J Appl Physiol 120: 702–710, 2016.
First published October 15, 2015; doi:10.1152/japplphysiol.00552.2015.

HIGHLIGHTED TOPIC | *Molecular Adaptations to Exercise, Heat Acclimation, and Thermotolerance*

Epigenetics and cytoprotection with heat acclimation

Michal Horowitz

Laboratory of Environmental Physiology, Faculty of Dental Medicine, The Hebrew University, Jerusalem, Israel

Submitted 2 July 2015; accepted in final form 5 October 2015

Horowitz M. Epigenetics and cytoprotection with heat acclimation. *J Appl Physiol* 120: 702–710, 2016. First published October 15, 2015; doi:10.1152/japplphysiol.00552.2015.—Studying "phenotypic plasticity" involves comparison of traits expressed in response to environmental fluctuations and aims to understand tolerance and survival in new settings. Reversible phenotypic changes that enable individuals to match their phenotype to environmental demands throughout life can be artificially induced, i.e., acclimation or occur naturally, i.e., acclimatization. The onset and achievement of acclimatory homeostasis are determined by molecular programs that induce the acclimated transcriptome. In heat acclimation, much evidence suggests that epigenetic mechanisms are powerful players in these processes. Epigenetic mechanisms affect the accessibility of the DNA to transcription factors, thereby regulating gene expression and controlling the phenotype. The heat-acclimated phenotype confers cytoprotection against novel stressors via cross-tolerance mechanisms, by attenuation of the initial damage and/or by accelerating spontaneous recovery through the release of help signals. This indispensable acclimatory feature has a memory and can be rapidly reestablished after the loss of acclimation and the return to the physiological preacclimated phenotype. The transcriptional landscape of the deacclimated phenotype includes constitutive transcriptional activation of epigenetic bookmarks. Heat shock protein (HSP) 70/HSP90/heat shock factor 1 memory protocol demonstrated constitutive histone H4 acetylation on hsp70 and hsp90 promotors. Novel players in the heat acclimation setup are poly(ADP-ribose)ribose polymerase 1 affecting chromatin condensation, DNA linker histones from the histone H1 cluster, and transcription factors associated with the P38 pathway. We suggest that these orchestrated responses maintain euchromatin and proteostasis during deacclimation and predispose to rapid reacclimation and cytoprotection. These mechanisms represent within-life epigenetic adaptations and cytoprotective memory.

heat acclimation; cross-tolerance; epigenetic and cytoprotective memory

DEFINITION AND GOALS

HEAT ACCLIMATION OR ACCLIMATIZATION improves heat dissipation and thermal tolerance and delays thermal injury, thereby enhancing endurance to exposure to heat. Acclimatory plasticity also manifests protection from nonthermal novel stressors. This trait has a within-life adaptive memory involving chromatin remodeling and regulation of gene expression. The primary goal of this review is to summarize recent insights into the molecular programs that affect acclimatory plasticity. Transcription factors underlying the acclimatory responses may bind/activate not only pro- but also antisurvival pathways. The research into the cross talk between conflicting signaling in heat acclimation is still in its infancy. Given the data available, we describe that, in acclimatory homeostasis, which presents a "compromised state," the ratio of helpful to deleterious effects favors the specific adaptagent. Future studies investigating this dilemma are needed.

PHENOTYPIC PLASTICITY

The term phenotypic plasticity has been employed in many biological fields, a widely accepted definition is "the capacity of a single genotype to exhibit a range of phenotypes in response to variation in the environment" (11). Evolutionary biologists often use this definition, which encompasses genetic contributions to plasticity. Another accepted meaning of phenotypic plasticity covers phenotypic responses to factors in the environment (7, 59). Environmental physiologists focus on the numerous outcomes noted in individuals in response to environmental fluctuations. Many studies have quantified and compared the mean values of traits in two or more environments to understand tolerance and survival in the new setting. Reversible phenotypic changes enable individuals to match their phenotype to environmental demands throughout life. The

Address for reprint requests and other correspondence: M. Horowitz, Laboratory of Environmental Physiology, Faculty of Dental Medicine, The Hebrew Univ., Jerusalem 91120, Israel (e-mail: m.horowitz@mail.huji.ac.il).

outcome of these changes is called acclimation if artificially induced or acclimatization when they occur in nature. Notably, the genotype (or adaptive reserves) influences the magnitude of the stimulus required to induce acclimation, and the ability to acclimate is important in comparative studies (19, 49, 50).

Cells respond to extra- and intracellular signals by changing gene expression patterns (25), and these processes play important roles in acclimation. In other words, the long-term physiological responses to acclimation are determined by the molecular programs that induce the acclimated transcriptome. For example, the abundance or sensitivity of postsynaptic receptors can be changed by differential transcription of receptor subunits. This affects organ responsiveness i.e., "(central command − peripheral organ) cross talk" and coordinates the adaptive physiological responses (17, 19). Evidence is available that acclimatory networks control the rate/magnitude of target gene activation, the timing of their activation, and, in turn, the function of their executor proteins. The changes may be transient or may become seemingly constitutive (memory affect), with epigenetic mechanisms maintaining gene expression signatures in the absence of the original signal (25).

A bioinformatics model of such a sequence of events, based on yeast strains, was recently published, and the interested reader is referred to Gitter et al. (13). Much evidence suggests that epigenetic mechanisms are powerful players that enable long-term phenotypic adaptation to transient signals without changes in DNA sequences. One of the main epigenetic mechanisms employed is posttranslational modifications to histone proteins, particularly histones H3 and H4, such as phosphorylation, acetylation, and mono-, di-, and tri-DNA methylation. These epigenetic mechanisms affect the accessibility of the DNA to transcription factors, thereby regulating gene expression. Studies on the role of these processes with respect to within-life adaptation to environmental stress in mammals are sparse, but more data are available regarding invertebrates and vertebrate ectotherms acclimated to temperature and/or osmolarity (e.g., Refs. 60, 62). In heat-acclimating mammals, Tetievsky and Horowitz (53) studied posttranslational modifications (acetylation, phosphorylation) in histone tails. Recently, Alvaredo et al. (2) used hibernators to demonstrate that genomic DNA methylation is dynamic across torpor-arousal bouts during winter hibernation. In humans, studies on the epigenetic involvement in reversible adaptive responses are limited to exercise training. DNA methylation status in response to acute and chronic exercises was assessed by Voisin et al. (58). Analysis of the available data led the authors to conclude that acute and chronic exercise affects DNA methylation levels significantly, in a tissue- and gene-specific manner. Lindholm et al. (32) demonstrated that training alters DNA methylation, along with other epigenetic changes in regulatory enhancer regions. Macgee and Hargreaves (37) studied histone modifications and the expression of metabolic genes in skeletal muscle. Although noncoding RNAs (42) research is in its infancy, they have been identified as integral components of epigenetic control. For example, micro-RNAs (miRNAs), an important class of small noncoding RNAs, seem to control about one-half of the protein coding genes in mammals, mostly by suppressing/silencing gene expression (26).

Our knowledge of the epigenetic control of gene expression in processes associated with within-life adaptations, such as acclimation, is sparse and sometimes derived from in silico studies. Here our discussion will be limited to heat acclimation.

PHENOTYPIC PLASTICITY: A FUNDAMENTAL OF HEAT ACCLIMATION

When heat acclimation homeostasis has been achieved, the increased endurance to heat stress stems primarily from a leftward shift (decrease) in the body temperature threshold (T-Tsh) for activation of heat dissipation effector organs and a rightward shift (an increase) of T-Tsh for thermal injury, thereby expanding the range of thermoregulatory control. The evolvement of the acclimated phenotype is a continuum of concerted, temporally changing processes at all levels of body organization. An apparent acclimated state is already seen at the onset of acclimation [short-term heat acclimation (STHA)]; however, acclimatory homeostasis is only achieved after long-term acclimation (LTHA). Different physiological adjustments are utilized during these phases. Examination of the profile of [central command − peripheral organ cross talk] reveals a transition from an early temporary inefficient state in which apparent acclimation depends on increased activity in the autonomic thermoregulatory control system, to efficient cellular performance. Physiological responses, such as central discharge/peripheral heat dissipation-to-organ response ratio, plasma volume and circulatory adjustments, or evaporative cooling, suggest that heat acclimation is biphasic (17, 19). Changes in transcript and encoded protein profiles of specific genes, i.e., cardiac sarcoplasmic (SR) Ca^{2+} turnover-related genes, such as SERCA (SR Ca^{2+}-ATPase) and phosphorylated phospholamban and cardiac contractility, or voltage-gated ion channels (9, 38, 45), match the biphasic physiological acclimation program. This confirms dynamic gene expression plasticity throughout the phases of heat acclimation.

A time course analyses of the global genomic response of rats during acclimation provided insight into the principle mechanisms involved in the development of the acclimated phenotype. The genomic analyses substantiated that short-term acclimation is a critical acclimatory checkpoint. The transcriptome map of the hypothalamus (45) matches the increased ratio of central discharge to effector organ response (Fig. 1*A*, *bottom*), suggesting decreased organ efficiency at that acclimation phase (STHA). The map shows a marked transient upregulation in the transcript of genes encoding voltage-gated ion channels, ion pumps, or transporters, as well as hormone or transmitter receptors and cellular messengers, collectively implying enhanced membrane depolarization, release of transmitters, and neuronal excitability. The transient downregulation of groups of genes participating in intracellular protein trafficking, metabolism, or phosphorylation suggests perturbations in cellular maintenance (45). The temporary nature of the genomic response to short acclimation is pronounced in the cardio-transcriptome. Significant upregulation of topoisomerase II, and ERCC1 (excision repair cross-complementation group 1), all involved in maintenance of DNA integrity-double-strand DNA repair/synthesis (22, 51, 52), support the notion that double-strand DNA damage occurs at the onset of acclimation. The up/downregulation of groups of transcripts and epigenetic markers suggests that epigenetic mechanisms are involved in the reprogramming gene expression at the onset of heat acclimation. Among these altered transcripts, it is

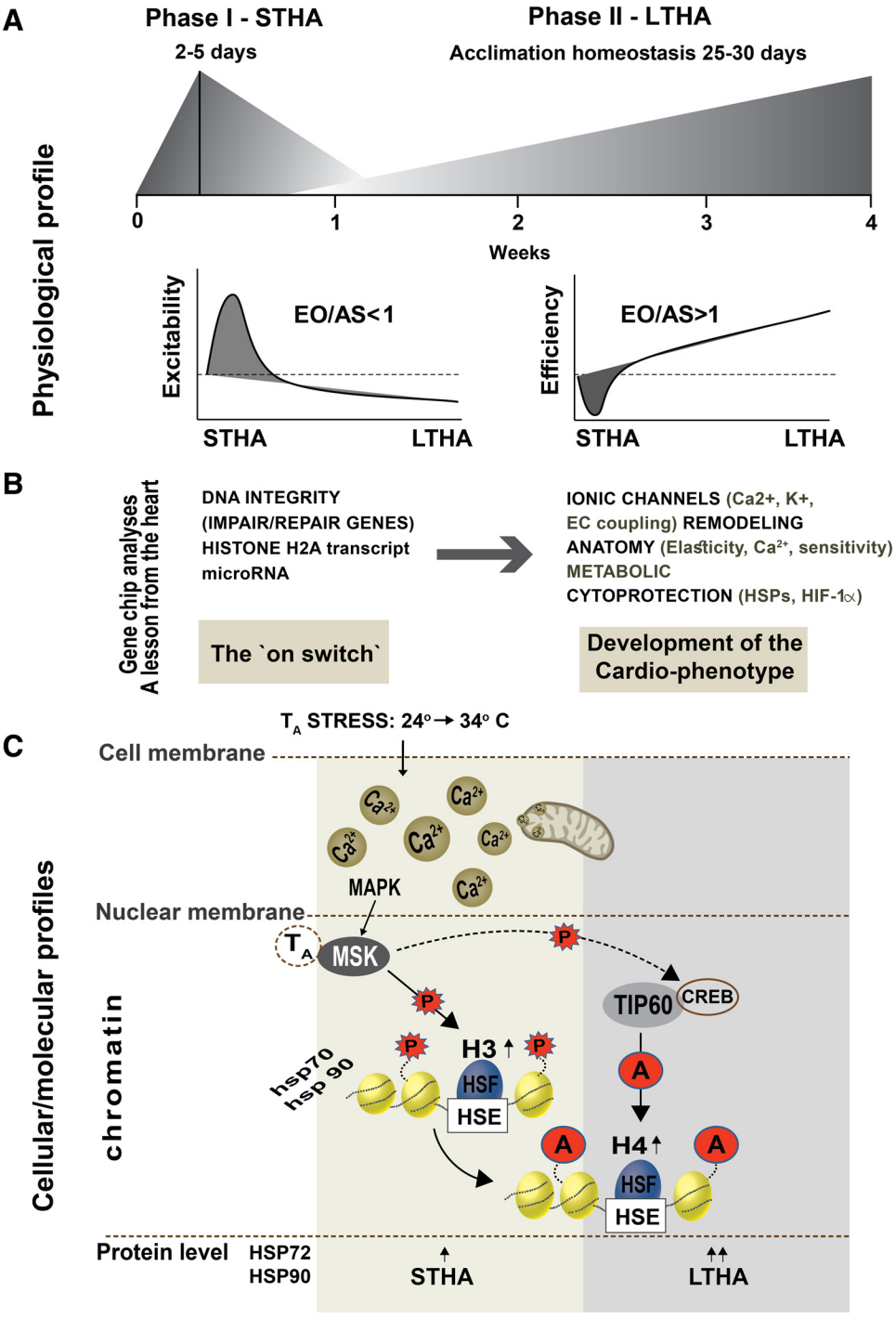

Fig. 1. Heat acclimation's dynamic. *A*: the physiological profile of heat acclimation. At the onset of heat acclimation [short-term heat acclimation (STHA)], the autonomic nervous system compensates for impaired cellular performance. Increased efficiency, a signature of long-term heat acclimation (LTHA), diminishes the need for accelerated autonomic excitability. *B*: STHA is the "on switch" of heat acclimation. Hierarchal clustering of genes representing global genomic responses during the evolvement of heat acclimation revealed upregulation of genes associated with maintenance of DNA integrity, altered linker histones, and profound downregulation of miR-1 and miR-206, leading to heat shock protein (HSP) 70 synthesis. *C*: conceptual model of epigenetic regulation of the induction of HSP reserves in the heart. Upon STHA, elevated basal body temperature and cellular Ca^{2+} increase the recruitment of mitogen- and stress-activated protein kinase downstream of P38 signaling cascade (MSK) 1/2 to the *hsp70 and hsp90* promoters to phosphorylate histone H3, which tags for histone H4 acetylation. Histone H4 is acetylated when acclimation homeostasis has been achieved. HSF1 (heat shock factor 1) binds to the heat shock elements (HSE) on the promoters of the *hsp* genes, initiating protein transcription/translation. Acclimation temperature (T_A) is 34°C. EO/AS, effector organ-to-autonomic signal ratio; CREB, cAMP response element-binding protein; TIP60, histone acetyltransferase (HAT)-interactive protein; EC, excitation-contraction; HIF-1α, hypoxia-inducible factor-1α. [Adapted from Horowitz (19) with permission and from Tetievsky et al. (51–53).].

important to note that the chromatin modifiers, such as histone species from the H1 cluster (Hist1h1a; Hist1h1b), linker histones associated with nucleosomal spacing and chromatin condensation, are significantly downregulated. An additional significant group of involved epigenetic markers is miRNAs (Horowitz M, Tetievsky A, and Izik-Radom S, unpublished observations) with upregulation of miRNA 297 (stress associated) and significant downregulation of miRNAs miR-1, miR-133, and miR-206. Of relevance to our heat acclimation model is the role of these miRs in the regulation of heat shock protein (HSP) 70 expression. Decreased miR-1 and miR-206 promotes HSP70 synthesis (27, 29, 43), and these miRs also control muscle mass. The concerted reprogramming of gene expression, based on the heart and the brain transcriptomes, in tandem with the physiological data, leads us to conclude that STHA is the "on switch "of acclimation (Fig. 1, *A* and *B*).

Recent publications regarding the role of gut permeability in thermal tolerance raise the question of whether STHA disrupts intestine permeability. When permeability is altered, LPS leaks and proinflammatory cytokines are released, which may affect the initiation of heat acclimation. LPS leakage has not been studied during STHA; however, the rise in rectal temperature

(T_{re}) at that phase (0.5–1°C) does not conform to the changes seen in T_{re} due to LPS leakage. Lambert et al. (31) demonstrated disruptions in intestinal permeability when T_{re} is above 41°C. In the hypothalamus, a transient downregulation of pyrogenic cytokines concomitantly with a decrease in T-Tsh for heat dissipation effectors during STHA was found (45), implying that LPS may not be involved. Furthermore, HSP72 levels, which may be elevated in response to LPS leakage, were stable at this acclimation phase (35).

In summary, the physiological survival strategy of the STHA phenotype is to massively increase heat dissipation. In the autonomic nervous system, this is displayed by a marked upregulation of the voltage-gated ion channels. Major stress signatures are miRs involved with HSP72 induction downregulation, as well as with activation of genes involved in the maintenance of DNA integrity. Changes in epigenetic markers can thus be considered as the "on switch" of acclimation, initiating the subsequent adaptive processes. Similar principles of dynamics in other species (e.g., Refs. 28, 62) suggest that this adaptive principle may be evolutionarily conserved.

The genomic responses of the LTHA phenotype includes enhanced stores of cytoprotective molecules in both the hypothalamus and the heart [e.g., HSPs, hypoxia-inducible factor (HIF)-1α, and the antioxidative glutathione *S*-transferase-p subunit] in a tissue-specific manner. The acclimated transcriptome of the heart demonstrates upregulation of genes encoding ion channel subunits linked to the excitation-contraction (EC) coupling cascade, Ca^{2+} binding/release features in the SR reticulum, and genes linked to sarcomere organization, which also affect Ca^{2+} sensitivity and ventricle elasticity. These correlate with the physiological features of the acclimated heart, including *1*) increased contractile efficiency, namely, production of greater pressure with lower oxygen consumption (achieved by changes in myosin isoform ratios and qualitative changes in EC coupling); and *2*) greater compliance and venous return, which improves the ability to cope with high thermal loads under sedentary and exercising conditions (20, 24).

By examining the changes in specific acclimation indicators (e.g., cardiovascular performance) during the phases of acclimation, we deepened our understanding of the molecular basis of the observed physiological trait. Considering that *1*) a profound feature of acclimatory homeostasis is the establishment of enhanced HSP72 reserves (35); *2*) heat stress induces selective histone H4 acetylation and histone H3 phosphorylation (53, 54); and *3*) gene chip hierarchal clustering shows that *hsps*, *Hsf1* (heat shock factor 1), and chromatin remodelers, are included in related gene clusters (22), we established a HSP72/HSP90 model to examine epigenetic processes throughout acclimation (Fig. 1*C*). Our results showed histone H3ser10 phosphorylation during STHA and recruitment of histone acetyltransferase TIP60 and histone H4 acetylation on the promotors of HSP72 and HSP90 of the acclimated phenotype (LTHA) (53). These processes remodeled chromatin to euchromatin (open chromatin), allowing the transcription factor HSF1 to bind to and initiate HSP translation. Evidence of the essential role of P-H3Ser10 in the induction of hsp70, without regard to acetylation state, was provided by Labrador and Corces (30). This supports the notion that P-H3Ser10 is needed to create a recognition site for future acetylation (shown by the conservation of arginine 164 in GCN5). This is yet to be demonstrated in our model.

An intriguing question is of course the upstream trigger for heat acclimation. Key upstream "initiators" include transducers of environmental stressors from the cell surface to the nucleus, such as the mitogen-activated protein kinases. A network graph structure analysis, as well as expression data, highlighted interactions during and after acclimation and revealed that the P38 MAPK [which activates the nuclear kinase mitogen- and stress-activated protein kinase (MSK) (48)] is important in acclimation and probably transmits environmental signals to the cells. Ca^{2+} overload and temperature, both players in our model, activate this pathway. Therefore, we examined MSK recruitment on HSP72 and HSP90 promoters and found significant upregulation during STHA. Thus it seems that epigenetic control of HSP cytoprotective reserves occurs in heat acclimation. The profound reduction of miR-1 and miR-206 during STHA (as mentioned above) is another example of epigenetic regulation of HSP70. Examination of the epigenetic regulatory control of HSP reserves is useful as a prototype acclimatory model. Furthermore, the variety of epigenetic markers detected during STHA and LTHA infer that epigenetic regulation of gene expression is well integrated in the acclimation repertoire and affects somatic memory formation, as described below.

HEAT ACCLIMATION CONFERS PROTECTION TO NOVEL STRESSORS

An inseparable outcome of acclimation is the mechanism of "cross-tolerance," whereby exposure to one stressor induces protection from a novel stressor. Such cross-reinforcement raises the possibility of inducing protection to a stressor without prior exposure to it. Protection from ischemic-reperfusion injury in the heart, and from brain hyperoxia, as well as from hypoxia and TBI (traumatic brain injury) have all been substantiated (3, 18, 47).

In contrast to classical preconditioning, where exposure to a short sublethal stress renders protection for approximately 2 days (39), heat acclimation-mediated cross-tolerance (HACT) confers protection for almost 3 wk. Sharing underlies cross-tolerance: innate protective networks enhanced by heat acclimation are exploited by novel stressors, and stress-specific signaling pathways evoked by the novel stressor work in concert with generic (protective) pathways and provide cross-tolerance. Hierarchal clustering by stress specificity (of samples from the hearts of acclimated animals, exposed to heat stress or to ischemic-reperfusion injury and hybridized onto cDNA atlas array of stress-associated genes) validated this hypothesis. In the heart, the essential cluster for HACT includes the upregulation of cytoprotective molecules, e.g., HSPs, antioxidative, anti-apoptotic, HIF-1α (22, 33). This allows a rapid protective response without the need for de novo transcription/synthesis of these molecules following exposure to a novel stress. The specific contribution of each of these molecules to HACT is yet to be evaluated. Studies from our laboratory (21, 55) demonstrated that *1*) the HSF1-HSP72 cascade (at least in the heart and in *C. elegans*) is regulated in a HIF-1α-independent manner; and *2*) HSP72 is essential but insufficient to confer protection in our models. Preliminary experiments using cardiac ischemia imply that

HIF-1α transcriptional activation (vs. HSP72) is the predominant player (21).

The buildup of cytoprotective reserves relies on transcriptional activation progressively induced during the course of acclimation. We found a paradox in STHA. The upregulation of HSF1 binding to the heat shock element (HSE) (52), and alteration of mitochondrial outer membrane integrity (i.e., maintenance of ψ delta membrane potential) (5), imply that membranal adaptive responses have been activated. However, STHA animals have lower tolerance (4, 61) to novel stressors (5). This suggests that HACT depends on long-term transcriptional/translational processes, which only establish cytoprotective protein reserves, or decrease ROS production in complexes I and III once acclimatory homeostasis has been achieved. This conclusion is further supported by the findings that daily administration of β-adrenergic blocker restricts HSP72 induction without affecting parasympathetic adjustments (18, 34), and that inhibition of HIF-1α transcriptional activation leads to loss of HACT, despite large HSP72 reserves (21).

HACT is a prolonged response, involving adaptive, long-lasting multiple tier networks, whereby intrinsic qualitative features, such as protein levels, their rate of induction, or isoform shifts, take place. Using the rat cardiac ischemia, whole body hypoxia, whole body hyperoxia, and mice TBI as models of immediate and long-term HACT responses, respectively, it is clear that HACT employs two major protective avenues, *1*) injury attenuation and *2*) postinsult release of help signals, which are enhanced in the acclimated phenotype.

Among the attenuating pathways are the fast-responding, large cytoprotective reserves, which have been discussed above. Of equal importance is the process of receptor remodeling, for which *N*-methyl-D-aspartate (NMDA) receptors (principal excitatory amino acid receptor permeable to calcium) are a model. Remodeling of the NMDA receptor subunit ratio to decrease opening probability and a decrease in the abundance of the receptor in the hippocampus and frontal cortex of heat-acclimated rats has been shown (61). Following exposure to hypoxic/ischemic stress, there is a massive glutamate discharge. The activated NMDA receptors accelerate the massive calcium influx, causing a calcium overload and oxidative stress. The now overactivated NMDA receptors enable the massive intracellular Ca^{2+} overload and the subsequent activation of endonucleases and kinases to cause neuronal death by necrosis or apoptosis (8, 10). The combination of fewer NMDA receptors in acclimated rats (under both basal and hypoxic conditions) and the increased receptor subunit ratio favoring decreased channel opening probability (GluN2B/GluN2A) reduce the Ca^{2+} overload on hypoxia (36). The nonacclimated phenotype shows a reciprocal effect. An additional avenue reducing Ca^{2+} flux via receptor remodeling is the significant increase in the GluA2 subunit of the α-amino-3-hydroxy-5-methyl-4-isoazole-propionic acid (AMPA) receptors (AMPA receptor subunit) in acclimated animals. This subunit has low calcium penetration; hence changes in its expression have a major impact on Ca^{2+} permeability. Yacobi et al. (61) reported a rapid decline in GluA2 in controls, but significant elevation in the acclimated ones. Heat acclimation-mediated receptor remodeling was also noted in cardiac muscle L-type Ca^{2+} channels (24).

In addition to injury attenuation, the employment of long-term posttraumatic help signals in the spontaneous recovery following TBI has been demonstrated. Recovery is determined by the balance between injury and repair mechanisms (40). In the heat-acclimated phenotype, AKT activation and enhanced transcriptional activation of HIF-1α targets (VEGF, nitric oxide, GLUT1) match the accelerated spontaneous recovery of the injured brain (56). Angiotensin receptor 2, which is elevated during heat acclimation, facilitates neurogenesis as well as neuroprotection [for details, the reader is referred to Umschweif et al. (57)].

HEAT ACCLIMATION MEDIATED CYTOPROTECTIVE MEMORY

Early sporadic physiological studies on heat acclimation, its decay (DeAC) and reinduction (ReAC) showed that reinduction of heat acclimation after a period of DeAC is much faster than the initial/original heat acclimation session (31). Various authors (12, 41) predicted that the physiological heat-acclimated phenotype decays at a rate of a 1-day loss of acclimatized status for every 2 days spent without heat exposure. Memory can be considered as a temporary regulator, and, once activated by a strong signal (from common signaling molecules), it remains "on" such that a second (weaker) signal is able to produce a rapid response. It was only in 2008 that an experimental model using rodents enabled examination of the mechanisms involved in heat acclimation memory. We tested physiological thermoregulatory central responses, EC coupling, and cardiac mechanics in rats. We confirmed that acclimation is memorized and can be regained after a 2-mo period of decline, by exposure to the original acclimating conditions for 2 days (which is clearly much shorter than the 30 days required to establish initial acclimatory homeostasis). Given that HACT is an integral feature of the acclimated phenotype, we expanded our memory protocol to explore whether cytoprotection has a memory. We used infarct size following cardiac ischemia-reperfusion insult, and the development of rigor contracture during cardiomyocyte hypoxia as physiological tests. Following LTHA, infarct size was significantly smaller than in nonacclimated controls, returned to preacclimation size following DeAC, and resumed the protected LTHA phenotype upon ReAC. Similarly, time to rigor contracture in the LTHA and the ReAC cardiomyocyte was markedly longer than in controls and the DeAC phenotype (52).

Interestingly, there was a dichotomy between the genotype and the physiological phenotype following the loss of acclimation (DeAC). Gene chip analysis of stress-associated genes detected that several gene clusters of the deacclimated transcriptome maintained their acclimated profile throughout the DeAC and ReAC phases. Therefore, it seems that a molecular program prolongs the availability of features acquired by the protected acclimated phenotype, enabling their rapid return upon ReAC. Maintained transcriptionally activated states of several chromatin remodelers (e.g., high-mobility group proteins, DNA polymerase), together with activated *hsf1* and *hsp70* genes implied that upstream epigenetic processes may play a role in memory formation. Using the HSP70/HSP90 model (see above), we demonstrated that, following 1 mo of acclimation decay, defined as a dormant memory phase (Fig. 2), acetyltransferase TIP60 and constitutively elevated acety-

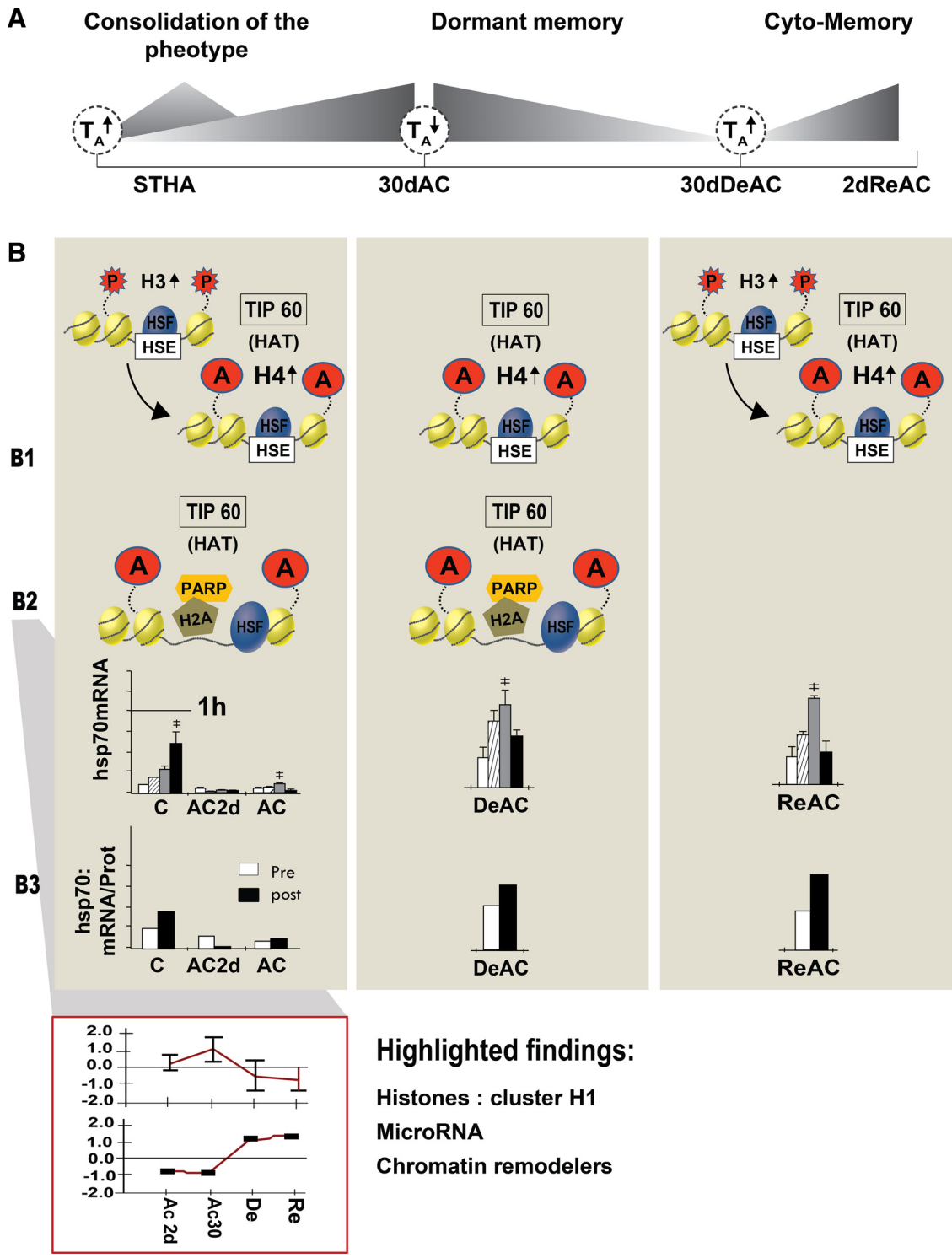

Fig. 2. Cues to the development of heat acclimation-mediated cytoprotective memory. During the decay of acclimation, the acclimated phenotype activates a variety of signaling pathways in an attempt to maintain acclimatory proteostasis. Activated kinases (e.g., MAPK P38), epigenetic markers, including chromatin modifiers, transcription factors, and microRNAs (51), are possibly among these mechanisms that then predispose rapid reinduction acclimation (ReAC). *A*: HSP70/HSP/HSF1 memory model time and temperature scales. *B*: epigenetic mechanisms regulate induction and maintenance of HSP70 reserves. *B1*: constitutive maintenance of euchromatin via histone H4 acetylation and HSF1 binding on the *hsp70* promotor during decay acclimation (DeAC) predisposes to rapid HSP reserve level and ReAC. *B2*: based on Tetievsky et al. (51) findings and Beneke (6), we hypothesized that poly(ADP-ribose)ribose polymerase 1 (PARP1) also plays a role in HSP72 accumulation. *Inset*: hierarchal clustering of affymetrix gene chip of the memory model. The profile of the clusters shows constitutive up/downregulation throughout DeAC-ReAC. *B3*: *hsp70* mRNA dynamics post-heat stress (at 41°C) and elevated hsp70 mRNA-to-HSP protein ratio on DeAC and ReAC before (pre) and during (post) heat stress at peak mRNA levels. The elevated ratio favors proteostasis. ‡Denotes time of peak mRNA level. Examples from hierarchal clustering of the rat genome showing continuous up/downregulation of transcript, including, e.g., chromatin modifiers, transcription factors, and MAPK P38 targeting HSP27 during DeAC and ReAC (for details, see text). C, control; AC2d, 2-day acclimation; AC30, 30-day acclimation; De, decay; Re, reinduction. [Adapted from Refs. 51–53.]

lation of histone H4 are recruited at the HSE of *hsp70* and *hsp90*. HSF1-HSE binding is only noted in the *hsp70* promoter. Upon reacclimation, the HSP70 and HSP90 epigenome returned to their acclimated state, and HACT resumed.

The constitutively acetylated histone H4 and the preserved euchromatin state found throughout heat acclimation-DeAC-ReAC seems to be a hallmark of heat acclimation mediated cytoprotective memory in the HSP72-HSP90 prototype. Furthermore, DeAC rats showed a significant increase in *hsp and atf3* mRNA levels compared with heat-acclimated animals and had a greater mRNA-to-protein ratio of HSP72 and HSP90 (calculated from Ref. 35). This implies that constitutive remodeling of gene transcription to maintain proteostasis is involved in cytoprotective memory formation (51–53). This conclusion is also supported by the finding that, as acclimation declines, eukaryotic translation initiation factor 2 subunit α (which initiates translation when dephosphorylated) increases the phosphorylated fraction of the kinase, i.e., delays protein translation. Concomitantly, *hsp72* transcript levels are elevated, despite stable HSP72 levels, suggesting HSP proteostasis (1).

THE DEACCLIMATED/REACCLIMATED TRANSCRIPTOME

Hierarchal clustering throughout "memory consolidation" (STHA → LTHA), the "dormant memory" (DeAC) and ReAC phases drew our attention to gene clusters that were only transcriptionally active during DeAC and ReAC. Similar to our stress gene associated array, the dichotomy between the molecular and the physiological characteristics of DeAC was noted. A large number of transcripts assigned to these clusters are epigenetic markers, such as chromatin modifiers and miRNAs. For detailed documentation, the reader is directed to Tetievsky et al. (51, 52). In the following text, we will highlight important epigenetic markers that have not previously been considered as players in our memory puzzle. *1*) Histone species from the H1 cluster (e.g., Hist1h1a; Hist1h1b) were significantly downregulated. Eight of the detected histones are linker histones, which bind to linker DNA (between nucleosome cores), thereby participating in nucleosome spacing, and, in turn, transcription factor accessibility, and gene expression control. *2*) PARP1 [poly(ADP-ribose)ribose polymerase 1] is a structural protein used by eukaryotic organisms to break through the chromatin barrier. When PARP1 is enzymatically inactive, it prevents gene transcription. However, when PARP1 is modified and activated by environmental (and developmental) signals, it not only modifies itself, it also alters other chromatin-associated molecules, which loosen chromatin and allow transcription.

PARP1 also poly(ADP-ribosyl)ates ATPase-chromatin remodelers such as SWI/SNF observed in our cardiac model, and loosens chromatin this way (14). PARP1 is dormant in the *Hsp70* promoter, and its activity is rapidly induced by TIP60, either by the histone H2A switch, namely PARP1 eviction/HSF1 insertion, or by direct acetylation (6). Large TIP60 recruitment on the hsp70 promotor and HSF1 binding during DeAC supports the notion of a similar scenario in our model, and PARP1 may be an additional predisposing factor in cytoprotective memory (Fig. 2*B2*). *3*) The MAPK superfamily responds to environmental stress more than other kinase families. Careful pathway analyses, taking into account significant pathway checkpoints and target genes, revealed that the P38 module is constitutively active during DeAC and ReAC (Fig. 2, *inset*) and plays a prominent role during these phases. Interestingly, in congruence with the miRNA identified in our analyses, 75% of these miRNA targets are connected to the P38 MAPK pathway [i.e., the transcription factors: cAMP response element binding protein (also function as histone acetyltransferase), activating transcription factor, or c-*jun*/c-*fos*], thereby highlighting the important role played by this pathway in our model (51).

Our recent data indicate that the induction of transcription is critical for memory formation associated with prolonged shifts in environmental conditions. There is often a dichotomy between the levels of cellular mRNA and protein expression and/or activity. Nevertheless, mRNA reacts to changes in the environment affecting the executioner protein pathways. The reproducibility of the results of several distinct bioinformatic analyses supports the concept that DeAC is a phase-dependent molecular program. The DeAC phase prolongs the availability of molecular features acquired by the protected acclimated phenotype, e.g., amplifies transcriptional responses critical to the cellular fight for survival and facilitates the return of these mechanisms upon ReAC. Stress induced transcriptional memory has been suggested regarding mental stress (23). Sailaja et al. (44) reported "that chromatin structure and histone modifications are causally involved in . . . transcriptional memory" during cholinergic neurotransmission. Here we suggest that transcriptional memory is a mode of adaptation to environmental challenges that are too persistent to be buffered by physiological homeostatic mechanisms alone, yet are too short-lived for the consolidation of genetic adaptations. The orchestrated responses maintain euchromatin, i.e., a reprogrammed transcriptome during DeAC, and are among the mechanisms that enable rapid ReAC and cytoprotective memory.

FUTURE PERSPECTIVE

The acclimated phenotype reflects a situation of "compromise." The ratio of helpful regulatory features to deleterious effects favors coping with fluctuating environments. Diverse pathways and transcription factors underlying the acclimatory responses can bind and activate the same genes. This activates pro-/anti-survival pathways to maintain long-term homeostasis. In heat acclimation, the examination of the "cross-over" between conflicting signaling is still in its infancy. Shared responses between fever and heat stress (16) draw our attention to HSF1, a key regulator of HSPs. HSF1 upregulates HSPs but also binds to several pyretic cytokines, which can affect heat acclimation. Nevertheless, in the heat-acclimated TBI model, acclimated mice show enhanced HSP72 reserves and an induction of anti-inflammatory cellular environment (46), despite possible HSF1 binding to pyretic cytokines and inducible nitric oxide synthase. Haddad and Horowitz (15) demonstrated inducible nitric oxide synthase attenuation of splanchnic vasoconstriction in tandem with peripheral vasodilation in heat-stressed acclimated rats. This effect was abolished when N^G-nitro-L-arginine was administered. An additional interesting conflict is posed by acclimation-induced HIF-1α upregulation, which activates aquaporine-4 and VEGF (potentially increasing blood-brain barrier permeability following TBI), yet there is less edema formation in heat-acclimated rats and mice (56). Taken together, it seems that, once acclimatory homeostasis is

achieved, following exposure to a specific adapting trigger, the ratio of helpful regulatory features vs. deleterious effects favors protection to the adaptagent. Future studies investigating this dilemma are needed.

ACKNOWLEDGMENTS

The author is indebted to Chen Fleishman for critical reading of the manuscript and very constructive comments.

GRANTS

The research of the author, cited in this review, was supported over the years by the Israel Science Foundation, founded by the Israel Academy of Sciences and Humanities, US-Israel Binational Fund, and German Israeli Foundation Research Grants.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: M.H. conception and design of research; M.H. performed experiments; M.H. analyzed data; M.H. interpreted results of experiments; M.H. prepared figures; M.H. drafted manuscript; M.H. edited and revised manuscript; M.H. approved final version of manuscript.

REFERENCES

1. **Abbas A, Horowitz M.** Changes in hsp mRNA dynamics are involved in HSP homeostasis during one month of heat acclimation decline (Abstract). In: *Proceedings of IUPS Birmingham, UK*, 2013, p. PCA334.
2. **Alvarado S, Mak T, Liu S, Storey KB, Szyf M.** Dynamic changes in global and gene-specific DNA methylation during hibernation in adult thirteen-lined ground squirrels, *Ictidomys tridecemlineatus*. *J Exp Biol* 218: 1787–1795, 2015.
3. **Arieli Y, Eynan M, Gancz H, Arieli R, Kashi Y.** Heat acclimation prolongs the time to central nervous system oxygen toxicity in the rat: Possible involvement of HSP72. *Brain Res* 962: 15–20, 2003.
4. **Assayag M, Gerstenblith G, Stern M, Horowitz M.** Long- but not short-term heat acclimation produces an apoptosis-resistant cardiac phenotype: a lesson from heat stress and ischemic/reperfusion insults. *Cell Stress Chaperones* 15: 651–664, 2010.
5. **Assayag M, Saada A, Gerstenblith G, Canaana H, Shlomai R, Horowitz M.** Mitochondrial performance in heat acclimation–a lesson from ischemia/reperfusion and calcium overload insults in the heart. *Am J Physiol Regul Integr Comp Physiol* 303: R870–R881, 2012.
6. **Beneke S.** Regulation of chromatin structure by poly(ADP-ribosyl)ation. *Front Genet* 3: 169, 2012.
7. **Bennett AF, Lenski RE.** An experimental test of evolutionary trade-offs during temperature adaptation. *Proc Natl Acad Sci U S A* 104: 8649–8654, 2007.
8. **Choi DW.** Excitotoxic cell death. *J Neurobiol* 23: 1261–1276, 1992.
9. **Cohen O, Kanana H, Zoizner R, Gross C, Meiri U, Stern MD, Gerstenblith G, Horowitz M.** Altered Ca^{2+} handling and myofilament desensitization underlie cardiomyocyte performance in normothermic and hyperthermic heat-acclimated rat hearts. *J Appl Physiol* 103: 266–275, 2007.
10. **Doble A.** The role of excitotoxicity in neurodegenerative disease: implications for therapy. *Pharmacol Ther* 81: 163–221, 1999.
11. **Fordyce JA.** The evolutionary consequences of ecological interactions mediated through phenotypic plasticity. *J Exp Biol* 209: 2377–2383, 2006.
12. **Garrett A, Rehrer N, Patterson M.** Induction and decay of short-term heat acclimation in moderately and highly trained athletes. *Sports Med* 41: 757–771, 2011.
13. **Gitter A, Carmi M, Barkai N, Bar-Joseph Z.** Linking the signaling cascades and dynamic regulatory networks controlling stress responses. *Genome Res* 23: 365–376, 2013.
14. **Gottschalk AJ, Timinszky G, Kong SE, Jin J, Cai Y, Swanson SK, Washburn MP, Florens L, Ladurner AG, Conaway JW, Conaway RC.** Poly(ADP-ribosyl)ation directs recruitment and activation of an ATP-dependent chromatin remodeler. *Proc Natl Acad Sci U S A* 106: 13770–13774, 2009.
15. **Haddad W, MH.** Heat acclimation alters endothelial nitric oxide activity in the splanchnic vascular bed. *J Therm Biol* 24: 403–408, 1999.
16. **Hasday JD, Thompson C, Singh IS.** Fever, immunity, and molecular adaptations. *Compr Physiol* 4: 109–148, 2014.
17. **Horowitz M.** Do cellular heat acclimation responses modulate central thermoregulatory activity? *Physiology* 13: 218–225, 1998.
18. **Horowitz M.** Heat acclimation and cross-tolerance against novel stressors: genomic-physiological linkage. *Prog Brain Res* 162: 373–392, 2007.
19. **Horowitz M.** Heat acclimation, epigenetics, and cytoprotection memory. *Compr Physiol* 4: 199–230, 2014.
20. **Horowitz M.** Matching the heart to heat-induced circulatory load: heat-acclimatory responses. *Physiology* 18: 215–221, 2003.
21. **Horowitz M, Alexander-Shani R.** Is Hif-1α Essential for Heat Acclimation and Cross-Tolerance (Abstract)? In: *Proceedings of Experimental Biology Boston 2015*. Bethesda, MD: FASEB, 2015, p. 994.16.
22. **Horowitz M, Eli-Berchoer L, Wapinski I, Friedman N, Kodesh E.** Stress-related genomic responses during the course of heat acclimation and its association with ischemic-reperfusion cross-tolerance. *J Appl Physiol* 97: 1496–1507, 2004.
23. **Reul JM.** Making memories of stressful events: a journey along epigenetic, gene transcription, and signaling pathways. *Front Psychiatry* 5: 5, 2014.
24. **Kodesh E, Nesher N, Simaan A, Hochner B, Beeri R, Gilon D, Stern MD, Gerstenblith G, Horowitz M.** Heat acclimation and exercise training interact when combined in an overriding and trade-off manner: physiologic-genomic linkage. *Am J Physiol Regul Integr Comp Physiol* 301: R1786–R1797, 2011.
25. **Kolybaba A, Classen AK.** Sensing cellular states-signaling to chromatin pathways targeting Polycomb and Trithorax group function. *Cell Tissue Res* 356: 477–493, 2014.
26. **Krol J, Loedige I, Filipowicz W.** The widespread regulation of microRNA biogenesis, function and decay. *Nat Rev Genet* 11: 597–610, 2010.
27. **Kukreti H, Amuthavalli K, Harikumar A, Sathiyamoorthy S, Feng PZ, Anantharaj R, Tan SL, Lokireddy S, Bonala S, Sriram S, McFarlane C, Kambadur R, Sharma M.** Muscle-specific microRNA1 (miR1) targets heat shock protein 70 (HSP70) during dexamethasone-mediated atrophy. *J Biol Chem* 288: 6663–6678, 2013.
28. **Kültz D.** DNA damage signals facilitate osmotic stress adaptation. *Am J Physiol Renal Physiol* 289: F504–F505, 2005.
29. **Kwon C, Han Z, Olson EN, Srivastava D.** MicroRNA1 influences cardiac differentiation in Drosophila and regulates Notch signaling. *Proc Natl Acad Sci U S A* 102: 18986–18991, 2005.
30. **Labrador M, Corces VG.** Phosphorylation of histone H3 during transcriptional activation depends on promoter structure. *Genes Dev* 17: 43–48, 2003.
31. **Lambert GP, Gisolfi CV, Berg DJ, Moseley PL, Oberley LW, Kregel KC.** Selected contribution: hyperthermia-induced intestinal permeability and the role of oxidative and nitrosative stress. *J Appl Physiol* 92: 1750–1761, 2002.
32. **Lindholm ME, Marabita F, Gomez-Cabrero D, Rundqvist H, Ekström TJ, Tegnér J, Sundberg CJ.** An integrative analysis reveals coordinated reprogramming of the epigenome and the transcriptome in human skeletal muscle after training. *Epigenetics* 9: 1557–1569, 2014.
33. **Maloyan A, Eli-Berchoer L, Semenza GL, Gerstenblith G, Stern MD, Horowitz M.** HIF-1α-targeted pathways are activated by heat acclimation and contribute to acclimation-ischemic cross-tolerance in the heart. *Physiol Genomics* 23: 79–88, 2005.
34. **Maloyan A, Horowitz M.** β-Adrenergic signaling and thyroid hormones affect HSP72 expression during heat acclimation. *J Appl Physiol* 93: 107–115, 2002.
35. **Maloyan A, Palmon A, Horowitz M.** Heat acclimation increases the basal HSP72 level and alters its production dynamics during heat stress. *Am J Physiol Regul Integr Comp Physiol* 276: R1506–R1515, 1999.
36. **Massey PV, Johnson BE, Moult PR, Auberson YP, Brown MW, Molnar E, Collingridge GL, Bashir ZI.** Differential roles of NR2A and NR2B-containing NMDA receptors in cortical long-term potentiation and long-term depression. *J Neurosci* 24: 7821–7828, 2004.
37. **McGee SL, Hargreaves M.** Histone modifications and exercise adaptations. *J Appl Physiol* 110: 258–263, 2011.
38. **Mirit E, Gross C, Hasin Y, Palmon A, Horowitz M.** Changes in cardiac mechanics with heat acclimation: adrenergic signaling and SR-Ca regulatory proteins. *Am J Physiol Regul Integr Comp Physiol* 279: R77–R85, 2000.

39. **Murry CE, Jennings RB, Reimer KA.** Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 74: 1124–1136, 1986.
40. **Neary JT.** Protein kinase signaling cascades in CNS trauma. *IUBMB Life* 57: 711–718, 2005.
41. **Pandolf KB, Burse RL, Goldman RF.** Role of physical fitness in heat acclimatisation, decay and reinduction. *Ergonomics* 20: 399–408, 1977.
42. **Place R, Noonan E.** Non-coding RNAs turn up the heat: an emerging layer of novel regulators in the mammalian heat shock response. *Cell Stress Chaperones* 19: 159–172, 2014.
43. **Radom-Aizik S, Zaldivar FP, Nance DM, Haddad F, Cooper DM, Adams GR.** Growth inhibition and compensation in response to neonatal hypoxia in rats. *Pediatr Res* 74: 111–120, 2013.
44. **Sailaja BS, Cohen-Carmon D, Zimmerman G, Soreq H, Meshorer E.** Stress-induced epigenetic transcriptional memory of acetylcholinesterase by HDAC4. *Proc Natl Acad Sci U S A* 109: E3687–E3695, 2012.
45. **Schwimmer H, Eli-Berchoer L, Horowitz M.** Acclimatory-phase specificity of gene expression during the course of heat acclimation and superimposed hypohydration in the rat hypothalamus. *J Appl Physiol* 100: 1992–2003, 2006.
46. **Shein NA, Doron H, Horowitz M, Trembovler V, Alexandrovich AG, Shohami E.** Altered cytokine expression and sustained hypothermia following traumatic brain injury in heat acclimated mice. *Brain Res* 1185: 313–320, 2007.
47. **Shein NA, Horowitz M, Shohami E.** Heat acclimation: a unique model of physiologically mediated global preconditioning against traumatic brain injury. *Prog Brain Res* 161: 353–63, 2007.
48. **Soloaga A, Thomson S, Wiggin GR, Rampersaud N, Dyson MH, Hazzalin CA, Mahadevan LC, Arthur JS.** MSK2 and MSK1 mediate the mitogen− and stress−induced phosphorylation of histone H3 and HMG−14. *EMBO J* 22: 2788–2797, 2003.
49. **Somero GN.** Comparative physiology: a "crystal ball" for predicting consequences of global change. *Am J Physiol Regul Integr Comp Physiol* 301: R1–R14, 2011.
50. **Taylor NA.** Human heat adaptation. *Compr Physiol* 4: 325–365, 2014.
51. **Tetievsky A, Assayag M, Ben-Hamo R, Efroni S, Cohen G, Abbas A, Horowitz M.** Heat acclimation memory: do the kinetics of the deacclimated transcriptome predispose to rapid reacclimation and cytoprotection? *J Appl Physiol* 117: 1262–1277, 2014.
52. **Tetievsky A, Cohen O, Eli-Berchoer L, Gerstenblith G, Stern MD, Wapinski I, Friedman N, Horowitz M.** Physiological and molecular evidence of heat acclimation memory: a lesson from thermal responses and ischemic cross-tolerance in the heart. *Physiol Genomics* 34: 78–87, 2008.
53. **Tetievsky A, Horowitz M.** Posttranslational modifications in histones underlie heat acclimation-mediated cytoprotective memory. *J Appl Physiol* 109: 1552–1561, 2010.
54. **Thomson S, Hollis A, Hazzalin CA, Mahadevan LC.** Distinct stimulus-specific histone modifications at Hsp70 chromatin targeted by the transcription factor heat shock factor-1. *Mol Cell* 15: 585–594, 2004.
55. **Treinin M, Shliar J, Jiang H, Powell-Coffman JA, Bromberg Z, Horowitz M.** HIF-1 is required for heat acclimation in the nematode *Caenorhabditis elegans*. *Physiol Genomics* 14: 17–24, 2003.
56. **Umschweif G, Alexandrovich AG, Trembovler V, Horowitz M, Shohami E.** Hypoxia-inducible factor 1 is essential for spontaneous recovery from traumatic brain injury and is a key mediator of heat acclimation induced neuroprotection. *J Cereb Blood Flow Metab* 33: 524–531, 2013.
57. **Umschweif G, Shabashov D, Alexandrovich AG, Trembovler V, Horowitz M, Shohami E.** Neuroprotection after traumatic brain injury in heat-acclimated mice involves induced neurogenesis and activation of angiotensin receptor type 2 signaling. *J Cereb Blood Flow Metab* 34: 1381–1390, 2014.
58. **Voisin S, Eynon N, Yan X, Bishop DJ.** Exercise training and DNA methylation in humans. *Acta Physiol (Oxf)* 213: 39–59, 2015.
59. **Whitman DW, Agrawal AA.** What is phenotypic plasticity and why is it important? In: *Phenotypic Plasticity of Insects: Mechanisms and Consequences*, edited by Whitman DW, Ananthakrishnan TN. Enfield, NH: Science, 2009, p. 1–63.
60. **Windisch HS, Frickenhaus S, John U, Knust R, Pörtner HO, Lucassen M.** Stress response or beneficial temperature acclimation: transcriptomic signatures in Antarctic fish (*Pachycara brachycephalum*). *Mol Ecol* 23: 3469–3482, 2014.
61. **Yacobi A, Stern Bach Y, Horowitz M.** The protective effect of heat acclimation from hypoxic damage in the brain involves changes in the expression of glutamate receptor a. *Temperature (Austin)* 1: 57–65, 2014.
62. **Yampolsky LY, Zeng E, Lopez J, Williams PJ, Dick KB, Colbourne JK, Pfrender ME.** Functional genomics of acclimation and adaptation in response to thermal stress in Daphnia. *BMC Genomics* 15: 859, 2014.