Stress-related genomic responses during the course of heat acclimation and its association with ischemic-reperfusion cross-tolerance

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Horowitz, Michal, Luba Eli-Berchoer, Ilan Wapinski, Nir Friedman, and Einat Kodesh. 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.—Acclimation to heat is a biphasic process involving a transient perturbed phase followed by a long lasting period during which acclimatory homeostasis is developed. In this investigation, we used cDNA stress microarray (Clontech Laboratory) to characterize the stress-related genomic response during the course of heat acclimation and to test the hypotheses that 1) heat acclimation influences the threshold of activation of protective molecular signaling, and 2) heat-acclimation-mediated ischemic-reperfusion (I/R) protection is coupled with reprogrammed gene expression leading to altered capacity or responsiveness of protective-signaling pathways shared by heat and I/R cytoprotective systems. Rats were acclimated at 34°C for 0, 2, and 30 days. 32P-labeled RNA samples prepared from the left ventricles of rats before and after subjection to heat stress (HS; 2 h, 41°C) or after I/R insult (ischemia; 75%, 45 min; reperfusion: 30 min) were hybridized onto the array membranes. Confirmatory RT-PCR of selected genes conducted on samples taken at 0, 30, and 60 min after HS or total ischemia was used to assess the promptness of the transcriptional response. Cluster analysis of the expressed genes indicated that acclimation involves a “two-tier” defense strategy: an immediate transient response peaking at the initial acclimating phase to maintain DNA and cellular integrity, and a sustained response, correlated with slowly developed adaptive, long-lasting cytoproteective signaling networks involving genes encoding proteins that are essential for the heat-shock response, antiapoptosis, and antioxidation. Gene activation was stress specific. Faster activation and suppression of signaling pathways shared by HS and I/R stressors probably contribute to heat-acclimation I/R cross-tolerance.

ischemia-reperfusion; stress genes; microarray; heart; heat stress

HEAT ACCLIMATION, A CONSERVED phenotypic adaptive response to a prolonged transfer to a higher ambient temperature, confers protection against acute heat stress and delays thermal injury. Conceptually, the process of heat acclimation in homeotherms can be delineated as a transition from an early transient, “inefficient” acclimation phase to an “efficient” state after acclimatory homeostasis has been reached (16, 17). In terms of controlling physiological mechanisms for heat dissipation, this process is characterized by an increased excitability of the autonomic nervous system when compensating for impaired cellular performance during short-term heat acclimation (STHA), followed by enhanced cellular function and decreased excitability on stable long-term acclimation (LTHA) (16, 17). In the latter phase, physiological mechanisms are “translated” into an expanded dynamic thermoregulatory range, characterized by decreased heat production and a reduced temperature threshold for the activation of heat-dissipation mechanisms and an elevated temperature threshold for the development of thermal injury (13). Acquiring data suggest that the induction of the heat-acclimated phenotype involves reprogramming the expression of genes encoding constitutive proteins and stress-inducible molecules (e.g., Refs. 6, 7, 25, 33). Data on changes in cellular constitutive proteins are sporadic and confined to the special interests of particular research groups. Alterations in Ca2+ regulatory and contractile proteins in various taxa and glycolytic enzymes, for example, have been the targets of several investigations (6, 7, 11, 12, 31). Among heat-inducible genes, those encoding the heat shock protein (HSP) 72 kDa and HSP 90 kDa have been the most extensively studied (2, 5, 24, 25, 41). These proteins confer cytoprotection via chaperoning the correct folding of other proteins or the degradation of abnormal proteins, or, alternatively, via their facilitatory interaction with the molecular signaling of cytoprotective pathways (27). The observation that heat acclimation sensitizes the HSP genes’ transcriptional response and increases the constitutive cellular reserve of these protein species (11, 24, 25) implies that cytoprotection can be accomplished without de novo HSP synthesis. The buildup of HSP cellular reserves is also intriguing. Because heat acclimation in mammals does not induce severe hyperthermia, other mediators are required to increase hsp gene transcription. The failure to build up HSP 72 kDa cellular reserves during acclimation in the presence of β-adrenergic blockade led us to postulate that the excitable sympathetic system, primarily during STHA, is a likely mediator of the transcriptional process (24). Taken together, the above findings place the HSP defense pathway as an integral part of the heat acclimation repertoire. An inseparable outcome of acclimation is that adjusting to one environmental stressor can, in addition to evolving primary adaptations, add to the amount of adjustment to additional stressors. Such cross-reinforcement raises the possibility of inducing adaptation to a stressor without prior exposure to that particular stressor (8) (exaptation). An important beneficial effect of heat acclimation observed in our laboratory is the development of “cross-tolerance” against oxygen supply-oxygen demand mismatching (17, 21, 22) and its consequences. In the heart, which has been studied more extensively than other organs, this phenomenon is manifested as improved mechanical and metabolic performance and reduced injury when sub-

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jected to ischemic-reperfusion (I/R) insult (7, 21, 22). With respect to cytoprotection, the ability of the heat-acclimated heart 1) to recruit a higher level of low-molecular-weight antioxidants than that recruited by nonacclimated hearts (1) and 2) to augment HSP72 on ischemic insult implies that heat acclimation enhances the responsiveness of shared protective pathways against both stressors.

Clearly, HSPs are important protective players during heat stress and I/R insult (17, 27, 44). Nevertheless, in view of the large number of inducible stress genes responding during acute heat stress (18, 34), we find it unlikely that the induction of HSP genes alone is sufficient to confer heat acclimation-induced thermostolerance or cross-tolerance to an organism. Furthermore, such thermostolerance can involve additional genes, including certain genes that are not among the consen-
sus of the heat-inducible set of genes. Our discovery of the essential role of the transcription factor hypoxia inducible factor-1 (HIF-1) in the induction of heat acclimation in Caenorhabditis elegans (42) strongly supports this notion. In the present investigation, we therefore decided to take a broad-
scale genomic approach and to gain the advantage of gene chip array technology to study a battery of stress-associated genes. This approach will expand our knowledge of gene families that have not yet been associated with heat acclimation and/or cross-tolerance and will distinguish between the general stress response and the acclimatory response.

The purpose of the present study was threefold: 1) to characterize the stress-related genomic response during different phases of heat acclimation; 2) to test the hypothesis that heat acclimation-I/R cross-tolerance is coupled with reprogram-
ing the expression of genes, leading to the altered capacity or responsiveness of cellular-signaling pathways shared by heat and I/R cytoprotective systems; and 3) to study the influence of heat acclimation on the threshold of protective molecular signaling.

MATERIALS AND METHODS

Animals and Experimental Protocols

All experiments were carried out on male Rattus norvegicus (Zabar strain, albino var), initially weighing 80–90 g, fed on Ambar labora-
tory chow and water ad libitum (7, 15). The left ventricle of the heart was used as the organ model. The large body of data accumulated on the heat-acclimated heart, including the HSP72 response, provided the rationale for performing genomic analyses on this organ.

The animals were randomly assigned to short-term heat-acclimated (STHA), long-term heat-acclimated (LTHA), and normothermic (control) groups (Fig. 1). The LTHA and the normothermic groups were further divided into 1) untreated animals serving as controls, 2)
heat-stressed rats, and 3) rats whose hearts were removed and subjected to I/R insult. The STHA group was divided into animals with no further treatment and animals undergoing heat stress. This design (Fig. 1) allowed us to follow 1) the expression levels of stress genes during the process of heat acclimation before and after heat stress (comparison of normothermic, STHA, and LTHA study groups), and 2) the cross-tolerance phenomenon between heat acclimation and acute environmental heat or I/R insults (comparison of normothermic and LTHA study groups before and after subjection to either heat stress or ischemia).

All experimental protocols were approved by the Ethics Committee for Animal Experimentation of the Hebrew University. The experimental evidence for the beneficial effects of heat acclimation on 1) heat endurance and 2) heat acclimation-I/R cross-tolerance has been studied in detail in many papers and are summarized in Table 1.

**Experimental Conditions**

The control group was held at an ambient temperature of 24 ± 1°C; heat acclimation was attained by continuous exposure to 34 ± 1°C and 30–40% relative humidity in a light-cycled room (12:12-h) for 2 (STHA) or 30 days (LTHA), as previously described (15). The rats were exposed for 2 h to 41°C for characterizing the heat stress response. The colonic temperature (Tc) was monitored online by a YSI 402 thermistor probe (YSI 402, Yellow Springs Instruments, Yellow Springs, OH) inserted 6 cm deep beyond the anal sphincter. The probe was attached to a computerized data-acquisition system (Biopac Systems, Santa Barbara, CA). After exposure to heat stress, the animals were returned to room temperature for 0-, 30-, and 60-min intervals poststress, thus allowing us to track transcript dynamics (25). The rats were then killed by cervical dislocation, and the hearts were rapidly excised and processed for mRNA analyses. For the ischemic challenge, the hearts were mounted on a Langendorff perfusion system and retrogradely perfused with Krebs-Henseleit buffer containing (in mM) 120 NaCl, 4.7 KCl, 1.2 MgSO4, 1.2 KH2PO4, 1.25 CaCl2, 25 NaHCO3, and 11 glucose, at pH 7.4, and then aerated with a mixture of 95% O2–5% CO2 at 37°C (6, 7, 21, 22). After 10 min of equilibration, the perfusion pressure was decreased by 75%, and the heart was subjected for 45 min to ischemic conditions, followed by 30 min of reperfusion, as previously described (21). In addition, to follow ischemic severity thresholds, we conducted an additional experimental series in which hearts were taken for RNA sampling after subjection to 50, 75, and 100% (global ischemia) reduction in perfusion pressure. Hearts from each group subjected to 100 cmH2O served as controls for this experimental group.

**Tissue Preparation for RNA Analysis**

The excised hearts were mounted on a Langendorff perfusion apparatus and retrogradely perfused for 2 min with Krebs-Henseleit buffer to wash out all remaining blood. The left ventricle was then carefully excised, frozen, and stored at –70°C until analysis. Ischemic-reperfused hearts underwent a similar procedure, but according to the I/R timing protocol.

**Stress-Genes Expression Using Clontech cDNA Atlas Array**

The Clontech rat’s stress atlas array containing genes spotted on a nylon membrane was chosen (Clontech Laboratory, Palo Alto, CA). The array includes 219 stress genes classified into five different functions. RNA samples from untreated, nonacclimated, and heat-acclimated hearts before and 60 min after heat stress, as well as hearts subjected to I/R insult, were used for this experimental series. In both control and heat-acclimated hearts, these protocols have previously proved to induce severe stress, leading to marked physiological changes, as well as to changes in the transcription of HSP 72 kDa (24, 25). Tri Reagent (Molecular Research Center) was used to extract the total RNA of left ventricles from nine hearts for each treatment group. For detection, we pooled the individual samples into mRNA preparations of three hearts each. The RNA samples were then treated with RNase-free DNase I to avoid genomic DNA contamination, and the purity of the RNA sample was confirmed by PCR. The quantity and quality of the sample were estimated from its absorbance at 260 and 280 nm, as well as by 1% agarose gel electrophoresis (25). The probes were labeled for 1 h by the reverse transcription of total RNA (3 μg) at 42°C in a primer mix (Clontech) containing [32P]dATP (Amersham Biosciences, Buckinghamshire, UK). The reaction was terminated by adding 0.1 M EDTA and 1 mg/ml glucogen (Sigma). The unincorporated 32P-labeled nucleotides were removed by Nucleo-Spin extraction columns (Clontech).

**cDNA Array Hybridization**

The membranes were prehybridized for 1 h at 68°C in a hybridization solution (Clontech) containing 0.1 mg/ml sheared salmon testes DNA (Sigma) to block nonspecific binding. The synthesized radiolabeled cDNA probe (5 to 15 x 106 cpm) was applied to each membrane and hybridized overnight at 68°C. Cot-1 DNA (Clontech) (1 μg/ml) was added to block nonspecific binding. Each membrane was used three times after stripping by boiling in 0.5% SDS, according to the Clontech manual. For analyses, the membranes were exposed to a phosphor screen for 15 and 24 h and detected by use of a Bio-imaging analyzer BAS2000. We used Atlas Image 2.01 software (Clontech) to grid the phosphor image, to record the pixel density of each spot, and to perform background subtraction. The background-subtracted data were then further analyzed.

**RT-PCR**

To confirm the results obtained for the arrays, we subjected the RNA samples used for gene chip array analysis to a separate RT-PCR detection system to measure the mRNA of specific genes. In addition, we tested RNA samples from hearts removed at several intervals after heat stress. This approach allowed us to test whether the process of acclimation affects the transcription dynamics of the tested genes.

**Table 1. Evidence for enhanced thermal endurance and I/R tolerance in heat-acclimated rats**

<table>
<thead>
<tr>
<th>Source</th>
<th>Nonacclimated</th>
<th>Heat Acclimated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat endurance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>During exposure at 39°C</td>
<td>247 ± 22 min</td>
<td>552 ± 65 min</td>
</tr>
<tr>
<td>I/R tolerance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to onset of ischemic contracture</td>
<td>6 ± 1.3 min</td>
<td>8 ± 2 min</td>
</tr>
<tr>
<td>Diastolic/recovery, ventricular pressure recovery after I/R</td>
<td>35%</td>
<td>80%</td>
</tr>
<tr>
<td>Infarct size, % of risk area</td>
<td>30 ± 7%</td>
<td>4.3 ± 1%</td>
</tr>
</tbody>
</table>

Values are means ± SE. I/R, ischemic-reperfusion insult.
Total RNA (1 μg) was reverse transcribed in a 20-μl reaction mixture containing 0.5 μg of oligo(dT)15 as primer and 200 U of Moloney murine leukemia virus reverse transcriptase, according to the manufacturer’s instructions (MBI Fermentas). For PCR, 1 μl of the cDNA was used for a 20-μl volume of PCR reaction mixture containing BioMix RED (Bioline) with 1.5 mM MgCl₂ and 1 pM of each specific primer. The sequence of the various primers used and the PCR program are presented in Table 2. The PCR products were resolved on 1.0% agarose gel, stained with ethidium bromide, and visualized under ultraviolet (UV) light. The density of the bands was computer analyzed with TINA Software (Raytest, Straubenhardt, Germany).

**Calibration and Normalization**

To avoid problems related to short- or long-term exposure to the phosphorimager (resulting in underestimation or saturation effect), we recorded the intensity of phosphorimages at different exposure times (between 5 and 60 h). To standardize different recordings of the same hybridization experiment, we performed a linear regression of the separate time points and averaged their regressed values to the recorded intensity at the middle time point. The individual data sets were used for comparisons between different experimental groups. To make the measurements from different hybridizations comparable, we rescaled each measured expression value by the average expression of all probes in that hybridization. We then transformed these values to make the control conditions a “baseline” for estimating the relative changes in expression. Specifically, we computed the log ratio of a gene’s (rescaled) expression value in a particular hybridization to the geometric mean of that gene’s rescaled expression values in all the hybridizations in the control group. We used a base-2 log so that a relative expression of 1 or −1 would indicate a twofold change over the average control conditions.

**Correlations and Comparisons**

We clustered the resulting data set using an agglomerative probabilistic method that groups genes together on the basis of similar expression profiles (9) (ScoreGenes package, http://compbio.cs.huji.ac.il/scoregenes/). This method iteratively assembles the genes such that the resulting set of clusters optimizes the likelihood that its genes come from the same distribution.

We clustered two sets of data: 1) for analysis 1, we used the clustered data from control, STHA, and LTHA control and heat-shock treatments to reveal groups of correlated genes in the course of heat acclimation before and after heat stress; 2) for analysis 2 we used the control and LTHA rats in control, heat-shock, and I/R conditions to interpret the cross-tolerance phenomenon. Before clustering, we removed the genes whose expression was not considered present (visible by the phosphorimager; see also below) in over half the treatments to reduce the effects of noisy data.

The selection of presence/nonpresence of a particular gene based on 50% presence in the total number of samples in the compared group in a series might be too conservative for within-treatment comparison because of a stress-specific gene evocation. Therefore, we conducted within-treatment comparisons in which presence/nonpresence was based on intensity threshold (above background) as well. A threshold of 1,200 pixels, which was compatible with spot visibility, was chosen. The specific cases in which this analysis was done are stated in RESULTS.

**Statistics**

A commercial statistics package (Sigmastat version 2, SPSS) was used for determining statistical significance. Significance proof for the kinetics of transcriptional response over time of acclimation or for heat acclimation-I/R cross-tolerance was provided initially by analyzing genes as groups (cluster analysis), using two-way and three-way ANOVA, respectively, followed by multiple comparisons. Acclimation and heat stress were the fixed effect for the first analysis, and acclimation, heat stress, and I/R for the second. For the stress severity threshold tests of selective genes, a two-way ANOVA was applied, with acclimation and time poststress (heat or ischemic) as the independent variables and gene expression the dependent variable. For paired single-gene comparisons, we used the nonparametric Mann-Whitney test. The pooled animal samples were assumed to be random samples from the animal population. A P value of <0.05 was considered statistically significant.

**RESULTS**

**Analysis of Genes as a Group**

Alteration of gene expression during the course of heat acclimation before and after heat stress (based on cluster analysis 1). Ninety-five genes matched our criterion of 50% presence in the heat-stressed group. With heat acclimation, 65 of the analyzed genes were clearly grouped into clusters in which the maximal average expression vs. that of the control group (in the most responsive treatment) was not altered by >1.5-fold. When compared with control, the expression of the other 30 genes was altered >1.5-fold, at least in the most

<table>
<thead>
<tr>
<th>GenBank Accession No.</th>
<th>Sequence</th>
<th>Annealing Temp/Cycles</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Actin: NM 031144</td>
<td></td>
<td>55 °C 23 cycles</td>
<td>Present study</td>
</tr>
<tr>
<td>HSP70: NM 031971</td>
<td></td>
<td>64 °C 40 cycles</td>
<td>Maloyan et al. (25)</td>
</tr>
<tr>
<td>HSP90: S45392</td>
<td></td>
<td>55 °C 28 cycles</td>
<td>Present study</td>
</tr>
<tr>
<td>HSFI X83094</td>
<td>55 °C 30 cycles</td>
<td>Clontech</td>
<td></td>
</tr>
<tr>
<td>bcl-2 (BAD): AF003523</td>
<td>55 °C 30 cycles</td>
<td>Clontech</td>
<td></td>
</tr>
<tr>
<td>bcl-xL: U72350</td>
<td>55 °C 30 cycles</td>
<td>Clontech</td>
<td></td>
</tr>
<tr>
<td>Nucleoplasmin (NPM): I03969</td>
<td>55 °C 28 cycles</td>
<td>Clontech</td>
<td></td>
</tr>
<tr>
<td>GST-P: X02904</td>
<td>55 °C 30 cycles</td>
<td>Present study</td>
<td></td>
</tr>
</tbody>
</table>

HSP, heat shock protein; GST-P, glutathione S-transferase P subunit.
responsive treatment. These genes, grouped into four different clusters (of ten) with their expression either upregulated or downregulated, differed in 1) the response to heat acclimation with time, and/or 2) the response to heat stress at each acclimation phase. Thus we could decipher whether acclimation affects the basal steady-state level of the expressed genes or influences the heat-stress response, as shown in Fig. 2. Genes categorized under cluster A8 encompass 11 genes, inducible by heat acclimation per se. Over the course of heat acclimation, these genes demonstrated a biphasic profile (2-way ANOVA, \( P < 0.0002 \), Fig. 2A), in which transcript steady-state levels showed a profound elevation on STHA and then stabilized at a lower expression level on reaching acclimation homeostasis (LTHA). The genes assigned to this cluster are linked with functions such as maintaining DNA integrity (damage/repair, 34%), free radical scavenging (33%), and stress regulators and effectors (33%) (for further details of genes associated with DNA damage/repair and antioxidant, see Table 3). In both STHA- and LTHA-treated groups, heat stress decreased mRNA expression of these genes to their preacclimation level (\( P < 0.001 \) and 0.03 for 2 and 30 days, respectively). In contrast, cluster A10 (Fig. 2B) includes three genes that did not change their steady-state transcript level in response to acclimation per se, yet the additional challenge of heat stress induced a marked upregulation of these transcripts compared with their levels in the nonacclimated state. This upregulation tended to be greater after 30 days of acclimation (average ratio \( > 2.65 \) and \( > 3.5 \) for STHA and LTHA, respectively, \( P < 0.001 \)). This cluster comprises the following genes: 1) DnaJ protein homolog 2 (DNAJ2), classified as a chaperon (36); 2) apoptosis regulator bcl-xL, an antiapoptotic member of the Bcl-2 superfamily (26); and 3) nucleophosmin, a nuclear-

Table 3. Genomic response over the course of heat acclimation: changes in the expression of genes assigned to cluster A8 before and after subjection to heat stress

<table>
<thead>
<tr>
<th>Gene</th>
<th>GenBank Accession No.</th>
<th>Function</th>
<th>C-HS</th>
<th>STHA</th>
<th>STHA-HS</th>
<th>LTHA</th>
<th>LTHA-HS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stress response regulators and effectors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSPB2</td>
<td>U75899</td>
<td>Heat shock protein</td>
<td>↑×3.1</td>
<td>↓×−1.2</td>
<td>↑×1.67</td>
<td>↑×1.3</td>
<td></td>
</tr>
<tr>
<td>Ribosomal protein S30 40S subunit</td>
<td>X62671</td>
<td>Protein turnover</td>
<td>↑×1.2</td>
<td>↑×4.1</td>
<td>↑×2.65</td>
<td>↑×1.3</td>
<td></td>
</tr>
<tr>
<td>DNA damage response repair and recombination</td>
<td>L08814</td>
<td>DNA binding &amp; chromatin protein</td>
<td>↓×1.4</td>
<td>↑×1.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Structure-specific recognition protein 1</td>
<td>Z12020</td>
<td>Transcription activators and repressors</td>
<td>↑×1.5</td>
<td>↑×2.5</td>
<td>↑×1.3</td>
<td>↑×2.4</td>
<td>↑×1.2</td>
</tr>
<tr>
<td>mdm2 protein</td>
<td>D10862</td>
<td>Inhibitor of DNA binding</td>
<td>↑×2.2</td>
<td>↑×1.3</td>
<td>↑×1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA binding protein D1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug and xenobiotic metabolism</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GST-P</td>
<td>X02904</td>
<td>Metabolism of cofactors</td>
<td>↓×2.1</td>
<td>↑×1.2</td>
<td>↑×2.2</td>
<td>↑×1.25</td>
<td></td>
</tr>
<tr>
<td>NADH-cytochrome b5 reductase</td>
<td>J03867</td>
<td>Metabolism of cofactors, enzymes</td>
<td>↑×2.0</td>
<td>↑×1.2</td>
<td>↑×1.5</td>
<td>↑×1.25</td>
<td></td>
</tr>
<tr>
<td>Micronutritional glutathione S-transferase 1</td>
<td>J03752</td>
<td>Xenobiotic metabolism</td>
<td>↑×1.3</td>
<td>↑×2.3</td>
<td>↑×3.0</td>
<td>↑×1.25</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as fold change relative to nonacclimated basal steady state level. C-HS, nonacclimated heat-stressed rats; STHA, heat acclimation for 2 days; LTHA, heat acclimation for 30 days. HS, heat stress. Genes responding to the stressor, not related to pathways discussed in this investigation, were not inserted into the table.
Cluster A7, which consists of three molecular chaperons (HSP90, HSP70, and HSC 70), had a related protein B23 (43). Cluster A7, which consists of three molecular chaperons (HSP90, HSP70, and HSC 70), had a different profile (Fig. 2C). The average expression within this cluster was upregulated twofold in response to acclimation alone. Exposure of the animals to heat stress during each assigned acclimatory phase, as well as before acclimation, raised the average expression of the transcripts even further (>4-fold, 2-way ANOVA, P < 0.0001), regardless of the acclimatory response, suggesting that the acute heat-stress response is not affected by acclimation. An additional noteworthy change is that genes assigned to cluster A6 [13 genes, mostly stress regulators and effectors (92%), Fig. 2D, Table 4], which showed relative underexpression and were not responded to the stressor not related to pathways discussed in this investigation were not inserted into the table. Values are presented as fold change relative to nonacclimated basal steady state level. Relative changes in gene expression 1 are not shown. Genes responding to ischemia was shown in four of seven clusters showing at least average per cluster of 1.5-fold change (log base 2 ratio ≥0.6). An additional three clusters, 40% of the total number of the analyzed genes, included genes that did not respond or showed no significant difference in their expression in response to the insults applied. The percentage of genes responding to each stress, I/R and/or heat shock, is depicted in Fig. 3.

Testing the hypothesis of cross-tolerance (based on cluster analysis 2). Analysis 2 includes 117 genes of the following treatment groups: control, control-heat shock, control-ischemia, acclimated 30 days, acclimated 30 days + heat shock, acclimated 30 days + ischemia (Figs. 3 and 4). Because the 30-day-acclimated groups included genes that were excluded because they were not “present” in over half of the control and the STHA series, a repeat analysis was conducted for this series using the 127 present genes. We asked the following questions: 1) Is the stress response specific?, namely, do heat-shock and I/R insults activate similar or different genes? 2) Does heat acclimation influence the I/R stress response? Analysis 2 provided evidence for a stress-specific response. A divergence between the genes responding to heat stress and those responding to ischemia was shown in four of seven clusters showing at least average per cluster of 1.5-fold change in transcript expression to the most responsive insult (above log base 2 ratio 0.6). An additional three clusters, 40% of the total number of the analyzed genes, included genes that did not respond or showed no significant difference in their expression in response to the insults applied. The percentage of genes responding to each stress, I/R and/or heat shock, is depicted in Fig. 3.
The number of genes responding to I/R was the highest, whereas that of the nonspecific genes was the smallest. I/R response specificity can be clearly seen in Fig. 4, which shows the gene map according to their cluster assignment. Cluster CT4, for example, includes 15 genes, of which most were upregulated twofold and above when hearts were subjected to I/R insult in both nonacclimated and LTHA samples. The resemblance between the magnitude of the response of nonacclimated and heat-acclimated groups was not statistically significant. Among the genes assigned to this cluster, seven were also influenced by heat acclimation per se, but that influence did not affect the magnitude of upregulation in response to I/R insult. Genes assigned to this cluster belong to three functional categories: stress-response regulators and effectors (47%), maintenance of DNA integrity (40%), and genes relating to the drug and xenobiotic metabolism group, all antioxidants (13%). Genes responding to heat acclimation too were mostly stress regulators or effectors. For further details, see Table 5.

Clusters CT2 (12 upregulated transcripts), CT7 (10 downregulated transcripts), and CT9 (7 upregulated transcripts, not shown) showed similar I/R specificity, yet the change in the averaged transcript level per cluster in the latter two clusters, although significant, did not exceed 1.4- to 1.5-fold. In contrast, cluster CT3 (P < 0.001, Fig. 4A) includes 15 genes responding significantly to heat stress only, whereas the genes assigned to cluster CT6 (not shown) responded to both heat stress and ischemia. The same was true for cluster CT10, which consists of only one gene, HSP70, which was upregulated by 16-fold in both treatments (Fig. 4).

Fig. 3. The number of genes responding to I/R was the highest, whereas that of the nonspecific genes was the smallest. I/R response specificity can be clearly seen in Fig. 4, which shows the gene map according to their cluster assignment. Cluster CT4, for example, includes 15 genes, of which most were upregulated twofold and above when hearts were subjected to I/R insult in both nonacclimated and LTHA samples. The resemblance between the magnitude of the response of nonacclimated and heat-acclimated groups was not statistically significant. Among the genes assigned to this cluster, seven were also influenced by heat acclimation per se, but that influence did not affect the magnitude of upregulation in response to I/R insult. Genes assigned to this cluster belong to three functional categories: stress-response regulators and effectors (47%), maintenance of DNA integrity (40%), and genes relating to the drug and xenobiotic metabolism group, all antioxidants (13%). Genes responding to heat acclimation too were mostly stress regulators or effectors. For further details, see Table 5.

**Does heat acclimation influence the I/R stress responses?**

The criterion for the exclusion of nonpresent candidate genes for analysis did not take into account the exclusion of genes expressed only in the 30-day groups. Therefore, to test whether LTHA influences specific pathways shared by heat and ischemic challenges, we reanalyzed the LTHA groups separately. The second analysis yielded an additional 10 genes whose transcriptional response to both heat and I/R insults was enhanced profoundly after heat acclimation. Genes assigned to this category (60%) include chaperons and other stress effectors or regulators, among which are two members of the Bcl-2 superfamily, those maintaining DNA integrity (20%) and ROS scavenging (20%). Table 6 lists genes that show such an acclimatory response and are referred to in this investigation.

**Analysis of Individual Genes**

To validate the results obtained in the gene chip array and to initiate a preliminary basis for testing whether heat acclimation can alter transcriptional dynamics, we also checked the following genes for temporal variations in their transcript level in basal as well as after either heat stress or ischemia: 1) two HSP genes, HSP70, already reported to respond on heat acclimation...
in rats (25), and HSP90 [overexpressed in various acclimated species (5)]; 2) two members of the Bcl-2 superfamily (26), BAD and bcl-xL; 3) glutathione-S-transferase P subunit (GST-P) associated with antioxidation and cellular redox state, and reported previously as heat-stress inducible (45); and 4) nucleoplasmin, which plays a role in the regulation of nucleolar functions of differentiation, growth, and apoptosis and was recently shown to be an essential stress response regulator during UV stress (43).

A good correlation was found between the results obtained for the individual gene RT-PCR and the arrays. Representative RT-PCR images for heart samples obtained from controls and heat-acclimated rats before and 1 h recovery after heat stress are presented in Fig. 5. The HSP genes and GST-P were upregulated in response to acclimation, bcl-2 (BAD) was downregulated, and both nucleoplasmin and bcl-xL did not change. Interestingly, after heat stress, the cytoprotective genes (HSPs and antioxidants) were less responsive in the acclimated phenotype, whereas the two bcl genes (which responded reciprocally to heat stress) and nucleoplasmin showed markedly enhanced transcript levels. The findings agree with both the arrays probed in the present investigation and those in the few available previous studies (1, 25). A noteworthy finding emerging from our analysis of individual genes was the earlier activation threshold of HSP70 and GST-P to ischemic insult and bcl-2 (BAD) to heat stress after heat acclimation (Fig. 6). Whereas both HSP70 and GST-P of the acclimated samples responded to lower ischemic insult threshold (perfusion pressure 50%), bcl-2 (BAD) and HSP70 responded 20 min after heat stress compared with 60 min in the control samples (data not shown).

### DISCUSSION

This investigation delineates for the first time the broad-scale genomic response of stress-related genes during the

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**Table 5. Genomic response of hearts of nonacclimated and long-term heat-acclimated rats before and after subjection either to HS or I/R insults: changes in the expression of genes assigned to cluster CT4**

<table>
<thead>
<tr>
<th>Gene</th>
<th>GenBank Accession No.</th>
<th>Function</th>
<th>C-HS</th>
<th>C-(I/R)</th>
<th>AC</th>
<th>AC-HS</th>
<th>AC-(I/R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apoptosis regulator bcl-xL</td>
<td>U72350</td>
<td>Antiapoptosis</td>
<td>↑×1.9</td>
<td>↑×1.5</td>
<td>↑×2.0</td>
<td>↑×2.0</td>
<td></td>
</tr>
<tr>
<td>HSC70 interacting protein (HIP)</td>
<td>X82021</td>
<td>HSP chaperon</td>
<td>↑×2.3</td>
<td>↑×1.75</td>
<td>↑×2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40S ribosomal protein S3</td>
<td>X51536</td>
<td>Protein turnover</td>
<td>↑×3.5</td>
<td>↑×2.6</td>
<td>↑×3.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>94-kDa glucose-regulated protein (GRP94)</td>
<td>X15187</td>
<td>HSP</td>
<td>↑×2.8</td>
<td>↑×2.3</td>
<td>↑×2.0</td>
<td>↑×2.8</td>
<td></td>
</tr>
<tr>
<td>150-kDa oxygen-regulated protein (ORP150)</td>
<td>U41853</td>
<td>stress response protein</td>
<td>↑×2.8</td>
<td>↑×2.0</td>
<td>↑×2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA binding protein D1</td>
<td>D10862</td>
<td>Transcription activator &amp; repressor</td>
<td>↑×2.3</td>
<td>↑×1.6</td>
<td>↓×1.7</td>
<td>↑×2.0</td>
<td></td>
</tr>
<tr>
<td>DNA damage response repair and recombination</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mdm2 protein</td>
<td>M92424</td>
<td>Transcription activator &amp; repressor</td>
<td>↑×3.2</td>
<td>↑×2.4</td>
<td>↑×2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thymine-DNA glycosylase (TDG)</td>
<td>U51166</td>
<td>DNA damage/repair signaling</td>
<td>↑×2.3</td>
<td>↑×1.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HMG2</td>
<td>D84418</td>
<td>Transcription activator &amp; repressor</td>
<td>↑×2.0</td>
<td>↑×1.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S3PR1</td>
<td>L08814</td>
<td>Chaperone</td>
<td>↑×2.1</td>
<td>↑×2.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Helicase II</td>
<td>D64059</td>
<td>Chromatin proteins</td>
<td>↑×2.3</td>
<td>↑×1.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug and xenobiotic metabolism</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GST-P</td>
<td>X02904</td>
<td>Xenobiotic metabolism</td>
<td>↑×2.1</td>
<td>↑×2.0</td>
<td>↑×2.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P450 oxidoreductase</td>
<td>M12516; M10068</td>
<td>Protein modification enzyme</td>
<td>↑×2.3</td>
<td>↑×1.8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as fold change relative to nonacclimated basal steady-state level. NC, no change. Bold type marks genes whose transcriptional response to both heat and I/R insults was enhanced profoundly only after heat acclimation (22).

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**Table 6. Genes whose transcriptional response to both heat stress and ischemic/reperfusion insults was enhanced profoundly after heat acclimation**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>Control</th>
<th>LTHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>bcl-2 (BAD)</td>
<td>Proapoptosis</td>
<td>NC</td>
<td>↑×3*</td>
</tr>
<tr>
<td>bcl-xL</td>
<td>Antiapoptosis</td>
<td>NC</td>
<td>↑×2</td>
</tr>
<tr>
<td>JNK1</td>
<td>Intracellular kinase network</td>
<td>NC</td>
<td>↑×1.5</td>
</tr>
<tr>
<td>JNK2</td>
<td>Stress response protein</td>
<td>NC</td>
<td>↑×4</td>
</tr>
<tr>
<td>Flavin containing monoxygenase 4 (FMO4) no. Z11737</td>
<td>Xenobiotic metabolism</td>
<td>NC</td>
<td>↑×1.5</td>
</tr>
<tr>
<td>DNAJ2</td>
<td>Heat stress proteins, general trafficking proteins, chaperones</td>
<td>↑×1.7</td>
<td>↑×2.6</td>
</tr>
<tr>
<td>Nucleoplasmin (NPM) no. J03969</td>
<td>DNA-binding and chromatin protein translation</td>
<td>NC</td>
<td>↑×4-5</td>
</tr>
<tr>
<td>DNA mismatch repair protein no. J04810</td>
<td>DNA synthesis, recombination and repair proteins</td>
<td>NC</td>
<td>↑×4</td>
</tr>
</tbody>
</table>

Values are presented as fold change relative to nonacclimated basal steady state level. NC, no change. Bold type marks genes whose transcriptional response to both heat and I/R insults was enhanced profoundly only after heat acclimation (22).
process of heat acclimation. It also describes how the acclimatory state affects the transcriptional response of these genes after heat stress or I/R insult. From our analyses, we outline the dynamics of the genomic response of different sets of stress-related genes over the time of acclimation, thus allowing some perception of the global acclimatory molecular strategy, 2) highlight genes that have not yet been associated with heat acclimation, and 3) discuss stress specificity in stress-gene activation and the likely pathways leading to the cross-tolerance conferred by heat acclimation.

Stress-Gene Dynamics Over Time of Heat Acclimation and Molecular Physiological Linkage

We previously provided evidence that heat acclimation induces molecularly based physiological and biochemical adaptations (6, 25). Given the biphasic acclimation profile, during which temporal perturbations in cellular homeostasis take place (16), correlative changes in stress-gene dynamics are likely to occur. The results of the present investigation confirm this view and support our hypothesis that STHA is a critical acclimatory checkpoint.

When interpreting the cluster analysis, the average expression per cluster of 1.5-fold, at least in the most responsive treatment over the course of acclimation, was taken (2- to 4-fold change for the majority of the genes). However, because the global acclimatory response of the intact animal stems from the additive effect of many, sometimes even small, changes (15–17), it is likely that the 1.5-fold change in the genomic response has a biological significance as well. It is notable that in the intact animal (as opposed to cultured cells), the response reflects not only direct cellular effects to the applied stress but is an integrated outcome of conflicting neurohumoral signals. We should also take into account that the “single sampling” protocol does not provide information on the peak response, which could be different for each acclimation phase (25).

Among the genes showing biologically meaningful changes in expression, four different gene profiles are delineated: 1) genes for which acclimation influences only their basal transcript level; 2) genes for which acclimation affects only their response to heat stress; 3) genes for which acclimation affects both their basal steady-state level and their response to heat stress; and 4) genes responding to heat acclimation and heat stress independently. The magnitude of change in expression of these gene groupings shows temporal variation over the

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**Fig. 5.** Confirmatory RT-PCR. Left: representative RT-PCR images for heart samples obtained from controls (C) and LTHA rats before and 60 min after heat stress. AC, acclimated; BAD, bcl-2-associated death promoter; bcl-xL, apoptosis regulator; GST-P, glutathione-S-transferase P subunit; NPM, nucleoplasmin; HSP, heat shock proteins. Right: qualitative changes in expression of the studied genes in respect to the C group: ↑, upregulation; ↓, downregulation. Number of arrows denotes magnitude of change.

**Fig. 6.** A: percent change in BAD steady-state transcript level 0, 30, and 60 min after heat stress (2 h, 41°C). B: percent change in GST-P (top) and HSP70 (bottom) steady-state transcript level during progressively graded ischemia. Transcriptional response of the heat-acclimated groups was earlier than in the nonacclimated ones. Each point is the mean ± SE of 3–4 individual RNA samples. Each sample was detected 3 independent times and was normalized to β-actin. *Significant difference from the matched control at that time point (P < 0.05, P < 0.02).
course of acclimation. The genes assigned to category 1 (cluster A8 and analysis 1a) include those representing a transient upregulation, peaking at STHA. Most genes assigned to this category are associated with maintaining DNA integrity and free radical scavenging. Furthermore, several genes assigned to this category show biologically meaningful expression only during the STHA phase, with only faint visibility before acclimation and after LTHA. The enhanced expression of topoisomerase II and ERCC1, respectively, engaged in double-stranded DNA repair and DNA synthesis (10, 23) (analysis 1a), for example, implies that the onset of heat acclimation leads to double-stranded DNA damage. Additional support for this type of damage is noted in the upregulation of genes associated with free radical scavenging (e.g., cytochrome b reductase, GST-P, Table 3), implying the presence of ROS, a major cause of DNA damage (20, 46). Similar upregulation of these genes was also shown by Sonna et al. (38) and Yellon and Latchman (44) after acute heat stress. Both damaged DNA and damaged proteins (14) are likely inducers of the cellular transient stress response via the activation of target genes and perhaps acclamatory responses. That neither of the transiently induced genes responded to acute heat stress suggests that, during the acclimation process, sustained heat strain (such as that imposed at the initial phase of heat acclimation) is required for their induction. Activating these genes could provide an initial line of defense for alleviating any genomic damage resulting from the strain associated with the onset of the acclimation process. This hypothesis is supported by the findings of Kultz (19), who reported that the onset of adaptation to hypertonic stress is accompanied by an upregulation of these genes encoding proteins associated with the maintenance of DNA integrity to allow different sets of adaptations to develop.

The genes classified under categories 2 and 3 include those that on acclimation, particularly after LTHA (with or without a change in their steady-state transcript level), respond to heat stress by a more profound transcriptional activation than that found in the nonacclimated phenotype. A high percentage of genes belonging to these categories are stress-response regulators and effectors, and their activation possibly enhances a heat-acclimation-specific cytoprotective signaling network to cope with perturbations in the acclimatory homeostasis achieved after LTHA.

All responding genes assigned to category 4 are chaperons whose interaction is required for the cytoprotective activity of HSP70. The response of these genes to heat as well as to other stressors has been well documented (for review, see, e.g., Refs. 14, 18, 27, 38). In the present investigation, the finding that subjecting the animals to acute heat stress per se led to a fourfold increase in the transcript level of these genes, irrespective of the acclimation phase, suggests that heat acclimation and heat stress induce two independent responses. The response obtained in our experiments for heat stress is similar to that reported in many previous publications (e.g., Refs. 18, 25, 29, 38).

The delineated gene profiles obtained in this investigation indicate a two-tier defense strategy. Whereas the immediate transient response is associated with the maintenance of DNA and cellular integrity during the strain developed at the onset of acclimation, the sustained response is correlated with adaptive, long-lasting cytoprotective signaling networks. Such adaptive plasticity correlates with our perception of the physiological acclimatory response. STHA has been shown to be a transient phase during which accelerated autonomic excitability overactivates heat-defense mechanisms to compensate for impaired cellular performance and to alleviate the initial heat strain, even at the expense of homeostasis. With progression of the acclimation process, these mechanisms are replaced with long-lasting ones that are characterized by enhanced cellular efficiency (for more details see Introduction and Refs. 16, 17). In sum, the invoked physiological strain at the initial phases of heat acclimation switches on a molecular/cellular and physiological “emergency” defense line to alleviate the initial strain, thus allowing “endurance” stressor-specific adaptations to develop (to be maintained until conditions change again).

Unmasking Heat-Acclimation-Mediated Cytoprotective Networks

The results of the present study call our attention to stress-associated genes that have not yet been linked with heat acclimation in mammalian species. After LTHA, these genes show enhanced transcriptional activation during heat stress, and certain genes also show an altered basal steady-state transcript level. From their known function in other stressful conditions, a possible role in heat acclimation can be deduced. Overall, the results of the present study verify a sustained activation of the HSP70 cytoprotection and point to the activation of genes representing additional cytoprotective pathways, a few of which will be discussed below. Consistent with our previous studies on HSP72 kDa (25), the upregulation of DNAJ2 is noteworthy: DNAJ homologs are considered one of the most highly conserved stress-response genes (19). Their products are required for stabilizing the interaction of HSP70 with unfolded proteins and enhancing the protective role of the HSPs (36). Hence, DNAJ upregulation in heat-stressed LTHA hearts can provide the molecular confirmation for the sustained cytoprotective role of HSP 72 kDa and HSP 90 kDa by augmenting their cellular constitutive reserves after LTHA in this tissue (24, 25). The increase in protein reserves together with the elevated steady-state transcript levels of hsp 70 and 90 and HSPB2 (small HSP associated with the protection of the mitochondrial membrane and widespread in cardiac tissue; Ref. 28) suggests an architected activation of these genes. An additional cytoprotective category of interest emerging in this investigation is associated with two members of the Bcl superfamily. One of these, bcl-xL, an antiapoptotic member of this superfamily (26), is upregulated during heat stress, whereas the other, bcl-2 (BAD), a proapoptotic member of the superfamily, shows profound downregulation, particularly after LTHA. Functionally, the protein products of these two genes are associated with the opening of mitochondrial voltage-dependent anion channels, the failure of which leads to a mitochondria-induced apoptotic cascade in the cell (26). The reciprocal profile of these two genes (together with no change in caspase 3, a downstream checkpoint of the apoptotic pathway) points to a heat-acclimation-induced cytoprotective pathway involving these genes [e.g., restriction of heat-induced apoptosis in bcl-xL overexpressed cell line (32), an enhanced role for mitochondria function in Ca regulation (26)]. We also detected the induction of additional genes, all connected with antiapoptotic or proapoptotic signaling [e.g., HSPs (40)], nucleophosmin, a UV-inducible oncogene associated with an
initial step in the regulation of cellular and genomic responses to external environmental stimuli (43) murine double minutes 2, which, when upregulated, attenuates the proapoptotic p53 pathway (35) and marked downregulation of JNK (30). Interestingly, cross-talk between mdm2 and the hypoxia-inducible factor HIF-1α has been also documented (4). Because HIF-1 has been shown to play an essential role in heat acclimation (42), further studies along this line can shed light on the role of HIF-1 in the acclimation process. The altered activity of these genes implies an intricate antiapoptotic network in the heat-acclimated phenotype. It is noteworthy that all these genes are also associated with other cytoprotective networks, but this issue is beyond the scope of the present investigation. LTHA is also characterized by the enhanced activation of antioxidants (e.g., GSTs, cytochrome b reductase), which fits well with our unpublished observations of an increased antioxidants-to-ROS ratio (Canaana H, Kohen R, and Horowitz M, unpublished observations). The activation of GST-P and cytochrome b reductase after heat stress has been reported in the past (18). The results of the present investigation show that heat acclimation leads to upregulation and faster activation.

Heat Acclimation Renders I/R Cross-Tolerance (Cluster Analysis 2)

An intriguing issue raised in this investigation is the stress-response aspect of the cross-tolerance phenomenon. We argued that cross-tolerance emerges from an enhanced capacity or responsiveness of molecular signaling shared by adaptation to the primary stress (heat tolerance) and the secondary stress (oxygen shortage) and its consequences. Evident from analysis 2 is that the expressed genes in the array used in this investigation showed significant specificity. The finding that induced or suppressed transcription of most genes responding to I/R was independent of the heat acclimation response, with a few genes also significantly responding to heat stress, suggests that the number of stress-associated genes shared by the two stressors is limited. Sonna et al. (37), in a publication on the effect of hypoxia on gene expression by human hepatocytes, reported relatively little overlap (11–22%) among genes altering their expression after heat stress (although in other systems). Thus the stress specificity found among the stress-related genes in our in vivo experimental model confirms Sonna’s observations (37). Most of the induced/suppressed genes reported here are those associated with maintaining DNA integrity, stress regulators, and stress effectors (as detailed in Table 5). Yet, in accordance with our hypothesis of “shared signaling cascades” as the underlying mechanism of cross-tolerance, we sorted eight genes (derived from cluster A8 and analysis 2b, Table 6) showing an enhanced response to heat stress and/or to I/R insult, but only after heat acclimation. The cellular functions associated with these genes represent J) antiapoptosis and the maintenance of protein integrity networks [e.g., decreased bcl-2 (BAD), JNK1, and JNK2, and flavin-containing monoxygenase] and upregulation of DNAJ2 (23, 26, 36, 39), and 2) maintenance of DNA and chromatin integrity [e.g., upregulation of DNA mismatch-repair protein, downregulation of M-phase inducer phosphatase 2, which inhibits damaged DNA signaling after genotoxic stress (3)] (Table 6). These conclusions are highly congruent with our analysis 1 (see Unmasking Heat-Acclimation-Mediated Cytoprotective Networks), which implies that these functions, at least in terms of molecular response, are enhanced in response to heat acclimation. We hypothesize that the profound change in the expression of these genes reinforces inherent coordinated cardioprotective mechanisms and contributes to the cytoprotective aspect of the cross-tolerance phenomenon.

An additional acclimation-mediated mechanism, not less important and possibly complementary, that can contribute significantly to the cross-tolerance phenomenon is the earlier threshold for gene activation on stress application. This rapid response was evident in our individual gene analysis for GST-P and HSP70 (Fig. 6), which attained their peak expression at 50% ischemia in heat-acclimated hearts compared with global ischemia in nonacclimated hearts. GST-P is one example of an ischemic-specific responder, whereas HSP70 is a universal player in the stress response. Both our previous and our present investigations have shown a faster HSP70 response during heat stress. Collectively, our data indicate that heat-acclimation-induced cross-tolerance involves the reinforcement of cytoprotective signaling. In previous reports, our laboratory showed that the redistribution of ATPase isoforms and changes in glycolytic flux lead to energy sparing in the ischemic acclimated heart (7, 16, 17). Hence, we now suggest that heat acclimation-I/R cross-tolerance is the outcome of orchestrated, enhanced, rapid cytoprotective, and sustained metabolic responses.

In summary, the results of this investigation indicate that the process of heat acclimation involves a continuum of activation and suppression of different sets of stress-related genes. The results confirm our previous hypothesis, based on physiological findings, that STHA is a critical phase in the switch of the acclimated phenotype and that acclimatatory homeostasis involves an altered expression of genes encoding networks of cytoprotective proteins. This investigation reconfirms the universality of HSP70 as a stress-responding gene but shows that the truly nonspecific component of the stress response is quite small. However, reinforcement of gene networks (which are possibly multipotential in their utilization) or different rates of response of various genes enables the adaptation to a stressor without prior exposure.

GRANTS

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