Dynamic Transcriptional and Metabolic Responses in Yeast Adapting to Temperature Stress

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Abstract

Understanding the response processes in cellular systems to external perturbations is a central goal of large-scale molecular profiling experiments. We investigated the molecular response of yeast to increased and lowered temperatures relative to optimal reference conditions across two levels of molecular organization: the transcriptome using a whole yeast genome microarray and the metabolome applying the gas chromatography/mass spectrometry (GC/MS) technology with *in vivo* stable-isotope labeling for accurate relative quantification of a total of 50 different metabolites. The molecular adaptation of yeast to increased or lowered temperatures relative control conditions at both the metabolic and transcriptional level is dominated by temperature-inverted differential regulation patterns of transcriptional and metabolite responses and the temporal response observed to be biphasic. The set of previously described general environmental stress response (ESR) genes showed particularly strong temperature-inverted response patterns. Among the metabolites measured, trehalose was detected to respond strongest to the temperature stress and with temperature-inverted up- and downregulation relative to control, midtemperature conditions. Although associated with the same principal environmental parameter, the two different temperature regimes caused very distinct molecular response patterns at both the metabolite and the transcript level. While pairwise correlations between different transcripts and between different metabolites were found generally preserved under the various conditions, substantial differences were also observed indicative of changed underlying network architectures or modified regulatory relationships. Gene and associated gene functions were identified that are differentially regulated specifically under the gradual stress induction applied here compared to abrupt stress exposure investigated in previous studies, including genes of as of yet unidentified function and genes involved in protein synthesis and energy metabolism.

Introduction

CHANGES OF AMBIENT TEMPERATURES are among the most ubiquitously occurring environmental challenges virtually all living organisms have to cope with. When exposed to increased or lowered temperatures, also microbial organisms such as yeast have evolved mechanisms enabling them to adapt immediately after temperature alterations. With the advent of whole genome microarray technology, the study of system-wide responses to environmental perturbations at the transcriptional level has become the main focus of stress-response molecular biology including the response of yeast to temperature shock (Becerra et al., 2003; Causton et al., 2001; Gasch et al., 2000; Sahara et al., 2002; Sakaki et al., 2003; Schade et al., 2004). Although many genes were observed to

alter their expression levels specifically to temperature stress conditions, a subset of genes and their associated transcripts were also found to be induced or repressed upon exposure to a wide range of other environmental perturbations (Causton et al., 2001; Gasch et al., 2000), suggesting the existence of specific and general stress-response mechanisms.

Transcriptional changes constitute only one domain of molecular organization and the complexity of dynamic cellular interactions between all levels including proteins, metabolites, and other molecular species has not yet been adequately addressed (Sauer et al., 2007). It is well possible—in fact, very likely—that environmental changes are first perceived at levels other than transcripts; for example, proteins with modified catalytic properties or changes at the metabolite level. Recently, the coordinated response between

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transcripts and metabolites in response to nutrient starvation conditions has been described over periods of up to 8 h in *Saccharomyces cerevisiae* (Bradley et al., 2009). Similar temporal response patterns of both transcripts and metabolites were shown to be connected via their functional and metabolic pathway relationships.

In this study, we report results from an integrated study of microarray-based whole-genome gene expression profiling with metabolite level data using the gas chromatography/mass spectrometry (GC/MS) technology for yeast cells exposed to elevated or lowered temperature compared to optimal growth conditions. Our goal has been to integrate as well as to compare both the transcriptional and metabolic responses over a time period of up to 24 h.

Previous studies on the response of yeast to high (Gasch et al., 2000) or low (Sahara et al., 2002) temperature cues have focused on sudden temperature shocks rather than a gradual adaptation of yeast cells to suboptimal growth conditions. Temperature shock may induce composite overlaying molecular responses. First, the general stress response, which, via stress-induced signal perception and damage repairs, allows the system to cope with imminent cell death; and second, the specific adaptive responses to—in our case—high and low temperature stress. Nisamedtinov and coworkers (2008) demonstrated that the rate of change of environmental conditions is an important factor in the stress response, and that under gradually changing conditions, yeast cells adapt with significantly reduced stress protein expression indicating less immediate stress compared to sudden changes. To dissect the specific adaptive aspects from the general stress responses, we decided to apply a slow temperature equilibration regime. As we will demonstrate, we thereby avoided cell lethality caused by a rapid change of environment and respective general responses. Although representing similar environmental cues, the two different temperature regimes caused distinct molecular response patterns at both the metabolite and the transcript level.

Materials and Methods

Yeast strain and growth conditions

The *Saccharomyces cerevisiae* strain used in this study was S288C, genotype MAT α SUC2 mal mel gal2 CUP1 flo1 flo8-1 (Brachmann et al., 1998; Mortimer and Johnston, 1986). Cultures were grown in synthetically defined (SD) medium as 250-mL batch cultures using chicane Erlenmeyer flasks agitated at 35–40 rotations per minute. SD-medium was supplemented with 2% (w) glucose and 0.67% (w) Difco yeast nitrogen base, YNB (Becton Dickinson GmbH, Heidelberg, Germany).

Temperature stress conditions

All cultures were pregrown to early log-phase under optimal conditions at 28°C, OD₆₀₀ = 1.0 ± 0.1, prior to temperature stress induction. Subsequently, the cultures were shifted to ambient temperatures of 37°C for heat treatment, and 10°C for the cold stress treatment. In parallel, cultures were kept under optimal conditions at 28°C as control. Yeast growth was monitored by determination of optical density OD₆₀₀. Samples were collected at 0 min, immediately prior to the temperature shift, and subsequently after 15 min, 30 min, 1 h,

2 h, 4 h, 8 h, and 24 h. The temperature equilibration inside the flasks was monitored by a digital thermometer (Testo AG, Lenzkirch, Germany).

Metabolite sample preparation and metabolite profiling

Internal standardization using mass isotopomer ratios. For using ¹³C-labeled metabolites as compound-specific internal standards of each metabolite (Mashego et al., 2004; Wu et al., 2005), samples were spiked by sequential quenching, as is described below, using 2.5 mL of a fully ¹³C-labeled yeast cell suspension. For *in vivo* labeling, yeast cells were grown under optimum conditions in SD medium batch culture using chicane Erlenmeyer flasks. The SD-medium was supplemented with 2% (w) pure U-¹³C-glucose (99 atom%, Sigma, St. Louis, MO, USA) (Birkemeyer et al., 2005).

Quenching. Metabolic activity was stopped and cells separated from the medium using the described quenching cold-methanol method (de Koning and van Dam, 1992; Gonzalez et al., 1997). The yeast cell suspension was sprayed rapidly into 70% methanol kept at –40°C in a dry ice/methanol bath. A volume of 2.5 mL of each sample was quenched into 25 mL of precooled methanol. The quenched samples were washed two times with 70% methanol. The samples were kept at –20°C during all washing and centrifugation steps. For mass isotopomer standardization, first 2.5 mL of a fully ¹³C-labeled reference culture grown to OD₆₀₀ = 1.0 ± 0.1 were harvested, centrifuged, washed, and resuspended in fresh, cold 70% methanol, then an amount of nonlabeled cells estimated by volume and optical density was harvested into the same tube. Exact volumes and optical densities of labeled and nonlabeled cultures were recorded for final numerical correction.

Methanol/chloroform extraction and metabolite profiling. The extraction of metabolites was performed by a conventional method of methanol/chloroform extraction as described previously (Erban et al., 2007). After washing and centrifugation of the quenched samples, 500 μ L extraction solution containing 488 μ L methanol and 12 μ L 0.2 mg/mL ribitol (CAS: 488-81-3), 1 mg/mL 2,3,3,3-d₄-alanine (CAS: 53795-92-9), and 0.5 mg/mL d-isoascorbic acid (CAS: 89-65-6) dissolved in methanol and water was added and the combined ambient and ¹³C-labeled cell pellet was resuspended. After adding 200 μ L chloroform, samples were incubated for 15 min at 70°C. A total of 500 μ L of each extract was dried without separation of a chloroform phase by vacuum centrifugation and subsequently processed by routine GC/MS profiling analysis.

Metabolite data processing. After baseline correction of GC/MS chromatogram files performed by the Leco-ChromaTof 1.61 software (LECO, St. Joseph, MI, USA), data were exported in NetCDF file format. This file format was used for TagFinder processing (Luedemann et al., 2008). In short, peak apex height data of all mass traces, *m/z* 70–600 amu, were retrieved, aligned, and compiled into a tabular matrix. Metabolites were identified by mass spectral matching of ambient and ¹³C-labeled reference spectra from the Golm Metabolome Database (Kopka et al., 2005). Representative mass fragments and monoisotopic masses were as

reported earlier (Huege et al., 2007). Response data of metabolite specific mass isotopomer pairs were manually retrieved from the above tabular data matrix and ratios calculated. To obtain metabolite level data that represent metabolite pool size changes relative to the ^{13}C -labelled standard, mass isotopomer response ratios were corrected numerically for minor OD_{600} fluctuations to obtain ratios of equal amounts of ^{13}C -labeled and ambient cells in each combined sample. If not stated otherwise, metabolite level data sets were normalized to the respective 28°C control measurements at each time point and further normalized to t_0 . All ratios entered the analyses as log-2 transformed values.

RNA sample preparation and transcript profiling

For purification of total RNA, the MasterPure™ Yeast RNA Purification Kit (Epicentre Biotechnologies, Madison, WI, USA) was used. Subsequent RNA clean-up was performed using the RNeasy MinElute Cleanup Kit (Qiagen, Hilden, Germany). Affymetrix gene chip hybridization and initial transcript analysis was performed by the German Resource Center for Genome Research (RZPD, Berlin, Germany) using the Affymetrix Yeast Genome 2.0 chip information covering 5,716 *Saccharomyces cerevisiae* transcripts. The Affymetrix probeset was provided in CEL file format and was subjected to quantile normalization using the “quantile probeset normalization” function of the affyPLM Bioconductor package (Bollstad et al., 2005). If not stated otherwise, transcript level data sets were normalized to the respective 28°C control measurements at each time point and further normalized to t_0 . All ratios were log-2 transformed before analysis.

Gene functional annotations, enrichment analysis

Functional annotations associated with the transcript microarray probes and their respective genes were obtained from the *Saccharomyces* Genome Database, SGD (<http://www.yeastgenome.org/>), and the gene ontology annotation information as provided by Affymetrix Yeast Genome 2.0 microarray. Enrichment analysis was performed using the EASE tool (Hosack et al., 2003) as available under the Multi-experiment Viewer software suite (<http://www.tm4.org/>).

Correlation analysis, minimum range criterion

Pairwise correlation computations were based on the Pearson correlation coefficients. Where stated, a transcript expression or metabolite level range threshold criterion was introduced. Only transcripts or metabolites with a range between the maximal and minimal value across all time points greater than a specified threshold (twofold difference, in most cases) were considered in the computations. This range criterion was introduced to enrich for correlations between molecules that exhibit significant temporal changes.

Results

Temperature adaptation of yeast was investigated across two molecular systems levels: the transcriptional response using standard Affymetrix hybridization gene-chip technology, and the metabolite-level response by application of GC/MS-based profiling technology (Erban et al., 2007; Fiehn et al., 2000) to a metabolite fraction of intercellular yeast primary metabolites. The GC/MS-based profiling technology

was optimized to obtain improved precision of relative quantifications achieved by applying mass isotopomer ratio profiling and *in vivo* stable isotope labeled standardization technology as described in (Birkemeyer et al., 2005).

First, we will describe the phenomenological response of yeast to changing temperatures as signified by altered growth behavior. Then, the molecular response is investigated at both the transcriptional and metabolomics systems level.

Yeast growth response during slow temperature equilibration

Shifts of ambient temperature from 28°C to $\sim 37^\circ\text{C}$ and $\sim 10^\circ\text{C}$ with an equilibration half time of 27.0 min (Fig. 1A, heat) and 25.5 min (Fig. 1B, cold), respectively, were compared for yeast cultures growing in synthetically defined medium with glucose as the major carbon source. Under these conditions, the yeast culture growth rate was unchanged up until 6 h after exposure to heat, whereas the growth rate was reduced

FIG. 1. Growth behavior of the yeast cultures at 28°C (open black circles) compared to cultures that were pregrown to $\text{OD}_{600} \sim 1.0$ and then shifted to high $\sim 37^\circ\text{C}$ (A, filled red circles) or low $\sim 10^\circ\text{C}$ (B, filled blue circles) temperatures. Experimental variance, $n > 4$, was minimal. Half times of temperature equilibration were observed as 27.0 and 25.5 min, respectively. Although yeast growth continued unchanged up until 6 h after stress exposure at 37°C , cold adaptation resulted in early reduction of cell growth rate after 1 h of exposure to $\sim 10^\circ\text{C}$.

TABLE 1. PERCENTAGE OF VIABLE YEAST CELLS AFTER EXPOSURE TO HIGH- AND LOW-TEMPERATURES STRESS

Time of exposure (h)	Control conditions (28°C kept at 28°C)	Cold stress (28°C shifted to 10°C)	Heat stress (28°C shifted to 37°C)
2	97.2%	96.3%	97.6%
4	97.6%	97.0%	97.1%
8	98.9%	96.8%	95.8%
16	81.0%	95.2%	82.0%
21	80.3%	92.0%	68.9%
24	75.0%	77.6%	32.1%

Bold font indicates viability >90%.

dramatically as early as 30 min after exposure to cold conditions. The yeast cultures reached different final optical densities depending upon the set ambient temperature ($\pm$ standard deviation, $n \geq 4$, $t = 24$ h), with $OD_{600} = 5.3 (\pm 0.3)$ under 28°C control conditions, $OD_{600} = 2.1 (\pm 0.3)$, when shifted to 37°C, and $OD_{600} = 1.4 (\pm 0.1)$, when shifted to 10°C, demonstrating that both the chosen elevated as well as lowered temperature represent suboptimal growth conditions for yeast. However, vital staining and cell sorting analysis determined greater than 95% viable cells under all three regimes up to 8 h into the time courses (Table 1). We concluded that the observed reductions of growth rate were caused by reduced cell propagation and not by enhanced cell death. At 24 h after exposure to the temperature stresses, the percentage of viable cells was reduced to $\sim 75\%$ under control and cold conditions, however, it was significantly reduced to $\sim 32\%$ under heat stress.

Comparative principal component analysis of transcriptional and metabolomic systems responses

To reveal and illustrate common as well as differentiating trends between (1) heat and cold responses, (2) primary metabolism and gene expression, and (3) slow temperature adaptation compared to published data on temperature shock, we performed a series of comparative principal component analyses (PCA) (Fig. 2) after standard data processing including $\log_2$ -transformation, and normalization to t_0 of both data set types.

Compared to the high and low temperature stress conditions, metabolic and transcriptional changes over time were minimal under control conditions indicating near steady-state conditions of the batch cultivations under study. Only the 24-h time point differed from the preceding time points both at metabolic and transcriptional levels (Fig. 2), as was also indicated by the vitality assay (Table 1). This observation was interpreted as the transition of batch cultures into the stationary phase as is also evident from the growth curve of yeast cultures under control conditions (Fig. 1). Similarly, the 24-h time point of the heat stress experiment series was found to represent an outlier when compared to the preceding time course data points (Fig. 2A and C). Taken together, both the reduced cell viability and the altered systems level profiles signify the 24-h time points as fundamentally different from the earlier time points. Therefore, the 24-h time point data should not be used, when specific adaptive responses to applied temperature shifts are at the center of investigation. The molecular temperature stress responses followed a nonlinear

trajectory both at the metabolic and transcriptional levels (Fig. 2), when projecting the data associated with the samples at various time points onto the first principal components. These principal components capture the largest variance of the data and suggest the action of a series of responses of gene expression and primary metabolite levels, which may indicate a sequence of signaling events involving both system levels. The major trends of heat and cold responses appeared to be temperature inverted. We use the phrase “temperature inverted” to indicate that relative to control conditions, opposite expression patterns were observed. On a single gene basis, this would mean switching from up to downregulation (or vice versa) compared to reference conditions. At the multivariate level as investigated by PCA, temperature-inverted behavior would manifest itself by 180° rotations of sample points in principal component space. As evident from the 180° rotated PCA-patterns, opposite responses of identical system elements to high or low temperature relative to control conditions appeared to be dominating at both the metabolic and transcriptional level. However, at the global level, comparison of slow temperature adaptation at the transcriptional level (Fig. 2C) both to heat and cold shock (Gasch et al., 2000; Sahara et al., 2002) revealed general agreement between the fast and slow experimental regimes of this study, even though different yeast strains and full YPD medium were used in the previous studies. The time course trends following heat and cold exposure were almost identical except for the different timing of events. Except under heat stress, where the amplitude of the early changes appeared to differ strongly. We concluded that our experimental setup yielded results that can be generalized and may apply to diverse yeast strains, medium conditions, and time regimes. Note that for the metabolomic data, no equivalent datasets reported elsewhere were available for comparison.

Transcript-level changes in response to changing temperatures

Transcript analyses using standard Affymetrix gene chip technology allowed near comprehensive assessment of the full yeast gene inventory covering 5,716 common and valid yeast gene models (Additional File 1). Approximately 5,100 of these gene models were also assessed in previous studies using different microarrays (Gasch et al., 2000; Sahara et al., 2002). Most extensive changes in gene expression were observed between 15 and 120 min after initiation of both temperature treatments. These early changes were equally distributed with almost symmetrical numbers of up- and down-regulated genes as judged by K-means clustering (Fig. 3A and B). Most of the early events were transient and reverted to basal expression levels (relative to control conditions) after 8-h exposure time. Applying a twofold (fourfold) differential expression range threshold (see Materials and Methods), a total of 2,535 (565) genes were observed to be differentially regulated during the 8-h hour time interval under heat stress. A similar number of 2,592 (709) genes were cold responsive. Although under heat conditions most changes occurred early and appeared to be transient, cold adaptation showed a second phase of transcriptional responses consistent with previous reports (Schade et al., 2004).

The 427 genes induced early under heat stress (Fig. 3A, cluster 2) represent functions typically associated with this

FIG. 2. Comparative principal component analysis (PCA) of GC-MS-based metabolite profiling data of a primary metabolite fraction (**A–B**) and respective transcription profiles (**C**). The metabolite and transcriptional profiles at 28°C control conditions were in general unchanged compared to the treatment conditions, with the exception of the 24-h time point. By contrast, PCA applied to the treatment time series of metabolite (**A–B**) as well as transcript level data (**C**) results indicated nonlinear and in part opposite temperature-induced trends. Comparison to preexisting transcriptional profiles of diverse temperature shock experiments (Gasch et al., 2000; Sahara et al., 2002), demonstrated largely identical trends of temperature-induced transcriptional responses which, however, exhibited different amplitudes. Size of the circles indicates time after exposure to the stress cue with the 24-h time point being represented by the largest size. Control at 28°C (filled gray), cold shift to 10°C (dark blue), and heat shift to 37°C (red). Previous reports on heat stress treatment experiments, hs-1 (orange), hs-2 (yellow) were taken from Gasch et al. (2000), the cold stress response was reported in (Sahara et al., 2002). The genotypes of prior studies were YPH500, MAT α , ura3–52, lys2–801, ade2– Δ 101, trp1– Δ 63, his3– Δ 200, leu2– Δ 1 (Gasch et al., 2000), DBY7286, MAT α , ura3–52 GAL2 (Sahara et al., 2002). Cultivation medium of the previous studies was full YPD medium.

stress (Gasch et al., 2000), but also with the response to the related desiccation stress. In addition, the functional categories: protein folding, protein-bound amino acid phosphorylation, cell wall organization, and biogenesis were dominating in this gene cluster as determined by an enrichment analysis of Gene Ontology terms (GO see Methods) (Fig. 3).

In contrast to heat response, the 652 transiently upregulated genes during cold adaptation (Fig. 3B, cluster 4) comprise gene functions enriched in rRNA processing, ribosomal large subunit biogenesis, ribosomal large subunit assembly and maintenance, processing of 20S pre-rRNA and 27S pre-rRNA, ribosome assembly, rRNA modification, and ribosome export from the nucleus. Following this early response, the second wave of gene induction comprises 17% of all genes (Fig. 3B, cluster 1). This cluster represents gene functions associated with ubiquitin-dependent protein catabolism, protein-vacuolar targeting, protein catabolism, and protein ubiquitination, Rho protein signal transduction as well

as fatty acid, lipid and phospholipid metabolism, and transport. Gene expression associated with amino acid and protein biosynthesis, ribosome assembly and development as well as mitosis and cell cycle were observed to be concomitantly reduced. These functions were represented by a gene cluster of equal size (Fig. 3B, cluster 3). The above observations were in agreement with the strong reduction of cell propagation during cold adaptation.

Differences between the transcriptional responses under abrupt and slow temperature stress exposure

With regard to the overall transcriptional response to increased or lowered temperature, we found general agreement between exposure to sudden temperature shock (Gasch et al., 2000; Sahara et al., 2002) and the slow temperature adaptation reported here (Fig. 2). Although the timing of the differential response is stretched out in the slow regime, similar functions

FIG. 3. K-means clustering of transcription (left) and primary metabolite profiles (right) using five and four centroids, respectively, as empirically determined optimal cluster numbers. Heat (top) and cold (bottom) responses are compared, ratios are $\log_2$ transformed ratio values. The number and relative frequency (%) of systems elements are listed. Note that the transcriptional responses appear to be symmetrical with regard to up- and downregulation (A,B), whereas the metabolic responses seemed rather asymmetric (C,D). The latter observation can be attributed to the bias of the GC-MS profiling technology, which allowed monitoring of only 50 primary metabolites (42 metabolites were detected in the heat-stress samples (28 annotated), and 44 were found in cold samples (31 annotated)). Note that trehalose constitutes cluster 2 (C) and cluster 1 (D).

are induced or repressed under both experimental settings (see above). However, we also detected significant differences. We determined those functional categories that were specific to either one of the four experimental protocols (heat or cold/ fast or gradual) with regard to up- or downregulated gene functions. When searching for genes that were upregulated only under gradual temperature increase, a number of transcripts with as of yet undetermined functions were identified, making them interesting targets for further research, whereas transcripts upregulated only under fast heat exposure (Gasch et al., 2000) are primarily associated with energy metabolism, possibly explained by different (lower) oxygen concentrations in the preheated medium used in the temperature shock experiment. Under cold stress, nonoverlapping sets of transcripts involved in ribosomal and other protein synthesis processes were identified under sudden (Sahara et al., 2002) and slow temperatures changes. A complete list of GO terms from an enrichment analysis and associated transcript probe identifiers is provided as Additional File 4.

Analysis of temperature-reciprocal and unidirectional transcriptional responses to high and low temperatures

In a previous study on broad stress response patterns in yeast and based upon rapid stress exposure experiments, Gasch and coauthors (2000) proposed the concept of a stress

factor independent general and, in essence, common transcriptional stress response. This common stress response should always be unidirectional, in other words, either increase or decrease the transcription of involved, so-called environmental stress response (ESR) genes. Such a common stress response mechanism may act to initiate general stress signaling cascades, general cell damage repair, and to prevent imminent cell death caused by abrupt changes of the environment.

When comparing the time profiles of these ESR genes between heat and cold conditions, opposite trends were observed. Although ESR genes were upregulated early after exposure to heat, they were downregulated under cold conditions (Fig. 4). Overall, the median Pearson correlation between time profiles for all transcripts under heat and cold stress was determined as -0.29 (3,631 transcripts), whereas it was -0.79 for the set of 220 ESR genes meeting the range criterion indicating an even more pronounced temperature inverted response behavior for the ESR gene set than for transcripts in general (note: the 24-h time point was excluded—see above—and the range of more than twofold difference conditions in at least one of the two conditions was imposed). As already concluded by earlier studies, the early response to cold stress conditions are mediated by different processes than typical ESR (Schade et al., 2004).

Furthermore, we estimated the presence and influence of a general stress-response mechanism on our experimental de-

FIG. 4. Temporal normalized expression profile of the 244 environmental stress response (ESR) genes reported by (Gasch et al., 2000) to heat and cold ambient temperatures. Data points correspond to observed mean relative expression values and respective associated standard deviations.

sign aimed at elucidating temperature-specific adaptive responses from the complete set of yeast genes. Because heat and cold temperature equilibration was observed to fall on similar characteristic time scales (Fig. 1), we compared at each time point the number of genes exhibiting a common unidirectional response to opposite temperature regimes. Furthermore, we determined the number of genes showing temperature-reciprocal transcriptional response. Applying thresholds of twofold and less stringent 1.41-fold changes, we observed virtually no unidirectional responses during early responses. Instead, the observed changes were highly temperature-specific (Table 2). Only at 24 h after exposure to extreme temperatures, we found unidirectional responses prevailing.

We conclude that by application of slow temperature equilibration, the early temperature stress response is not accompanied by a safe guarding general stress response. By contrast, ESR responses appear to be inverted after exposure to cold. Thus, we believe our experimental design reveals temperature-specific adaptive responses. Reciprocal responses, which are proportional to the direction of the temperature shift relative to control conditions, are prevailing in our experiment. Exemplary genes, which exhibited the most extreme and early reciprocal responses were, YBR267W (REI1, Cytoplasmic pre-60S factor) and YOR028C (CIN5, a basic leucine zipper transcription factor) (Fig. 5).

Metabolite-level changes in response to changing temperatures

In contrast to the near comprehensive transcriptional analysis, mass isotopomer enhanced GC/MS-based profiling of yeast intercellular metabolites yielded a total of only 50 metabolites, of which 33 could be annotated as compounds that are associated primarily with central metabolism (Additional File 2). Thirty-six metabolites were detected under all

experimental conditions (26 of those annotated), whereas a total of 42 metabolites were detected in the heat-stress samples (28 annotated), and 44 were found in cold-stressed samples (31 annotated).

K-means clustering revealed the prevailing dynamic response patterns among the relative changes of metabolite pool sizes. The general trends were observed as strong increases under heat stress, and both increased as well as decreased metabolite levels under cold stress (Fig. 3C and D). The largest set of responsive metabolites was associated with relatively late response patterns both under heat and cold treatment. We caution that these patterns may not be generalizable, because the metabolic window of our GC/MS-based profiling approach is small and focused on primary metabolites. The distribution and pattern of metabolic responses may change upon reinvestigation using future metabolomic technologies with increased coverage and quantitative dynamic range.

However, integrative insight into selected metabolite responses or patterns of few primary metabolites may already shed light on adaptive metabolic systems responses. For example, the disaccharide trehalose, which may also be a crucial factor involved in other stress responses (Estruch, 2000), exhibited a strong differential response pattern to both heat and cold stress, which has not been reported previously in paired transcriptional and metabolomic analyses. Following heat stress, trehalose levels first increased strongly and then reverted to control levels at 60 min. The cold stress response of trehalose was exactly opposite. After an initial decrease, trehalose started to continuously increase after 2 h of exposure to cold. The late trehalose response to near-freezing stress has been reported previously (Kandror et al., 2004).

During heat stress, 31 of the measured metabolites accumulated with time (Fig. 3C). In addition to the strong changes of trehalose levels, the monosaccharides glucose and fructose as well as glutamate and aspartate increased immediately after exposure to heat. Other amino acids started to

FIG. 5. Exemplary genes exhibiting extreme and early reciprocal temperature responses. Genes were selected according to maximized differential temperature response 15–30-min after exposure to heat or cold and best reciprocal correlation in the time range 15 min to 8 h. Heat, 28°C shifted to 37°C (red), cold, 28°C shifted to 10°C (blue), 28°C control condition (white).

accumulate at later time points. Only proline, glycine, and phenylalanine exhibited an initial reduction of pool sizes and then returned to control levels. Some intermediates of the TCA cycle, such as succinate, fumarate, and malate, as well as glycerate, showed a remarkably similar transient reduction pattern at 15 min and then reverted back to control conditions. In total, 38 of the covered metabolites, including some not yet identified mass spectral tags, changed more than twofold (max–min range) in response to heat. Ten metabolites changed more than fourfold (range) including valine, fumaric acid, threonine, asparagine, arginine, and trehalose of the annotated metabolites.

During cold stress, only 19 metabolites displayed a range greater than twofold. Five metabolites including glucose and trehalose exhibited a range greater than fourfold. This set included most of the measured amino acids. A small number of metabolites including fumarate and malate decreased significantly during exposure to cold. The monosaccharides fructose and glucose as well as the compounds citrate and glutamate had a similar response behavior in heat and cold. These compounds increased substantially during the monitored time interval.

For metabolites measured under both heat and cold stress condition, the median pairwise Pearson correlation coefficient obtained from correlating both temporal profile data was 0.43 (excluding the 24-h time point; range more than twofold in at least one of the two conditions, 33 metabolites). Thus, compared to transcripts (see above, median correlation coefficient –0.29), the measured metabolites exhibited a greater preservation of response behavior when exposed to heat and cold.

In conclusion, we observed highly differential metabolic response patterns of heat and cold adaptation. These patterns indicate a temperature-specific, but in both cases extensive dynamic adjustment and adaptation of the central metabolism such that molecular interactions or regulatory relationships appear modified in response to the temperature

stress. The metabolic dynamic adaptation is biphasic in both cases. Early and late changes of metabolite pools may be either transient or continued. We detected indications of temperature-inverted metabolic responses to high- and low-temperature stress. However, we also discovered common metabolic responses of metabolites, such as fructose, glucose, glutamate, and citrate.

Pairwise transcript–transcript and metabolite–metabolite correlation

Pairwise linear correlation analysis as a measure of relatedness and pathway proximity between metabolites or transcripts is a frequently used approach to infer functional and metabolic pathway relationships between genes and metabolites from expression level (in case of gene transcripts) or concentration level (metabolites) data. If, indeed, pairwise correlations between metabolites and associated enzymes reflect functional relatedness mediated by metabolic, signaling, or transcriptional networks and, furthermore, these network relationships hold independently of external conditions as they follow biochemical laws, it appears reasonable to assume that pairwise correlations between transcripts or metabolites should be preserved even if external stimuli change if the underlying functional network linking the molecules remains the same. Alternatively, they change, if the metabolic pathway map (for metabolites) or transcriptional network (for transcripts) changes or the regulatory relationships within the networks are modified.

Indeed, for gene expression levels, we observed evidence for consistent pairwise correlation levels between gene transcripts under heat and cold stress conditions (Additional File 3, Supplementary Fig. 1). Expression levels between transcripts with a minimum range of at least twofold between the observed maximum and minimum expression value across the measured time points in the time series data were corre-

TABLE 2. NUMBER OF GENES EXHIBITING TEMPERATURE-RECIPROCAL OR UNIDIRECTIONAL TRANSCRIPTIONAL RESPONSES

	<i>Time after exposure to temperature stress</i>						
	<i>15 min</i>	<i>30 min</i>	<i>1 h</i>	<i>2 h</i>	<i>4 h</i>	<i>8 h</i>	<i>24 h</i>
2.0-fold changes							
unidirectional	0	0	15	27	37	110	669
cold_up and heat_up	0	0	14	26	26	87	390
cold_down and heat_down	0	0	1	1	11	23	279
temperature-reciprocal	188	350	248	42	23	48	71
cold_up and heat_down	129	211	142	14	8	23	33
cold_down and heat_up	59	139	106	28	15	25	38
>1.41-fold changes							
unidirectional	16	39	110	242	278	606	1672
cold_up and heat_up	14	28	97	196	190	400	898
cold_down and heat_down	2	11	13	46	88	206	774
temperature-reciprocal	612	945	783	325	210	345	370
cold_up and heat_down	339	471	368	171	106	169	189
cold_down and heat_up	273	474	415	154	104	176	181

Intersection analysis comparing heat and cold adaptations was performed using a twofold (1.41-fold) transcriptional induction threshold compared to control conditions and normalizing each time course to t_0 .

lated between the heat and cold treatment data series with the mean Pearson correlation coefficient obtained as 0.51, $p \ll 0.05$. The twofold range criterion was introduced to enrich for transcripts with significant differential responses during the course of the experiment and, thus, to avoid including correlations between genes that change insignificantly. Metabolites exhibited a weak, but significant conservation of pairwise correlation between heat and cold treatment conditions. When considering only metabolites that changed significantly during the course of the experiment (twofold range), thus again reducing the risk of including noise-level correlations, a mean correlation conservation level of $r = 0.26$ was obtained ($p = 0.01$, $N = 91$) (Additional File 3, Supplementary Fig. 2). Interestingly, pairwise correlations between metabolites that change significantly predominantly show positive correlations, suggesting an overall concordance of response between these metabolites.

Thus, conservation of network relationships among transcripts and metabolites is evident from the data. However, substantial dynamic adjustment and adaptation of metabolic and transcriptional pathways between the different temperature conditions can also be surmised as well as the conservation of pairwise correlation was not very stringent.

Discussion

Unicellular yeast has been the model organism of choice for the study of molecular response mechanisms of eukaryotic cellular systems to environmental perturbations and stress conditions. Stimulated by the early available whole genome gene chip microarrays, the study of transcriptional changes has been at the center of numerous research efforts, including the study of responses to altered ambient temperatures (Becerra et al., 2003; Causton et al., 2001; Gasch et al., 2000; Sahara et al., 2002; Schade et al., 2004). With technological advances, other layers of molecular organization including the proteome and metabolome are increasingly moving into the research focus (Bradley et al., 2009; Kresnowati et al., 2006). In the present study, we generated and analyzed paired

molecular profiling data covering transcriptomics and metabolomics data for yeast culture samples exposed to suboptimal ambient temperatures with the goal of acquiring an integrated as well as comparative view of molecular response mechanisms across different organizational levels.

Our experimental design differed from previous studies by applying a gradual temperature stress exposure rather than abrupt temperature shocks (Gasch et al., 2000; Sahara et al., 2002). We believe that this more natural protocol of introducing changing temperatures may have the advantage of eliciting the naturally evolved response thereby avoiding the onset of “emergency”-stress programs associated with sudden stress exposures. Indeed, high cell viability was preserved for a long time period (Table 1), while still observing early as well as late differential responses at both the transcript and metabolite levels (Fig. 3). At the same time, the slow temperature responses largely agreed with data obtained from abrupt temperature stress induction as judged by principal component comparison to transcript data of previous temperature shock experiments (Fig. 2). Thus, we conclude that representative temperature stress experiments were performed, which are well comparable to previous gene expression compendia. However, we also detected significant differences associated with particular cellular functions (Additional File 4) including gene transcripts of as of yet unidentified functions whose functional characterization appears indicated.

The heat and cold conditions applied here constitute suboptimal growth conditions and represent similar but inverse environmental cues relative to ambient control conditions. As a consequence, their associated molecular response patterns differed significantly (Fig. 2). In fact, for the majority of early temperature responsive genes, we observed temperature-inverted, “thermometer-like” behavior (Table 2). The differential, temperature-inverted response was specifically apparent among genes that were shown to respond to a broad spectrum of environmental conditions, the so-called environmental stress-response genes or ESR genes (Gasch et al., 2000). ESR genes displayed rapid downregulation rather than

the typical stress induction when exposed to cold stress. We observed, however, delayed upregulation of ESR genes late after cold stress initiation (Fig. 4). The temperature-inverted response patterns may reflect differential response mechanisms, but may also be caused by temperature acting purely as a thermodynamic parameter.

Pairwise correlations between both transcript pairs and metabolite pairs were found generally maintained across the cold and the heat conditions, but only as a trend and with many pair correlations differing significantly (Additional File 3, Supplementary Figs. 1 and 2). Assuming that the underlying metabolic, signaling and transcriptional networks are identical between the two states, the lack of a more stringent preservation of correlation is surprising. Obviously, one source for this observation may be the technical or biological noise. More fundamentally, however, this finding may also reflect the large extent of network rearrangement or reprogramming caused by changes in regulatory relationships relative to the comparatively static framework of metabolic reactions. For transcriptional regulatory networks, substantial plasticity has been shown previously (Luscombe et al., 2004). High plasticity has also been demonstrated for relationships between yeast metabolites and transcripts under different nutritional stress conditions (Bradley et al., 2009).

Current metabolomic and transcriptomic technologies provide information at different degrees of completeness. The available transcript microarray platforms allow the profiling of essentially all transcripts encoded in the yeast genome, and their analysis can thus be considered close to comprehensive. By contrast, only few metabolites can be monitored given any of the currently available routine metabolomic technology platforms. The metabolites covered by this study are predominantly from the central metabolism as the employed GC/MS technology was designed for primary, small, and stable metabolites (Fiehn et al., 2000; Huege et al., 2007). The complexity of covered metabolites is further reduced to those, which can be profiled accurately by mass isotopomer ratios. A recent effort to consolidate the various metabolic network reconstructions resulted in an estimated number of 911 unique chemical transformations (excluding transport reactions) operating on 1,168 predicted metabolites (Herrgard et al., 2008). Thus, because of technological constraints, the metabolite data reported in this study correspond to only as small portion of the complete yeast metabolome, highlighting the needed for a substantial expansion of reach of metabolomics technologies.

The generated dataset forms an ideal basis for applying more advanced analysis methods such as time-lagged correlation or Granger causality (Granger, 1980) to infer potential causal relationships between metabolites and transcripts as well as to discern the temporal dynamics of temperature stress response as described in the accompanying article (Walther, 2010).

Data availability

The microarray transcript profiling data have been uploaded to the Gene Expression Omnibus database (<http://www.ncbi.nlm.nih.gov/geo/>) and are available under GEO-ID GSE15352. All metabolite data are provided as Supplementary Material and are available at <http://bioinformatics.mpimp-golm.mpg.de/resources/files/supplementary-material/omics> or upon request.

Acknowledgments

The authors acknowledge the long standing support and encouragement by Prof. L. Willmitzer. We wish to thank Alexander Erban and Alexander Lüdemann for helpful discussions. This work was generously supported by the Max Planck Society.

Author Disclosure Statement

The authors declare that no conflicting financial interests exist.

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