

Heat-shock proteins and thermotolerance: linking molecular and ecological perspectives

How do organisms survive high-temperature episodes? Why is there so much variation among different organisms in their capacity to withstand high-temperature events? Answering these two questions is critical to understanding the underlying functional and ecological mechanisms of thermotolerance, and may help us to predict and mitigate the natural and agricultural consequences of global climate change. Higher plants and animals can employ a number of behavioral, morphological and physiological traits to minimize the probability that tissues and cells will be exposed to critically high temperatures¹. However, despite these adaptations, higher organisms may still need to employ metabolic responses that prevent cellular damage during the times that avoidance mechanisms are insufficient and cells are exposed to high temperatures. With their limited mobility and relatively simple morphology, most microorganisms must rely solely on biochemical mechanisms of thermotolerance. Thus, the discovery of heat-shock proteins (hsps; specific proteins expressed preferentially at high temperatures) and their occurrence in a wide range of organisms from bacteria to humans has led to a flurry of research on the functional significance of hsps, including their role in thermotolerance²⁻⁵. Induced hsps are thought to protect existing proteins and membranes during heat stress or facilitate repair or degradation of damaged proteins following a stressful event^{3,5}.

Recently, experiments with hsp-deficient mutant strains of microorganisms or eukaryotic cell cultures have shown that certain hsps (but not all) can be required for survival at elevated temperatures or for acquisition of thermotolerance^{3,6}. For example, *Saccharomyces cerevisiae* mutants lacking a functional gene coding for Hsp104 cannot survive an acute temperature event, and transforming these mutants with a functional *HSP104* gene restores thermotolerance⁶.

Are hsps involved in ecological variation in thermotolerance?

Although we are beginning to understand many of the underlying cellular mechanisms of thermotolerance³, we do not yet have a satisfying answer to the second question posed above – i.e. what is responsible for variation in thermotoler-

ance among organisms? For example, most research to date has focused largely on the relationship between hsp production and survival, leaving open the question as to whether more subtle variation in the fitness of organisms that survive a high-temperature event is related to variation in the type of hsp produced, or the pattern of production of a given hsp or set of hsps.

Gehring and Wehner⁷ provide an example of how evolutionary perspectives can be merged with molecular approaches to begin to understand the ecological role of hsps. They studied the heat shock response in desert ants (*Cataglyphis bombycina* and *C. bicolor*), which survive body temperatures of 50°C for at least 10 minutes, and compared their response to wood ants (*Formica polyctena*), which live in moderate climates. The results were somewhat surprising, but illuminating. Production of hsps in *Cataglyphis* was induced at temperatures only 2°C higher than in *Formica*, but continued at much higher temperatures. The induction of Hsp70 and Hsp83 at such relatively low temperatures in this desert-adapted species, however, was consistent with the ants' behavior. In these ants, an entire forager population leaves the nest in the middle of the day *en masse*. Thus, they can encounter a temperature shift from <30°C to >50°C in a few seconds, which is insufficient time to mount an effective heat shock response. Thus, the authors argue that the presynthesis of hsps at relatively low nest temperatures, coupled with continued production at higher temperatures, may provide the necessary thermoprotection when ants are suddenly exposed to midday desert conditions when emerging from the nest.

This ecological/evolutionary approach has also been incorporated in at least three other studies. Bosch *et al.*⁸ showed that *Hydra oligactis*, a species that inhabits relatively thermostable habitats and is very sensitive to high temperatures, was not able to induce the production of any detectable hsps in response to temperature stress, whereas individuals of a closely related species that lives in a broad range of habitats, *Hydra attenuata*, induced the production of an Hsp60-related protein in response to acute temperature and was able to survive. Similar results were found for two species of

Collisella limpets⁹. The limpet species that occupies the high intertidal zone and thus experiences greater temperature fluctuations is more tolerant to acute heat stress and produced a broader array of hsps than its low intertidal counterpart. White *et al.*¹⁰ observed (1) a diversity of isoforms of Hsp30 and Hsp70 among six closely related fish species of the genus *Poeciliopsis*, most of which live in habitats that have extreme fluctuations in temperature over seasonal and daily time-frames, and (2) a unique isoform of Hsp60 and Hsp100 only in *P. monarcha*, a species that lives in a relatively thermostable habitat in comparison to the other five species examined. They argue that these patterns of diversity in hsps are consistent with the view that hsps may be under selection because of their role in thermotolerance.

Why does this variation exist?

The results of the studies discussed above are consistent with the notion that hsps are not only important in thermotolerance but might also be responsible for some of the variation in thermotolerance among organisms. Yet one of the difficulties in hsp research is in linking the studies that document a large range of variation among organisms in hsp production with molecular studies explicitly showing the requirement and function of hsps in thermotolerance. In other words, given that hsps can be necessary for thermotolerance and that the capacity to produce hsps is strongly evolutionarily conserved^{2,3}, why don't all organisms make them in the same way and in the same pattern? For example, why did *Hydra oligactis* lose, or never develop, the ability to produce hsps in response to temperature stress? One possible answer to this question is that the ability to produce hsps in response to high temperatures comes at some kind of cost to other organismal functions. For example, in the case of *Hydra*, individuals that perhaps produced hsps in a population evolving in a thermally stable habitat might have been less fit than individuals that lost the ability to induce Hsp60 and/or other hsps because of an unfavorable cost/benefit ratio of that trait in such a stable temperature environment. If this were true, then those individuals would likely be eliminated from the population.

Whether the ability to induce the production of hsps confers a cost has only been addressed in three sets of studies that we know of. Krebs and Loeschcke¹¹ suggested that the heat shock response might have a fitness cost if expressed at normal temperatures. They induced a heat shock response with a sub-lethal high temperature event in *Drosophila* and observed reduced fitness of those individuals

in comparison to non-induced controls. Feder *et al.*¹² showed that *Drosophila* cells that over-expressed Hsp70 at normal temperatures grew slower than normal cells, suggesting that accumulating Hsp70 at normal temperatures confers a significant cost to *Drosophila* cell growth. Sanchez *et al.*¹³ observed that a mutant strain of *S. cerevisiae* that was unable to synthesize Hsp104 grew faster than its wild-type counterpart under aerobic conditions.

If producing hsp is costly for organisms, and natural selection has worked towards optimizing the cost/benefit ratio of hsp, then we might also expect variation in hsp production to occur as a function of developmental stages or environmental conditions. For example, organisms might invest in more-effective heat shock responses as a function of the likelihood of a given developmental stage to experience a heat shock, or as a function of the importance of a given developmental stage to overall organismal fitness (e.g. leaves with the highest assimilative value to the plant). It is well known that hsp are developmentally regulated in animals and plants^{3,4}, but whether developmental variation is related to the cost of hsp is unknown. One example that might suggest that developmental variation in hsp may too be under selection owing to costs is that most plant species studied do not produce hsp in pollen, but sorghum – a plant adapted to hot, dry environments – has been reported to accumulate hsp in germinating pollen¹⁴. Thus, in this species, whose pollen might be exposed to extremely high temperatures, the benefit of maintaining hsp production in pollen might exceed the cost of producing these proteins. There is, however, no evidence that hsp represent any benefit or cost in pollen during temperature stress.

Organisms also require resources for *de novo* production of stress-proteins, so producing hsp might also involve a resource cost. For example, there are indications that total cellular hsp accumulation might represent 10–15% of total soluble protein in a given tissue¹⁵, although actual measurements of the total nitrogen (N) cost of hsp have not been made. In organisms that are often N-limited (e.g. plants, herbivorous insects) the diversion of N-based resources into hsp at the expense of other processes could confer significant costs, so we should expect there to be variation in hsp production as a function of resource availability. This variation should also be correlated with thermotolerance. There is also evidence in *S. cerevisiae* that patterns of Hsp70 production in response to heat stress are greatly influenced by the physiological status of the organisms as regulated by nutrition¹⁶. However, far more work needs

to be done to understand whether environmental and developmental variation in hsp production might result from the optimization of cost and benefits.

Linking function, cost and variation: the challenge for the future

To date, the vast majority of studies of induced hsp have attempted to determine their mechanism of action in stress tolerance at the molecular, biochemical and cellular level^{2–5}. One goal of this research is to use this information to potentially breed or genetically engineer greater stress resistance in organisms that provide benefits for humans (e.g. agricultural plants⁴). The few ecologically based studies of hsp suggest that the ability of organisms to live in stressful habitats might be related to the ability to produce hsp in response to stress⁸, and they also suggest that different organisms may use the same hsp in different manners⁷ or different hsp^{3,9} to solve problems of thermotolerance in different habitats. These patterns may arise because hsp production incurs significant costs along with significant benefits^{11–13}. These costs might also be exacerbated under different environmental conditions or at different developmental stages⁴.

Understanding the nature of the costs of hsp production at the molecular, biochemical and physiological level may provide the necessary link to allow us to determine the critical information regarding an organism's evolutionary history and ecological conditions that would lead to the enhancement of hsp being truly beneficial in stress tolerance. At the very least, they would provide us with a way to link questions addressed at the functional level (what do hsp do and how do they do it?), with questions at the ecological level (why is there so much variation in the ability of even closely related organisms to survive stressful conditions?). Additionally, given that there is a great interest in manipulating other induced responses, such as damaged-induced induction of plant defenses¹⁷, then hsp could be a good model system to link functional and ecological perspectives.

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