N-terminal dimerization reaction during the atpase cycle. J. Biol. Chem. *278*, 10328–10333.

Ritossa, F.M. (1962). A new puffing pattern induced by a temperature shock and DNP in Drosophila. Experientia *18*, 571–573.

Rodriguez, F., Arsène-Ploetze, F., Rist, W., Rüdiger, S., Schneider-Mergener, J., Mayer, M.P., and Bukau, B. (2008). Molecular basis for regulation of the heat shock transcription factor sigma32 by the DnaK and DnaJ chaperones. Mol. Cell *32*, 347–358.

Rohlin, L., Trent, J.D., Salmon, K., Kim, U., Gunsalus, R.P., and Liao, J.C. (2005). Heat shock response of Archaeoglobus fulgidus. J. Bacteriol. *187*, 6046–6057.

Rospert, S., and Chacinska, A. (2006). Distinct yet linked: chaperone networks in the eukaryotic cytosol. Genome Biol. *7*, 208.

Rudolph, B., Gebendorfer, K.M., Buchner, J., and Winter, J. (2010). Evolution of Escherichia coli for growth at high temperatures. J. Biol. Chem. *285*, 19029–19034.

Saidi, Y., Finka, A., Muriset, M., Bromberg, Z., Weiss, Y.G., Maathuis, F.J., and Goloubinoff, P. (2009). The heat shock response in moss plants is regulated by specific calcium-permeable channels in the plasma mebrane. Plant Cell *9*, 2829–2843.

Schaupp, A., Marcinowski, M., Grimminger, V., Bösl, B., and Walter, S. (2007). Processing of proteins by the molecular chaperone Hsp104. J. Mol. Biol. *370*, 674–686.

Schirmer, E.C., Glover, J.R., Singer, M.A., and Lindquist, S. (1996). HSP100/Clp proteins: a common mechanism explains diverse functions. Trends Biochem. Sci. *21*, 289–296.

Schrader, E.K., Harstad, K.G., and Matouschek, A. (2009). Targeting proteins for degradation. Nat. Chem. Biol. *5*, 815–822.

Sharma, S., Chakraborty, K., Müller, B.K., Astola, N., Tang, Y.C., Lamb, D.C., Hayer-Hartl, M., and Hartl, F.U. (2008). Monitoring protein conformation along the pathway of chaperonin-assisted folding. Cell *133*, 142–153.

Shivaji, S., and Prakash, J.S. (2010). How do bacteria sense and respond to low temperature? Arch. Microbiol. *192*, 85–95.

Singer, M.A., and Lindquist, S. (1998a). Multiple effects of trehalose on protein folding in vitro and in vivo. Mol. Cell *1*, 639–648.

Singer, M.A., and Lindquist, S. (1998b). Thermotolerance in Saccharomyces cerevisiae: the Yin and Yang of trehalose. Trends Biotechnol. *16*, 460–468.

Smith, D.F. (1998). Sequence motifs shared between chaperone components participating in the assembly of progesterone receptor complexes. Biol. Chem. *379*, 283–288.

Spiess, C., Beil, A., and Ehrmann, M. (1999). A temperature-dependent switch from chaperone to protease in a widely conserved heat shock protein. Cell *97*, 339–347.

Stetter, K.O. (2006). Hyperthermophiles in the history of life. Philos. Trans. R. Soc. Lond. B Biol. Sci. *361*, 1837–1842, discussion 1842–1843.

Sundar, S., McGinness, K.E., Baker, T.A. and Sauer, R.T. (2010). Multiple sequences signals direct recognition and degradation of protein substrates by the AAA+ protease HslUV. Published online September 15, 2010.

Szalay, M.S., Kovács, I.A., Korcsmáros, T., Böde, C., and Csermely, P. (2007). Stress-induced rearrangements of cellular networks: Consequences for protection and drug design. FEBS Lett. *581*, 3675–3680.

Tabuchi, Y., Takasaki, I., Wada, S., Zhao, Q.L., Hori, T., Nomura, T., Ohtsuka, K., and Kondo, T. (2008). Genes and genetic networks responsive to mild hyperthermia in human lymphoma U937 cells. Int. J. Hyperthermia *24*, 613–622.

Tachdjian, S., and Kelly, R.M. (2006). Dynamic metabolic adjustments and genome plasticity are implicated in the heat shock response of the extremely thermoacidophilic archaeon Sulfolobus solfataricus. J. Bacteriol. *188*, 4553–4559.

Taipale, M., Jarosz, D.F., and Lindquist, S. (2010). HSP90 at the hub of protein homeostasis: emerging mechanistic insights. Nat. Rev. Mol. Cell Biol. *11*, 515–528.

Takai, K., Nunoura, T., Sako, Y., and Uchida, A. (1998). Acquired thermotolerance and temperature-induced protein accumulation in the extremely thermophilic bacterium Rhodothermus obamensis. J. Bacteriol. *180*, 2770–2774.

Thibault, G., Yudin, J., Wong, P., Tsitrin, V., Sprangers, R., Zhao, R., and Houry, W.A. (2006). Specificity in substrate and cofactor recognition by the N-terminal domain of the chaperone ClpX. Proc. Natl. Acad. Sci. USA *103*, 17724–17729.

Todd, M.J., Viitanen, P.V., and Lorimer, G.H. (1994). Dynamics of the chaperonin ATPase cycle: implications for facilitated protein folding. Science *265*, 659–666.

Toivola, D.M., Strnad, P., Habtezion, A., and Omary, M.B. (2010). Intermediate filaments take the heat as stress proteins. Trends Cell Biol. *20*, 79–91.

Van Montfort, R., Slingsby, C., and Vierling, E. (2001). Structure and function of the small heat shock protein/alpha-crystallin family of molecular chaperones. Adv. Protein Chem. *59*, 105–156.

Vigh, L., Los, D.A., Horváth, I., and Murata, N. (1993). The primary signal in the biological perception of temperature: Pd-catalyzed hydrogenation of membrane lipids stimulated the expression of the desA gene in Synechocystis PCC6803. Proc. Natl. Acad. Sci. USA *90*, 9090–9094.

Vigh, L., Nakamoto, H., Landry, J., Gomez-Munoz, A., Harwood, J.L., and Horvath, I. (2007). Membrane regulation of the stress response from prokaryotic models to mammalian cells. Ann. N Y Acad. Sci. *1113*, 40–51.

Viitanen, P.V., Gatenby, A.A., and Lorimer, G.H. (1992). Purified chaperonin 60 (groEL) interacts with the nonnative states of a multitude of Escherichia coli proteins. Protein Sci. *1*, 363–369.

Voellmy, R., and Boellmann, F. (2007). Chaperone regulation of the heat shock protein response. Adv. Exp. Med. Biol. *594*, 89–99.

Vogel, J.L., Parsell, D.A., and Lindquist, S. (1995). Heat-shock proteins Hsp104 and Hsp70 reactivate mRNA splicing after heat inactivation. Curr. Biol. *5*, 306–317.

Voit, E.O., and Radivoyevitch, T. (2000). Biochemical systems analysis of genome-wide expression data. Bioinformatics *16*, 1023–1037.

Walter, S., and Buchner, J. (2002). Molecular chaperones—cellular machines for protein folding. Angew. Chem. Int. Ed. Engl. *41*, 1098–1113.

Walsh, N.P., Alba, B.M., Bose, B., Gross, C.A., and Sauer, R.T. (2003). OMP peptide signals initiate the envelope-stress response by activating DegS protease via relief of inhibition mediated by its PDZ domain. Cell *113*, 61–71.

Wandinger, S.K., Richter, K., and Buchner, J. (2008). The Hsp90 chaperone machinery. J. Biol. Chem. *283*, 18473–18477.

Weber-Ban, E.U., Reid, B.G., Miranker, A.D., and Horwich, A.L. (1999). Global unfolding of a substrate protein by the Hsp100 chaperone ClpA. Nature *401*, 90–93.

Welch, W.J., and Feramisco, J.R. (1982). Purification of the major mammalian heat shock proteins. J. Biol. Chem. *257*, 14949–14959.

Welch, W.J., and Suhan, J.P. (1985). Morphological study of the mammalian stress response: characterization of changes in cytoplasmic organelles, cytoskeleton, and nucleoli, and appearance of intranuclear actin filaments in rat fibroblasts after heat-shock treatment. J. Cell Biol. *101*, 1198–1211.

Welch, W.J., and Suhan, J.P. (1986). Cellular and biochemical events in mammalian cells during and after recovery from physiological stress. J. Cell Biol. *103*, 2035–2052.

Welker, S., Rudolph, B., Frenzel, E., Hagn, F., Liebisch, G., Schmitz, G., Scheuring, J., Kerth, A., Blume, A., Weinkauf, S., et al. (2010). Hsp12 is an intrinsically unstructured stress protein that folds upon membrane association and modulates membrane function. Mol. Cell *39*, 507–520.

Westerheide, S.D., Anckar, J., Stevens, S.M., Jr., Sistonen, L., and Morimoto, R.I. (2009). Stress-inducible regulation of heat shock factor 1 by the deacetylase SIRT1. Science *323*, 1063–1066.

Winkler, J., Seybert, A., König, L., Pruggnaller, S., Haselmann, U., Sourjik, V., Weiss, M., Frangakis, A.S., Mogk, A., and Bukau, B. (2010). Quantitative and spatio-temporal features of protein aggregation in Escherichia coli and consequences on protein quality control and cellular ageing. EMBO J. *29*, 910–923.

Wong, P., and Houry, W.A. (2004). Chaperone networks in bacteria: analysis of protein homeostasis in minimal cells. J. Struct. Biol. *146*, 79–89.

Wu, B.J., Kingston, R.E., and Morimoto, R.I. (1986). Human HSP70 promoter contains at least two distinct regulatory domains. Proc. Natl. Acad. Sci. USA *83*, 629–633.

Wu, C. (1984). Two protein-binding sites in chromatin implicated in the activation of heat-shock genes. Nature *309*, 229–234.

Yam, A.Y., Xia, Y., Lin, H.T., Burlingame, A., Gerstein, M., and Frydman, J. (2008). Defining the TRiC/CCT interactome links chaperonin function to stabilization of newly made proteins with complex topologies. Nat. Struct. Mol. Biol. *15*, 1255–1262.

Yost, H.J., and Lindquist, S. (1986). RNA splicing is interrupted by heat shock and is rescued by heat shock protein synthesis. Cell *45*, 185–193.

Yura, T., Tobe, T., Ito, K., and Osawa, T. (1984). Heat shock regulatory gene (htpR) of Escherichia coli is required for growth at high temperature but is dispensable at low temperature. Proc. Natl. Acad. Sci. USA *81*, 6803–6807.

Zeuthen, E. (1971). Synchrony in Tetrahymena by heat shocks spaced a normal cell generation apart. Exp. Cell Res. *68*, 49–60.

Zhu, X., Zhao, X., Burkholder, W.F., Gragerov, A., Ogata, C.M., Gottesman, M.E., and Hendrickson, W.A. (1996). Structural analysis of substrate binding by the molecular chaperone DnaK. Science *272*, 1606–1614.