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Variation in the heat shock response and its implication for predicting the effect of global climate change on species' biogeographical distribution ranges and metabolic costs

L. Tomanek

Center for Coastal Marine Sciences and Environmental Proteomics Laboratory, Department of Biological Sciences, California Polytechnic State University, San Luis Obispo, CA 93407, USA

ltomanek@calpoly.edu

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Summary

The preferential synthesis of heat shock proteins (Hsps) in response to thermal stress [the heat shock response (HSR)] has been shown to vary in species that occupy different thermal environments. A survey of case studies of aquatic (mostly marine) organisms occupying stable thermal environments at all latitudes, from polar to tropical, shows that they do not in general respond to heat stress with an inducible HSR. Organisms that occupy highly variable thermal environments (variations up to >20°C), like the intertidal zone, induce the HSR frequently and within the range of body temperatures they normally experience, suggesting that the response is part of their biochemical strategy to occupy this thermal niche. The highest temperatures at which these organisms can synthesize Hsps are only a few degrees Celsius higher than the highest body temperatures they experience. Thus, they live close to their thermal limits and any further increase in temperature is probably going to push them beyond those limits. In comparison, organisms occupying moderately variable thermal environments (<10°C), like the subtidal zone, activate the HSR at temperatures above those they normally experience in their habitats. They have a wider temperature range above their body temperature range over which they can synthesize Hsps. Contrary to our expectations, species from highly (in comparison with moderately) variable thermal environments have a limited acclimatory plasticity. Due to this variation in the HSR, species from stable and highly variable environments are likely to be more affected by climate change than species from moderately variable environments.

Key words: heat shock proteins, heat shock response, transcriptomics, global climate change, biogeography, metabolic costs, acclimation, intertidal zone, subtidal zone.

Introduction

The heat shock response (HSR) has been characterized for a wide range of species and found to exhibit a high degree of conservation in its basic properties from bacteria to animals (Feder and Hofmann, 1999). The HSR is characterized by the preferential synthesis of a group of proteins, the heat shock proteins (Hsps), that are molecular chaperones, which help proteins fold correctly during translation and facilitate their transport across membranes under non-stressful conditions (Frydman, 2001; Hartl and Hayer-Hartl, 2002). Under stressful conditions, molecular chaperones stabilize denaturing proteins and refold proteins that have already been denatured. If proteins are irreversibly denatured, molecular chaperones help hand them over to the proteolytic machinery of the cell, mainly along the ubiquitin–proteasome pathway (Glickman and Ciechanover, 2002). The molecular chaperone role of Hsps reflects the fact that protein conformation is a thermally sensitive weak-link in the macromolecular machinery of the cell that contributes to setting thermal tolerance limits (Somero, 2004). The HSR is thus an important biochemical indicator to assess levels of thermal stress and thermal tolerance limits. While important in facilitating tolerance of heat stress, operation of the HSR does not come without considerable cost. Production of Hsps and their function in ATP-consuming protein folding reactions can add considerably to the ATP demands of the cell. Increased levels of Hsps when conditions are not stressful have been shown to be maladaptive (Feder et al., 1992; Krebs and Loeschke, 1994; Silbermann and Tatar, 2000). Furthermore, there is evidence that organisms from thermally distinct habitats vary in their HSR in a way that suggests that some use the response more frequently than others (Tomanek, 2008). How this variation among species from different thermal habitats can help us understand how global climate change will affect these organisms will be the focus of this review.

Variation in the HSR

The HSR of species from stable thermal environments

There are a great number of HSR comparisons in the terrestrial and the marine environment. The terrestrial studies have overwhelmingly, but not exclusively, focused on *Drosophila*, and a number of excellent reviews are available (Hoffmann et al., 2003; Sørensen et al., 2003). Here I will focus my attention on studies from the aquatic, mainly marine, environment. In the following overview I will compare how the HSR differs in animals that occupy different thermal environments with respect to absolute temperature and range of temperature, and suggest insights we can gain from a broad comparison of how global climate change could affect these organisms.

The HSR has been described as being an almost universal response to heat and a variety of other stresses (Parsell and Lindquist, 1993). This statement is based on two observations: first, the HSR has been observed in almost all organisms studied to date (important exceptions will be reviewed below). Second, enzymes demonstrate almost universal kinetic properties, which depend on an evolved balance

between flexibility and stability of the conformational changes that are necessary for their catalytic activity (Fields, 2001). As a consequence, at higher temperatures enzymes unfold and expose hydrophobic side chains due to an increase in molecular movement. This leads to interactions between hydrophobic side chains of different proteins and to protein denaturation, which universally requires the activity of molecular chaperones, e.g. Hsps.

However, as with every rule in biology, there are exceptions that tell an interesting story. The first observation of an organism lacking the HSR was found in a comparison of congeneric temperate freshwater cnidarian species of the genus *Hydra* (Bosch et al., 1988; Gellner et al., 1992). While *Hydra vulgaris* is able to tolerate a much greater range of temperatures, *Hydra oligactis* is extremely sensitive to even minor thermal variations and lacks an inducible response to heat stress. Antarctic marine organisms that live at extremely stable temperatures (–1.9°C) are another example (Clark and Peck, 2009). Several Antarctic and Arctic fish species fail to display a HSR when exposed to temperatures 5–6°C above those of their respective waters (Afonso et al., 2008; Buckley and Somero, 2009; Buckley et al., 2004; Clark et al., 2008a; Hofmann et al., 2000; Zakhartsev et al., 2005). This has also been shown to be the case for some Antarctic marine invertebrates and ciliates (Clark et al., 2008c; LaTerza et al., 2001). However, some intertidal Antarctic invertebrates do show an inducible response at temperatures 10–12°C above those they inhabit, although they will probably never experience these temperatures under natural conditions (Clark et al., 2008b). Another interesting example is a recent study on the HSR of a warm stenothermal coral reef fish (Kassahn et al., 2007). A survey of heat-induced mRNA, using a non-species specific microarray, showed no induction of any of the typical inducible Hsps, e.g. Hsp70, Hsp90, Hsp40 and small Hsps. Together these studies suggest that strongly stenothermal organisms, whether from cold or warm environments, may lack the HSR.

The mechanistic bases for the lack of an inducible HSR vary among taxa. In the case of *H. oligactis* it was shown that rapid degradation of Hsp mRNA is responsible for the lack of an inducible response (Brennecke et al., 1998). In other cases, e.g. in the Antarctic notothenioid fish *Trematomus bernacchii*, heat-induced transcription of HSP-encoding genes may be absent (Buckley and Somero, 2009). Another potential reason for a lack of an inducible response is based on the transcriptional regulatory model of Hsp synthesis, the so-called ‘cellular thermostat’ model (Craig and Gross, 1991; Morimoto, 1998). The model assumes that the heat shock transcription factor 1 (HSF1) is bound and thereby inactivated by a multi-chaperone complex consisting of at least Hsp70, Hsp40 and Hsp90. The complex dissociates from the transcription factor in response to thermal or other proteotoxic stress because the chaperones are required to stabilize denaturing proteins. HSF1 monomers are then free to form an active trimeric protein that binds to the promoter region of Hsps genes, specifically the heat shock element, initiating the transcription of *Hsp* message. Following the synthesis of Hsps, the HSF1 is again sequestered by a multi-chaperone complex and inactivated. Thus, higher steady-state levels of Hsps preceding a thermal insult will at least delay if not prevent the activation of the HSR (Buckley and Hofmann, 2002; Buckley et al., 2001; Tomanek and Somero, 2002). It follows that the lack of an inducible response in Antarctic fish could in part be due to constitutively high levels of the inducible isoforms of Hsp70 (Clark et al., 2008a; Place et al., 2004). These high levels have been conjectured to be due to increasing levels of protein cold denaturation, which are based on the thermodynamically favorable tendency of hydrophobic groups to be hydrated at lower temperatures, e.g. the hydrophobic effect (Tsai et al., 2002), exposing hydrophobic side chains that are normally buried deep inside the protein and leading to denaturation. The loss of an inducible response in Antarctic fish has also been linked to a mutation in the binding region of the transcription factor, HSF1 (Buckley et al., 2004).

The HSR of species from highly variable thermal environments

In contrast to the stable thermal environment of the Southern Ocean, where annual temperature variation never exceeds approximately 3–4°C, the intertidal zone of the temperate latitudes is characterized by great temperature fluctuations that raise body temperatures of marine organisms by more than 20°C during low tide, sometimes on a daily basis (Denny and Harley, 2006; Helmuth, 1998). Bivalves, gastropods, crustaceans and fish that inhabit the rocky intertidal zone have long fascinated physiologists with their ability to cope with temperature changes that are five to six times as great as when we feel a bad fever (4°C above human body temperature) – and they survive! This is especially impressive because subtidal congeners of intertidal dwellers are often incapable of surviving in the thermal environment of the intertidal zone, although living only centimeters away.

A number of studies have shown that it is likely that intertidal organisms activate the HSR during frequent periods of recurring thermal stress when low tides occur during the middle of the day. Thus, the HSR is part of their biochemical strategy to cope with the extreme thermal fluctuations that are typical for the intertidal environment (Berger and Emlet, 2007; Dong et al., 2008; Hofmann and Somero, 1995; Miller et al., 2009; Sanders et al., 1991; Todgham et al., 2006; Tomanek, 2005; Tomanek and Sanford, 2003; Tomanek and Somero, 1999; Tomanek and Somero, 2000). A particularly comprehensive study by Gracey et al. (Gracey et al., 2008) showed that while transcript levels of several Hsps are upregulated in specimens of the California ribbed mussel *Mytilus californianus* that were collected from the field at 1.64 m above mean low low water (MLLW) and that experienced a body temperature of 30.5°C, they were not upregulated in mussels that were collected at 1.52 m above MLLW, or only 0.12 m below the higher site, and had only experienced body temperatures as high as 22.5°C due to partial shading during the same low tide episode. Thus, activating the HSR within the typical range of body temperatures they experience is part of the strategy of intertidal organisms to cope with thermal stress. Also, the upper limits of the temperature range of Hsp synthesis are close to the highest body temperatures that these organisms experience under natural conditions (Tomanek and Somero, 1999). Fig. 1 illustrates this point: the species of the snail genus *Chlorostoma* (formerly *Tegula*) that occupies the low- to mid-intertidal zone experiences body temperatures of up to at least 33°C, well above the onset temperature (T_{on}) of Hsp70 synthesis in this species (27°C), suggesting that it frequently induces the HSR under natural conditions. Interestingly, these high body temperatures in *Chlorostoma funebralis* coincide with the temperature at which the synthesis of Hsps reaches its peak (T_{peak}). Temperature exposures above this temperature lead to a HSR that does not match the increased thermal insult. Furthermore, the temperatures at which Hsp synthesis (and protein synthesis in general) cease (T_{off}) are within a few degrees Celsius of the highest body temperatures of *C. funebralis*, suggesting that the translational machinery contributes to setting an upper limit to Hsp synthesis during acute heat stress. These studies suggest that first, the HSR is relatively frequently employed as a defense strategy towards thermal stress in this highly variable thermal environment and second, the thermal insults against which an intertidal species can defend itself are close to the

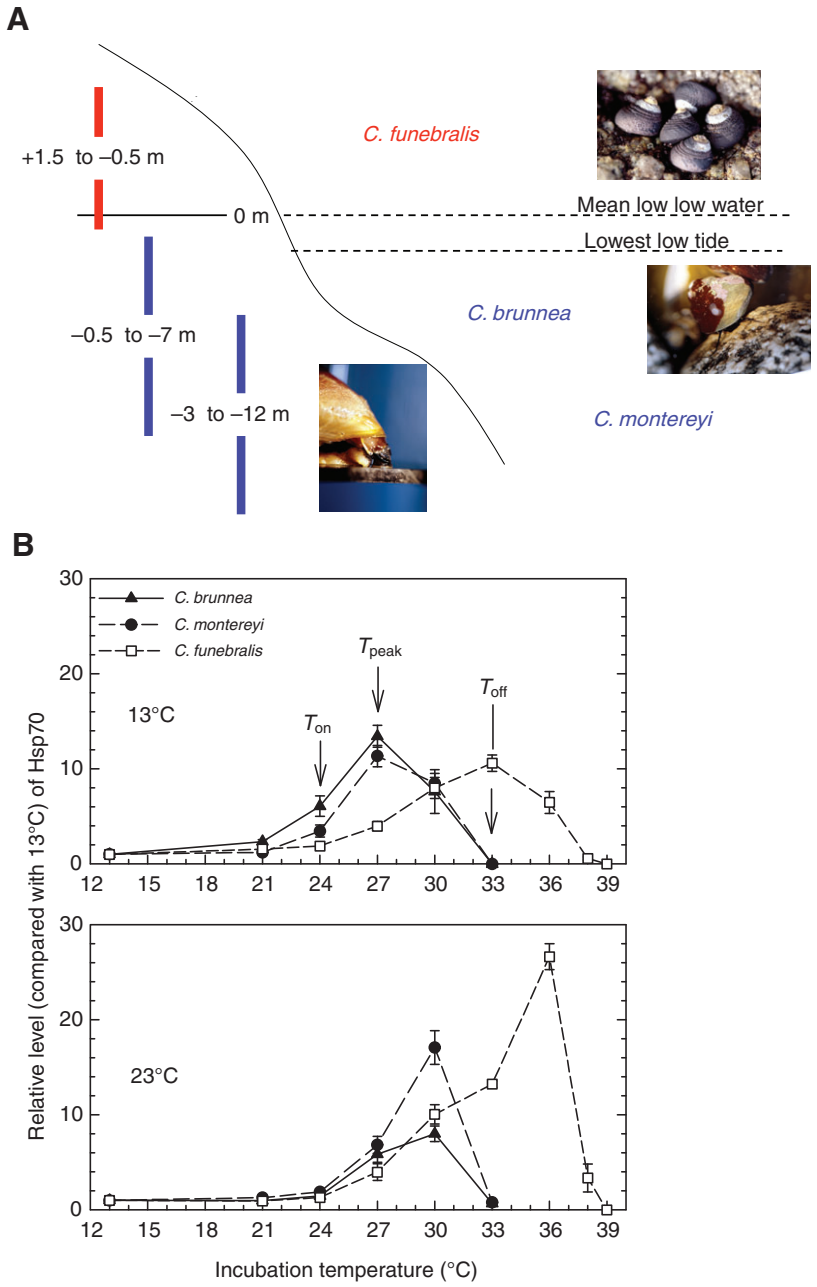

Fig. 1. (A) Vertical distribution ranges of three temperate snail species (red for inter- and blue for subtidal species) of the genus *Chlorostoma* (formerly *Tegula*) along the physical gradient from the subtidal to the mid-intertidal zone in Pacific Grove, CA, USA [after Riedman et al. (Riedman et al. 1981) and Watanabe (Watanabe, 1984)]. (B) Relative induction (compared with the 13°C control) of Hsp70 in the three temperate *Chlorostoma* congeners *C. funebralis*, *C. brunnea* and *C. montereyi* after acclimation to 13°C and 23°C for 30–34 days; T_{on} indicates the onset temperature, T_{peak} indicates the temperature of maximal induction and T_{off} indicates the cessation temperature of heat shock protein (Hsp) synthesis. Data are means ± 1 s.e.m. (N=5) for all data points except for 13°C-acclimated *C. funebralis* at 36°C, 23°C-acclimated *C. funebralis* at 33°C and *C. brunnea* at 13°C (all N=4) (modified from Tomanek and Somero, 1999).

upper body temperatures these organisms currently experience in their habitats. In other words, these organisms live close to their thermal limits and any further increase in body temperature may drive local extinctions of the affected populations. Thus, when organisms belonging to the extreme opposites of the spectrum of thermal environments, very stable *versus* highly variable, are compared, they both are likely to be greatly affected by even a slight increase in temperatures, one due to a lack of an inducible response and the other due to living close to the upper thermal limits of Hsp synthesis, which it maximally relies on in order to occupy this niche.

The HSR of species from moderately variable thermal environments

How do these extremes compare with the relationship between body temperatures and Hsp synthesis in moderately variable thermal environments, e.g. the subtidal zone, where temperatures can fluctuate over several degrees (<10°C) but not to the extent as in the intertidal zone (>20°C)? A comparison of the heat shock responses between subtidal and intertidal snail species of the genus *Chlorostoma* showed that the onset of the response in the subtidal species, *Chlorostoma montereyi* and *Chlorostoma brunnea*, following acclimation to a typical sea surface temperature (SST) of 13°C occurs at temperatures slightly above the range of body temperatures they experience (Fig. 1), which were measured to be as high as 24°C (Tomanek and Somero, 1999). Thus, subtidal *Chlorostoma* congeners are able to live in their environment without inducing the response, in contrast to the intertidal congener (Fig. 1). Other subtidal species have also been shown to induce Hsp synthesis above the body temperatures that these organisms typically experience in their respective thermal niche, e.g. goby fish of the genus *Gillichthys* (Dietz and Somero, 1993). However, should global warming be of sufficient magnitude, a higher frequency in

temperature extremes in the shallow subtidal may increase the occurrence of the response in these organisms, especially in those that are slow-moving or sessile. In this context it is curious to notice that the overall temperature range of Hsp70 synthesis from T_{on} to T_{off} is 11–12°C in the intertidal *C. funebralis* and 9°C in the two subtidal congeners. However, considering the maximum observed body temperatures, intertidal *C. funebralis* are limited to a temperature range of only 5–6°C over which they can mount a HSR, while the subtidal congeners have 9°C range of temperatures over which they can still expand their thermal range (if we assume that they are able to expand it to T_{off}, which is probably an overestimate). Thus, in contrast to the intertidal species, subtidal *Chlorostoma* congeners have a wider range of temperatures above those that they normally experience over which they can synthesize Hsps, suggesting that they may be more apt to cope with the predicted future climate scenario of warmer temperatures (IPCC, 2007).

As I did with the examples from the Antarctic I would like to briefly touch upon the molecular underpinnings that determine the variation in the HSR along the physical gradient from the subtidal to the intertidal zone that I have just described. In contrast to subtidal congeners, there is evidence that intertidal organisms have higher steady-state levels of inducible isoforms of Hsp70 but not Hsp90 (Clark et al., 2008d; Dong et al., 2008; Tomanek and Somero, 1999; Tomanek and Somero, 2002), and higher levels of HSF1, which would enable them to activate the response faster (although according to the cellular thermostat model this may also depend on the ratio of Hsp70 to HSF1). Also, a faster activation of Hsp70 synthesis has been shown to occur in intertidal *versus* subtidal species in response to a heat shock-inducing thermal stress (Tomanek and Somero, 2000; Tomanek and Somero, 2002).

In summary, the differences in when organisms activate the HSR in their thermal niche suggest that those from the most stable thermal environments may lack the option of inducing increased synthesis of Hsps, those from highly variable environments are already maximizing the protective effects of the response through high levels of expression of Hsps, and organisms from moderately variable thermal niches have the option to induce the response over a wide temperature range above the one they are currently experiencing (Fig. 2). This evolutionary variation seems to involve complex molecular changes that will be difficult to reverse or modify in order to retool for a rapidly changing world. Thus, it seems as if global climate change has a greater potential for affecting organisms from the former two thermal environments, an observation for which there is already some evidence (Barry et al., 1995; Harley et al., 2006; Helmuth et al., 2006).

Acclimation ability (plasticity) and thermal tolerance

The HSR is characterized by the preferential synthesis of mainly Hsps and suppression of the translation of other messages; the degree to which this is the case may depend again on the thermal environment of the organism (Tomanek and Somero, 1999; Tomanek and Somero, 2000). Over a longer-time period of weeks or months, acclimation (or acclimatization) results in an increase in steady-state levels either of the constitutive or the inducible isoforms of Hsp70 or other Hsps. Changes in levels of Hsps are therefore indicators of an organism's short- and long-term ability to cope with thermal stress, although it is clear by now that this role plays out within the network of multiple physiological processes at various levels of biological organization (Kassahn et al., 2009).

An example of the importance of plasticity in conjunction with thermal variation is a study on annual killifish (*Astrofundulus limnaeus*) that showed that gene expression patterns vary widely between constant and fluctuating thermal environments within the same species (Podrabsky and Somero, 2004). Fish were either kept at constant 20°C, 26°C and 37°C or fluctuating temperatures on a daily basis from 20°C to 37°C and a time course was taken over a two-week period. Transcripts of small Hsps were much more responsive to short-term thermal fluctuations than the cognate forms of Hsp70 or Hsp90. However, the latter two showed elevated levels in response to chronic heat stress.

Although an Antarctic intertidal limpet has been shown to induce a HSR at 15°C, a temperature well above the temperature range it

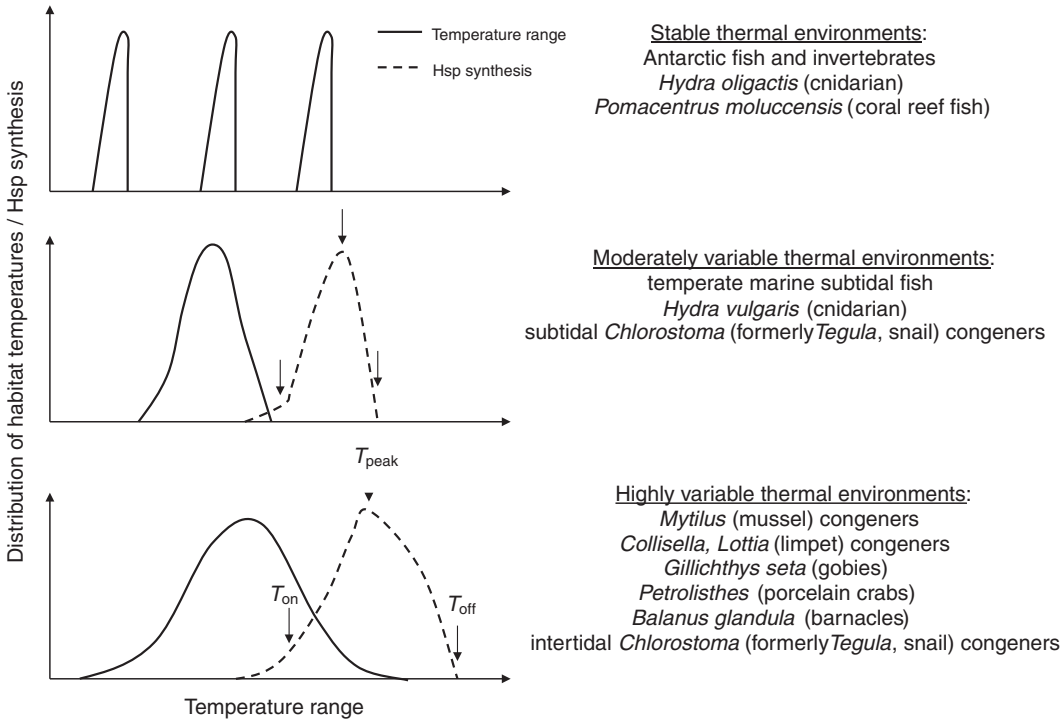

Fig. 2. Comparison of range of body temperatures and thermal range of heat shock protein (Hsp) synthesis in aquatic (mostly marine) animals from thermally stable, moderately and highly variable environments (for citations, see text); T_{on} indicates the onset temperature, T_{peak} indicates the temperature of maximal induction and T_{off} indicates the cessation temperature of Hsp synthesis (modified from Tomanek, 2008).

experiences, it did exhibit proteotoxic stress with an increase in steady-state levels of several Hsps in response to long-term acclimation to 2°C (Clark et al., 2008d). It is possible that these Antarctic limpets would experience 2°C according to predictions of rising temperatures; therefore, it may provide an estimate for when to expect thermal stress due to seasonal changes in temperature in the future climate of the Antarctic. A comparison between the temperate intertidal *versus* subtidal *Chlorostoma* congeners showed that an increase in acclimation temperature from 13°C to 23°C elicits a shift in T_{on} (Fig. 1) with concomitant changes in steady-state levels of Hsp70 and Hsp90 in the more heat-sensitive subtidal *C. brunnea* and *C. montereyi* but not in the heat-tolerant intertidal *C. funebralis* (Tomanek, 2002; Tomanek, 2005; Tomanek and Somero, 1999; Tomanek and Somero, 2002). The lack of acclimatory plasticity in more eurythermal intertidal species has also been observed for changes in heart rates of *Chlorostoma* congeners (Stenseng et al., 2005) and congeners of the crab genus *Petrolisthes* (Stillman, 2003). There is increasing evidence that the ability of eurythermal organisms to acclimate or readjust their physiology to increasing temperature is limited. The results of these studies seem to suggest that these organisms have maximized their biochemical safety factor, which does not allow for further adjustments to even higher temperatures. These organisms are living close to their thermal limits and any further increase in temperature is likely to push them beyond it.

Predicting the effect of global climate change

In order to predict how global climate change is going to differentially affect species from varying thermal environments we need to assess the ability of their current physiology to respond to a further increase in temperatures. The average predictions made by the Intergovernmental Panel for Climate Change are only of limited use for specifying how this increase will actually affect the body temperatures of organisms, because fine-scale spatial and temporal niche-specific temperatures matter more to organisms than broader-scale, habitat-specific ones (Helmuth, 2009). Simply using predicted air or water temperatures as a proxy for the expected changes in body temperature of organisms do not provide a realistic scenario (Gilman et al., 2006). Thus, I will not refer to them to estimate future physiological stress. The thermal signals that matter the most to organisms are auto-correlated because thermal extremes are overlaid on top of longer periods of warming, which allows the organism to ramp up its defense towards possible greater thermal extremes, the possible ultimate function of the acclimation response in animals (Huey et al., 1999). Thus, short- and long-term physiological (as well as evolutionary) responses need to be considered in unison to gain a realistic picture of the ability of the organism to cope with thermal stress.

Given the relationships between existing thermal variability in the environment and species employment of the HSR as well as their acclimatory plasticity, we would expect that species that currently live closer to their thermal limits, e.g. upper temperature of Hsp synthesis or heart rate, will be affected more by climate change. However, while organisms from moderately variable thermal environments may have a broader thermal range over which acclimatization can occur, these adaptive responses to rising temperatures will incur potentially significant costs. Rising temperatures may require these species to increase the frequency with which they activate the HSR, incurring a metabolic cost that is known to accompany the synthesis and function of increasing levels of Hsps (Feder et al., 1992; Krebs and Loeschke, 1994; Silbermann and Tatar, 2000). A similar trade-off occurs between populations of a single species that differ in how close they live to their temperature-dependent biogeographical range limits (reviewed by Hofmann, 2005). In order to weigh the costs and risks associated with current thermal niche occupancy we have to go beyond the typical indicators of heat stress and evaluate the metabolic consequences from a broader perspective. Several recent studies have obtained such a broader perspective of the changes associated with heat stress by quantifying the changes in mRNA levels of hundreds to thousands of genes, or the so-called transcriptome, using microarrays (Cossins and Crawford, 2005; Gracey and Cossins, 2003). In the following section I want to give a brief overview of the changes in gene expression that are heat-induced and provide insights into the systems response of the cell to heat stress.

Unity and diversity of the cellular stress response: a systems view of thermal stress

Transcriptomic and proteomic studies have revealed a number of cellular stress proteins that are part of the minimal stress response (Kültz, 2005; Petrak et al., 2008; Wang et al., 2009). Although the notion of a unifying set of stress proteins is helpful to identify the main molecular targets of environmental stresses, the ecological and evolutionary variation of the stress response is important for evaluating the biochemical strategies that organisms employ to cope with stress and for predicting the metabolic costs or constraints that will determine how climate change will affect organisms. Recent advances in the application of molecular tools to non-model organisms have given us an opportunity to survey changes in the expression of hundreds to thousands of gene messages in response to environmental stress (Gracey and Cossins, 2003). Here I am including the ones that are relevant for defining the consequences of heat stress in aquatic organisms in broader terms.

Place et al. compared the expression of about 2500 mRNAs from different populations of the ribbed mussel *Mytillus californianus* from four sites along the Pacific coast of North America that are known to vary in their thermal profiles (Place et al., 2008). The differences in thermal profiles of the sites do not follow a latitudinal gradient but instead show a mosaic pattern that depends on the occurrence of low tides during daylight hours (Helmuth et al., 2002). Their comparison of mussels from four sites showed that animals from Strawberry Hill, OR, USA, had greater levels of *hsc71* mRNA, the constitutive isoform of Hsp70, than those from the other sites, two much further south, where mussels were not emersed for as long and presumably did not experience as much heat stress. Thus, their data illustrate that ‘hot spots’ of physiological thermal stress do not have to be on the edge of a species’ distribution but instead can occur in a mosaic pattern, confirming previous work showing biogeographical heterogeneity in regions that did not follow a latitudinal temperature gradient of thermal stress within a species (Fangue et al., 2006; Osovitz and Hofmann, 2005; Sagarin and Somero, 2006).

The challenge of drawing conclusions from taking samples of the transcriptome of mussels from the intertidal zone at one time point and from one tidal height was illustrated by another study. Gracey et al. investigated the oscillatory changes of the transcriptome during the tidal cycle and the importance of tidal height in possibly entraining the oscillation (Gracey et al., 2008). They collected mussels (*M. californianus*) from the field every 4 h over a 72 h time period from two sites that were only 0.12 m apart in vertical height but the lower site was partially shaded during low tide (Fig. 3). The authors found transcriptional oscillations of genes that are associated with cellular metabolism and that were anti-correlated with the expression of genes that are activated during cell growth and

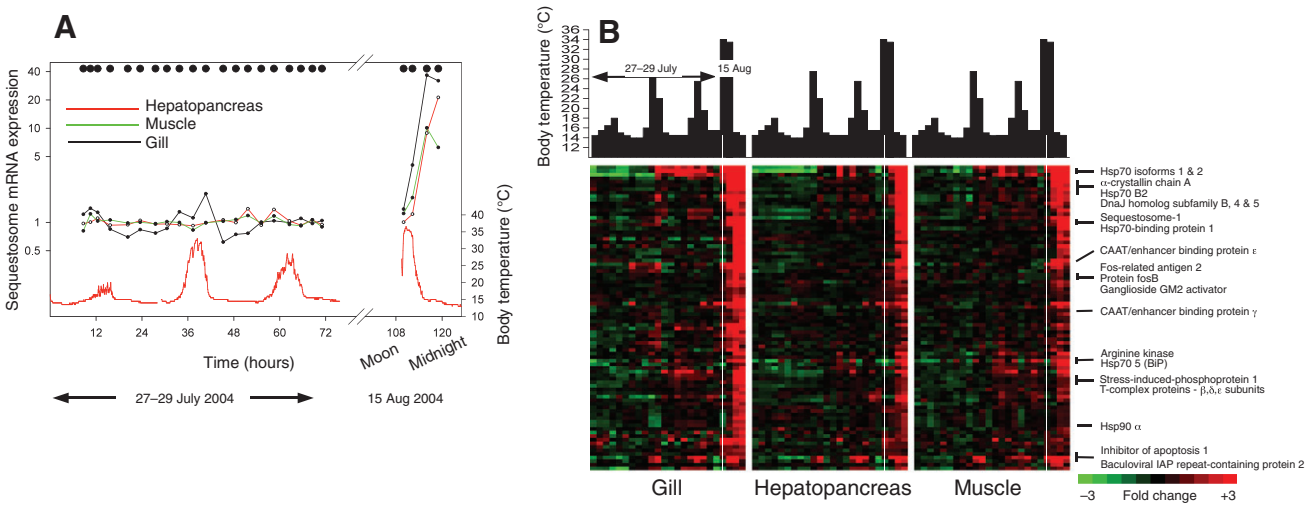

Fig. 3. Pattern of gene expression in the California ribbed mussel *M. californianus* over a 72 h time period plus an additional collection two weeks later during an unusually hot day: (A) body temperature of high-site mussels reached 38°C during an unusually hot day. Expression of sequestosome mRNA during this time period as an example of a gene expressed specifically during this extremely stressful event. (B) Heat map showing a K-means cluster of 85 genes whose expression profile was similar to that of sequestosome 1. Each row corresponds to a gene, with columns representing the relative expression of each gene across the 20 time points in the three-day study and the four time points from the *ad hoc* collected samples for gill, hepatopancreas and muscle tissue. The identity and expression of particularly relevant genes within the cluster are shown [after Gracey et al. (Gracey et al., 2008)].

proliferation. These oscillations were striking in mussels from the higher site but much attenuated in specimens from the lower site. Furthermore, these oscillations did not show any simple association with either a circadian, circatidal or body temperature rhythm, although they were more pronounced in mussels from the higher site. The rationale for the anti-correlation was suggested to be an incompatibility of metabolic processes that generate reactive oxygen species and growth processes dependent on molecular integrity, e.g. of DNA, and thus a need for compartmentalization of these processes in time.

Genes that are involved in protein folding set the other temporal-expression pattern detected by Gracey et al. (Gracey et al., 2008). These genes were consistently expressed at greater levels in mussels from higher sites regardless of body temperature and time of sampling, suggesting that those mussels may be 'preparing' for the greater likelihood of thermal extremes. Two weeks later the authors collected another set of mussels during an occasion when mussel temperatures increased up to 38°C (about 5°C higher than during the initial collection period), a temperature that mussels may experience on four to five days during the year (Fig. 3). Several genes that were not upregulated in the initial sampling were now highly induced, among them the genes encoding sequestosomal protein, which plays an important role during the formation of protein aggregates, and an α-crystalline, which is a member of the family of small Hsps that are involved in binding to denaturing proteins to stabilize them until Hsp70 can refold them (Fig. 3).

In addition, the Gracey et al. study contributes to understanding the effects of rising temperatures on energy generation in organisms (Gracey et al., 2008). Arginine kinase, a protein involved in transfer of high-energy phosphate groups from arginine phosphate to ADP to form ATP, was upregulated during this severe cellular thermal insult (Fig. 3B). Arginine phosphate is a way to store high-energy phosphoryl groups that can be quickly made available as ATP. The upregulation of arginine kinase at these very high body temperatures is therefore an indication of the immediate energetic requirements of the cells. The message of creatine kinase, the vertebrate analogue to arginine kinase, was upregulated in response to cold in a study on the effect of temperature fluctuations in annual killifish, possibly to increase the capacity to transfer high-energy phosphoryl-groups from phosphocreatine (Podrabsky and Somero, 2004). Both phosphagens, phosphocreatine and phosphoarginine, have been proposed to play an important role in increasing the tolerance of organisms to environmental stressful conditions, e.g. hypoxia and acidosis (Ellington, 2001). The role of enzymes involved in transfer of high-energy phosphoryl groups is in part to regulate the flux from ATP-producing to ATP-consuming cellular processes during periods of high-energy demand (Dzeja and Terzic, 2003). However, using a proteomics approach we have found that the arginine kinase protein is downregulated in response to heat stress that is milder (28°C and 32°C) in gill tissue of the two blue mussel congeners *M. trossulus* and *M. galloprovincialis* (L.T. and M. Zuzow, unpublished). Another transcriptomic study on the HSR in porcelain crabs of the genus *Petrolisthes* also showed a down- instead of an upregulation of the arginine kinase message in the hepatopancreas with relatively mild (30°C) heat stress (Teranishi and Stillman, 2007).

Certainly, the response of arginine or creatine kinase is only one example of how important metabolic enzymes change in response to extreme temperature stress. A comparison of seasonal and latitudinal acclimatization regimes in porcelain crabs showed that variation in environmental temperature is an important factor in determining gene expression profiles of a number of transcripts that are involved in energy metabolism (Stillman and Tagmount, 2009). Another group of proteins that showed differences between constant and fluctuating thermal conditions involved metabolic pathways that produce methylamines, e.g. glycine betaine, that act as 'chemical chaperones' and whose stabilizing effect on proteins in turn may affect the expression of major Hsps (Podrabsky and Somero, 2004). These examples illustrate the complexity we face when we apply a systems biology approach to the ecological setting of the organism and show that we will need to expand our comparisons to various ecological conditions

and a diverse set of species before we can confidently identify a general cellular pathway that can quantify the metabolic costs of heat stress.

What does the Gracey et al. (Gracey et al., 2008) study teach us about the thermal limits of intertidal organisms and the effect climate change will have on these organisms? First, mussels from higher sites experience higher levels of thermal stress and shorter feeding times. This correlates with lower growth rates of mussels from higher shore sites (Somero, 2002). Second, extreme heat stress under field conditions may directly require increasing costs in form of high-energy phosphate groups. Both observations suggest that increasing thermal extremes, regardless if long- or short-term, will incur metabolic costs and affect growth rates in animals closest to the edge of their thermal envelope, potentially leading to a contraction of the vertical distribution of intertidal organisms.

Further evidence that growth rates will be affected by thermal extremes comes from several transcriptomic studies that characterize the cellular response to environmental stress. They have observed a decrease in the expression of genes involved in promoting cell growth and proliferation during stressful conditions, illustrating that there are immediate fitness costs associated with such conditions (Gracey et al., 2008; Gracey et al., 2001). A study on the marine goby *Gillichthys mirabilis* by Buckley et al. introduces the complexity of the cellular growth response because the suppression of cell proliferation genes was observed in muscle but not in gill tissue (Buckley et al., 2006).

Transcriptomic studies that focus on the ecological context of the organism provide a crucial next step in elucidating the molecular underpinnings of thermal tolerance, from a scale of less than a meter along the physical gradient from the subtidal to high-intertidal zone to a scale of hundreds to thousands of kilometers across biogeographical range boundaries. These studies have so far shown the incredible power of a systems biology approach and of a microarray approach in general. They have also opened the door to a more careful search of other indicators of the metabolic costs of thermal stress. The patterns that have emerged are not always consistent though. Heat stress seems to upregulate genes of increasing ATP production in some, e.g. intertidal mussels, but not other species, e.g. porcelain crabs. Some patterns that seem to hold in several studies, the anti-correlation of the induction of genes involved in energy metabolism and cell growth and proliferation turn out to be tissue specific (Buckley et al., 2006). To evaluate the importance of any of these patterns of change in response to heat stress we will have to expand the transcriptomic studies to either more comprehensive field studies between populations of a species or between species that are known to respond differently to heat stress. Importantly, we need studies that characterize the molecular changes that indicate the metabolic costs that are associated with short- and long-term heat stress, e.g. during acclimation following short-term heat stress. We also need to evaluate how the transcriptomic response compares with the proteomic response. Although there is evidence that some of the mRNA messages that are upregulated will also lead to higher levels of the corresponding proteins (Buckley et al., 2006), it is unclear to date if this is the case for some, most or all of the genes. Furthermore, some messages will not change and yet protein levels may increase. Simply, we need to combine transcriptomic and proteomic approaches to expand our perspective of how the organism translates a changing environment into a molecular and biochemical response and what this response means for the fitness of the organism.

Conclusion

The results of a number of studies on the HSR in organisms from environments that differ in absolute temperature and thermal range suggest that the response (i) may be absent in organisms that occupy thermally stable environments, (ii) employed at maximal or near-maximal levels in species from highly variable thermal environments, and (iii) be a widely 'expandable option' in organisms from moderately variable thermal environments. Thus, whereas species from highly variable thermal environments may be relatively heat-tolerant, they may already be utilizing their HSR under current conditions at such a level that acclimatory plasticity in this trait is largely or entirely absent. Organisms from moderately variable environments can modify (acclimate and acclimatize) their constitutive levels of Hsps and shift their response to a higher onset temperature. This suggests that species from the extreme ends of the thermal spectrum, either from very stable or highly variable thermal environments, live closer to their thermal limits and that they will be more affected by global climate change than species from moderately variable thermal environments. However, increasing the incidence of HSR activation due to increasing temperatures in organisms from moderately variable thermal environments may incur costs that are detrimental to the long-term fitness of the species and restrictive of the thermal niches in which the organisms can occur.

Studies venturing into the systems response of cells to thermal stress have provided us with interesting results; one of them is the trade-off between increasing production of ATP and cell growth and proliferation. They have started to provide insights into possible indicators of the metabolic costs of thermal stress. Future comparative studies promise to provide a broader and more comprehensive picture of the molecular changes that distinguish species or populations that experience different levels of thermal stress.

Accurate predictions of the effect of climate change on species distributions will only come from collaborative efforts between research groups that work on the biophysical aspects of as well as the systems biology response of the organism to heat stress. However, as the contributions to this symposium show, it will be challenging to predict who will be the winners and losers in a changing and ever more complex world.

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