On Heat and Cells and Proteins

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Two principal forms of temperature-control strategies have evolved, i.e., poikilothermic and homeothermic life. Even in homeothermic animals, the temperature field of the body is not homogenous. These observed temperature differences can affect cellular function directly or via the expression of heat shock or cold shock proteins.

The received wisdom is that higher forms of life are governed by the ambient temperature, since virtually all biochemical processes are temperature dependent. The extreme ambient air temperatures on Earth range from −89.2°C (measured July 21, 1983, Wostock, Antarctica) to +58°C (measured September 13, 1922, Al Aziziyah, Libya). Despite these temperature extremes, homeothermic species, like humans, have the ability to regulate their core body temperature within a narrow range to 37°C due to autonomic temperature regulation mechanisms. The ability to maintain a relatively constant internal temperature has allowed homeothermic animals to be independent of the influence of fluctuations in environmental temperature. In addition, remarkable adaptations to heat and cold are described in homeotherms. Whereas heat acclimatization is achieved within weeks, for example, by simply increasing the sweat rate, acclimatization to cold is only achieved following exposure to a cold environment for years. The best-studied population regarding cold acclimatization are the traditional Korean and Japanese divers called Ama. During their diving work in 10°C cold sea water, rectal temperatures ranging from 37°C to 34.8°C have been observed (7). When the physiologist Suki Hong studied these women in the early 1960s, their basal metabolic rate during the winter months, when they were diving in very cold water, was significantly elevated above values observed during warmer months. To compensate for this caloric deficit, food consumption increased by −1,000 kcal compared with nondivers. In addition, the shivering rate in cold water was decreased compared with nondiving control women. However, when Hong repeated his studies in the 1990s, the Ama no longer showed the enhanced metabolic capacity, since (fortunately) they were no longer diving in their traditional cotton bathing suits but were provided with wet suits to combat the cold stress.

The core body temperature of cold-blooded animals, the so-called poikilothermic animals, depends on the ambient temperature. In poikilothermic animals, temperature is the most important environmental factor governing species distribution. Temperature extremes are achieved in poikilothermic aquatic animals. The Antarctic fish of the teleost suborder Notothenioidae is an extreme stenotherm, which lives in the cold, thermally stable waters of coastal Antarctica, where temperatures range from +0.3°C to −1.86°C. In 1998, the colony-dwelling polychaete worm Alvinella pompejana, inhabiting deep-sea hydrothermal temperatures above +80°C and having a thermal gradient of 60°C or more over its body length, was described.

Although the systemic mechanisms of adaptation to changes in temperature are well known, much less is known about the adaptation at the cellular or genetic level. Life, viewed from the cellular perspective, shows high temperature variations even in the bodies of homeotherms (Fig. 1). Temperatures as high as +45°C can be measured on the skin during sun exposure, which is equivalent to the temperature in the Sahara. Myocytes are exposed to temperatures up to 40°C in the working muscle. The lowest and highest core body temperatures, which were survived in cases of accidental hypothermia or hyperthermia, are −30°C and +43°C, respectively. Systemically, the temperatures are sensed via cold and heat receptors on specialized mammalian somatosensory neurons. Those receptors belong to the transient receptor potential family of ion channels, which convert temperature stimuli into electric potentials. At the cellular level, however, each cell is capable of reacting to a change in temperature with an enhanced or decreased production of proteins. Sonnhammer et al. (15) detected 227 upregulated and 168 downregulated genes (out of 12,600 investigated) by using a chip array approach after exposure of peripheral blood mononuclear cells to 43°C for 20 min. A subset of those proteins, the heat shock proteins (HSPs), are specialized for protecting cells against heat-induced damage. Similar to the specific heat shock response, each cell is capable of enhanced production of cold shock proteins (CSP) in response to a temperature decrease.

Discovery of the heat shock response

In 1962, Ritossa discovered the HSPs in his pioneering work. After an increasing the temperature in an incubator containing Drosophila cultures, he observed remarkable changes in the chromosomal puffing patterns, i.e., the gene activity patterns of the polytene chromosomes in larval salivary glands. Today, it is well known that in response to many stresses, including heat, oxidizing conditions, and exposure to toxic compounds, all cells produce a common set of HSPs. The name of these proteins, however, was derived from the first trigger (i.e., heat) that was identified as increasing their synthesis. Increased expression of HSPs is mediated at multiple levels: mRNA synthesis, mRNA stability, and translation efficiency. Experiments with various species have shown that increased expression of these proteins can protect the organism against stress-induced damage. Moreover, cells given a
nonlethal HSP-inducing preshock subsequently survive an otherwise lethal exposure to elevated temperatures. Strikingly, the expression patterns of HSPs show high levels of conservation among the different species. Thus the heat shock response is thought to be virtually universal among organisms. However, in two cases of fish exposed to cold, thermally stable environments (Hydra oligatis and Trematomus bernacchii), no heat shock response has been detected.

There are numerous examples showing that HSP expression can vary with environmental temperature in humans despite their ability to maintain a constant core body temperature. Increased HSP synthesis has been observed in vivo during exercise in the heart muscle depending on the ambient temperature affecting myocardial adaptations (6). Changes in HSP72 expression in leukocytes are associated with adaptation to exercise under conditions of high environmental temperature. The importance of the HSPs for environmental systemic adaptation is well demonstrated mostly in poikilothermic animals. These studies also show that the temperature at which the HSP genes are activated is subjected to thermal acclimatization as a function of season or other changes in the mean environmental temperature. Collectively, the HSPs evolved as general stress-inducible proteins to maintain cellular integrity (11). This resistance mechanism, however, not only takes place in pathophysiological situations but is also adapted in mammalian physiology, for example in osmotic resistance of keratinocytes or renal cells, where physiological osmotic conditions (via change in humidity or sodium chloride/urea accumulation, respectively) results in enhanced expression of HSPs (2).

**HSPs and molecular chaperones**

Under normal (nonstressful) conditions, molecular chaperones assist in the routine folding and compartmentalization of newly synthesized proteins, and they also take part in a variety of other cellular functions. During thermal or other forms of stress, heat-induced HSPs bind to denatured proteins thereby preventing their aggregation and aiding in their refolding into native, functional states following restoration of ambient temperature. HSPs have been classified in eukaryotic cells by their molecular weight. To date, there are six identified HSP families (HSP100, HSP90, HSP70, HSP60, HSP40, and the small HSPs). Since the expression of some members of the HSP families is increased upon glucose starvation, these proteins are called glucose-regulated proteins. Some HSPs, discovered independently of their role as stress proteins (like ubiquitin or αB-crystallin) carry their particular names. An overview of the chaperone protein families and their structure and function is given in Table 1. HSP90 and HSP70 in particular play an important role in maintaining cellular function under nonstress and stress conditions.

**HSP90 family**

HSP90s are highly conserved proteins, which represent 2% of the whole cellular protein content. HSP90 comprises three structural domains identified by proteolytic digestion. The ~25-kDa NH2-terminal domain is connected to a highly conserved ~55-kDa COOH-terminal region by a charged linker, which is variable in length and composition among species and isoforms. Two ATP-binding pockets have been described, one located in the NH2-terminal and one in the COOH-terminal region. Upon ATP binding or heat, HSP90 switches from a form in which the two NH2-terminal domains are separate to one in which they are associated generating a toroidal dimer structure. This conformational change is thought to be responsible for substrate binding. HSP90 acts as an ATP-dependent molecular chaperone involved in the folding and activation of an unknown number of substrate proteins, including steroid hormone receptors, protein kinases, and transcription factors (14). The complex formation of these client proteins with HSP90 is a prerequisite for their stability and functionality. Therefore, chaperones...
belonging to the HSP90 family are key players in cellular events, such as DNA replication, RNA transcription, protein folding, maturation, translocation through the endoplasmic reticulum and mitochondrial membranes, proteolysis, and cell signaling. HSP90 displays a strong specificity for its client proteins. HSP90 alone, however, is incapable of promoting the folding and/or activation of any of its known substrate proteins. For full activity, the interplay of HSP90 with other HSPs and co-chaperones is required. In the case of the progesterone receptor, at least seven additional proteins are involved (HSP70, Hip, Hop, immunophilins, and p23). On the basis of the progesterone receptor model, the substrate is cycling between an early complex (containing HSP70 and Hop), an intermediate complex (containing HSP70, Hop, and a HSP90 dimer), and the mature complex (containing a HSP90 dimer and p23) with the necessity of ATP hydrolysis. The importance of HSP90 for cellular functions is demonstrated by the use of specific HSP90 antagonists like geldanamycin (GA) that occupy the ATP binding pocket of HSP90, thereby preventing the cycling of the HSP90 heterocomplex. GA-treated cells show deficits in cell growth as well as in stability and activation of protein kinases and transcription factors.

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**HSP70 family**

The 70-kDa heat shock-related proteins comprise a family of highly conserved molecular chaperones that regulate protein folding during normal and stress conditions (3). HSP70, like HSP90, is one of the most abundant of these proteins, accounting for as much as 1–2% of total cellular protein. HSP70 proteins promote the folding of nascent chains on ribosomes, translocation of proteins across membranes, and protection at high temperatures via interaction with exposed hydrophobic surfaces of unfolded or partially folded proteins. HSP70 proteins contain two domains, an NH2-terminal ATPase domain and a COOH-terminal peptide-binding domain. The HSP70 peptide-binding domain binds a seven-residue peptide in an extended conformation between a β-sheet subdomain and an α-helical subdomain. It is thought that ATP binding to the ATPase domain triggers substrate release by causing the α-domain to bend upward at a flexible junction near the middle of the long helix that extends over the peptide. HSP70 co-chaperones, such as Hip, Hop, HSP40, and Bag-1, have been shown to play an important role

<table>
<thead>
<tr>
<th>Chaperone Family</th>
<th>Structure</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSP100</td>
<td>6- to 7-mer</td>
<td>Dissolution of protein aggregates or oligomers, inhibition of protein aggregation, thermoresistance</td>
</tr>
<tr>
<td>HSP90</td>
<td>Dimer</td>
<td>Control of folding and activity of protein kinases, steroid receptors, bHLH transcription factors, thermoresistance</td>
</tr>
<tr>
<td>HSP70</td>
<td>Monomer</td>
<td>De novo protein folding, protection against heat denaturation</td>
</tr>
<tr>
<td>HSP60</td>
<td>14-mer</td>
<td>De novo protein folding, immunogenic as a self-antigen</td>
</tr>
<tr>
<td>sHSP</td>
<td>8- to 24-mer</td>
<td>Protein folding, creates a reservoir of unfolded proteins for ATP-dependent HSP70 function, maintains solubility of cytoplasmic crystallins in the lens</td>
</tr>
</tbody>
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HSP, heat shock protein; sHSP, small HSP; bHLH, basic helix loop helix.

**TABLE 1. Overview of HSP chaperone families**

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HSPs and HSP90 (Fig. 2). Following heat shock, HSP70 and the cytosol as an inactive, monomeric protein that is bound by HSP genes. At nonstressful temperatures, HSF1 is present in HSF1 and the heat shock element found in the promoter of all of heat shock genes is mediated by the interaction between amino acid analogs. During gene expression, transactivation of HSP genes including HSP70 and HSP90. Subsequently, the activity of HSF1 is negatively regulated via increased binding of the newly synthesized HSP70 and HSP90 to HSF1. The central process for cellular temperature sensing therefore is the equilibrium between the binding of free HSPs to HSF1 or to stress-denatured proteins.

Cell death: apoptosis and necrosis

Exposing cells to heat can result in apoptosis or necrosis depending on the temperature applied. Most interestingly, the threshold temperature of apoptosis induction on the cellular level is equivalent to the safely tolerable upper threshold systemic core temperature in humans. For the induction of necrosis, higher temperatures than those inducing apoptosis have to be applied in vitro (10). The expression of small HSPs or the inducible HSP70 has been shown to enhance the survivability of mammalian cells exposed to numerous types of stimuli, including heat or other forms of apoptotic stress stimuli (1). Whereas apoptosis must be assumed that the antinecrotic function is connected to the chaperone function, especially of HSP70 and HSP90, the exact mechanism of the antiapoptotic function of the HSPs is not entirely clear. There seem to be multiple possibilities for HSP70 to interact with apoptotic pathways. HSP70 reportedly interacts with Apaf-1, thereby preventing its interaction with procaspase-9 and finally caspase-dependent apoptosis. Recently, it has been demonstrated that HSP70 also takes part in caspase-independent apoptotic events via a specific interaction with the apoptosis-inducing factor, which is released from mitochondria early in the apoptotic process.

The cold shock response

Cold stress changes the lipid composition of cellular membranes and suppresses the rate of protein synthesis and cell proliferation. However, a set of proteins called CSP is expressed at higher levels starting at a temperature of 32°C. Hypothermia induces the expression of RNA-binding proteins like cold-inducible RNA-binding protein (CIRP), the first CSP identified in mammalian cells, and RNA-binding motif protein 3 (4, 12). Reminiscent of HSP, CIRP is also expressed at 37°C and developmentally regulated, possibly working as an RNA chaperone. Cellular and molecular biology of mammalian cells at 32°C is a new area expected to have considerable implications for physiology, for example in testis and skin, where cell temperatures are typically 30–34°C and ~33°C, respectively.

HSPs are the link between the environmental temperature and cellular function

The HSPs and CSPs are evolutionarily the most conserved response to temperature changes. Although the proteins were discovered a long time ago and the molecular functions of these proteins are more and more understood, the link

**FIGURE 2.** Cellular temperature sensing. Under normothermic temperatures, the heat shock transcription factor 1 (HSF1) is bound to heat shock protein 70 (HSP70). After heat exposure, HSP70 is recruited to denatured proteins, leaving HSF1 in a nonrepressed conformation. HSF trimerizes, becomes phosphorylated, and binds to specific heat shock response elements in the promoter region of heat-inducible genes. The subsequent increased production of HSP70 leads to a negative feedback regulation of HSF1 activity via binding the HSF1.
between temperature/HSP/CSP-mediated effects and cellular functions is less explored. Exposing cells to elevated temperatures affects not only HSP expression and activity but also the interaction of HSPs with partner proteins. For example, increasing HSP90 expression and activity by elevated temperatures in vitro or in vivo affects the stability and activity of transcription factors like glucocorticoid receptor or the hypoxia-inducible factor-1α. The ambient temperature thereby affects gene expression initially via a direct activation of heat-inducible genes and subsequently indirectly via altered HSP expression and activity.

Further outlooks

Further studies are needed to determine the influence of temperature changes in the homeothermic body on cellular function and gene expression, since in most in vitro studies simply the core body temperature of 37°C is simulated. This temperature, however, represents just the core of our body and neglects the dynamic temperature changes in other parts under physiological and pathophysiological conditions. The importance of precise temperature regulation in homeotherms is best exemplified in the testis. Even slight elevations of the scrotal temperature (which is normally 30–34°C) are associated with infertility. This is partly attributed to higher expression of HSP90 proteins and p53-mediated cell death as well as decreased CSP expression. This is probably one of the most dramatic examples of the need for temperature adaptation to “fertilize” our planet.

References
1. Arrigo AP. sHsp as novel regulators of programmed cell death and tumori-