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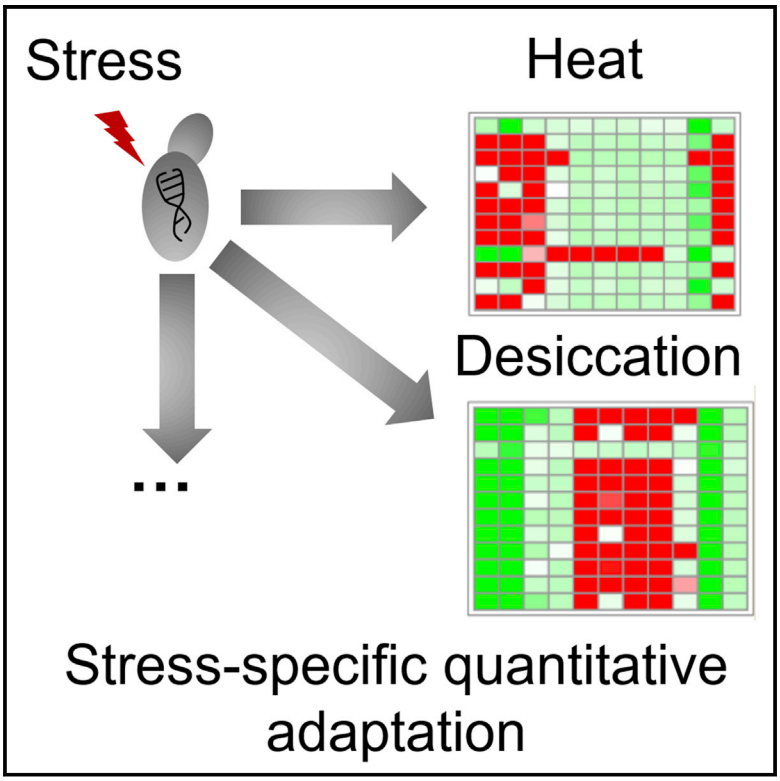
Quantitative Operating Principles of Yeast Metabolism during Adaptation to Heat Stress

Graphical Abstract



Authors

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In Brief

Evolution selects coordinated adaptive changes in gene expression and metabolism that ensure survival to stress challenges. Pereira et al. identify quantitative ranges for those changes in a set of genes and physiological variables (production of ATP, trehalose, NADH, etc.) that are specific for adaptation to heat stress, desiccation/rehydration, or pH.

Highlights

- A feasibility range for metabolic adaptation of yeast to heat stress is quantified
- Specific feasibility ranges for adaptation to desiccation and pH stress are found
- The feasibility ranges apply to more than a dozen different yeast strains

Data and Software Availability

GSE58319
GSE38478

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Quantitative Operating Principles of Yeast Metabolism during Adaptation to Heat Stress

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SUMMARY

Microorganisms evolved adaptive responses to survive stressful challenges in ever-changing environments. Understanding the relationships between the physiological/metabolic adjustments allowing cellular stress adaptation and gene expression changes being used by organisms to achieve such adjustments may significantly impact our ability to understand and/or guide evolution. Here, we studied those relationships during adaptation to various stress challenges in *Saccharomyces cerevisiae*, focusing on heat stress responses. We combined dozens of independent experiments measuring whole-genome gene expression changes during stress responses with a simplified kinetic model of central metabolism. We identified alternative quantitative ranges for a set of physiological variables in the model (production of ATP, trehalose, NADH, etc.) that are specific for adaptation to either heat stress or desiccation/rehydration. Our approach is scalable to other adaptive responses and could assist in developing biotechnological applications to manipulate cells for medical, biotechnological, or synthetic biology purposes.

INTRODUCTION

Microorganisms evolved adaptive responses that enable them to survive stressful challenges in ever changing environments (Darwin, 1859; Bidle, 2015; Cushman and Bohnert, 2000; Jayaraman, 2011; Nevo, 2001; Reusch and Wood, 2007; Seo et al., 2011; Vilaprinyo et al., 2010). Adaptation to those challenges is achieved by adjusting metabolism to new conditions, through the modulation of gene expression, protein levels and activity, and the flow of metabolites (Chen et al., 2015; Gasch, 2007; Jenkins et al., 1997; Seo et al., 2011; Vilaprinyo et al., 2010). Such adjustments integrate and balance the effects of stress with the physiological needs of the cell, ensuring that critical physiological parameters are fine tuned to guarantee survival (Curto et al., 1995; Nikerel et al., 2012; Sorribas et al., 1995; Vilaprinyo et al., 2006; Voit and Radivoyevitch, 2000). The ranges within which those parameters may fall to guarantee survival can be considered as quantitative operating principles for the response. Understanding those principles and the molecular determinants of successful stress responses (*successful phenotypes*) may have a significant impact in our ability to interpret evolution, treat diseases, and manipulate microorganisms for medical, biotechnological, or synthetic biology purposes.

Saccharomyces cerevisiae is well characterized at the genomic, proteomic, and metabolomic levels in a variety of environmental and physiological conditions making it an important model to study stress adaptation (Castells-Roca et al., 2011; Diezmann and Dietrich, 2011; Gibney et al., 2013; Malinovska et al., 2012; Molina-Navarro et al., 2008; Tirosh et al., 2011) The sets of yeast genes whose expression is modulated during adaptive responses to different types of stress only partially overlap (Berry and Gasch, 2008; Serra-Cardona et al., 2015). In addition, the changes in expression for ubiquitous stress responsive genes quantitatively depend on the type and intensity of the stress challenge, as can be seen by comparing various published experiments (Causton et al., 2001; Eisen et al., 1998; Gasch et al., 2000). These quantitative dependencies suggest the existence of specific ranges for those changes (operating ranges or feasibility regions) that lead to *successful phenotypes*, enabling cell survival (Curto et al., 1995; Nikerel et al., 2012; Sorribas et al., 1995; Vilaprinyo et al., 2006; Voit and Radivoyevitch, 2000). Investigating if such feasibility regions for gene expression changes exist and how and why they came about could allow us to understand their causal relationship with the physiological and metabolic requirements that are needed for cellular adaptation and survival. That understanding would identify quantitative operating principles for adaptation and enable the creation of genotype-to-phenotype mapping of stress adaptation at the molecular level (Coelho et al., 2010; Curto et al., 1995; Gjuvsland et al., 2011, 2013; Guillén-Gosálbez and Sorribas, 2009; Savageau et al., 2009; Sorribas et al., 1995, 2010; Vilaprinyo et al., 2006; Voit and Radivoyevitch, 2000; Wang et al., 2012; Zackrisson et al., 2016).

Here, we establish a systematic methodology that identifies quantitative operating principles underlying metabolic adaptation based on gene expression profiles and apply it to the analysis of stress responses in *S. cerevisiae*. We adapt a minimal model of yeast central metabolism previously used to study heat stress adaptation (Curto et al., 1995; Sorribas et al., 1995, 2010; Vilaprinyo et al., 2006; Voit and Radivoyevitch, 2000) and

Table 1. Physiological Variables Used to Identify Possible Operating Principles in the Adaptive Responses of Yeast to Heat Stress

Variable	Acronym	Rationale for Using This Variable[a]	Basal Condition	Quantitative Boundaries for Heat Stress Survival
V1	V_{ATP}	Changes in gene expression need to accommodate an increase in the rate of ATP production.	60	123.349 < V1 < 339.19
V2	V_{Tre}	Changes in gene expression need to accommodate an increase in the rate of trehalose synthesis.	0.0012	0.0076 < V2 < 0.094
V3	V_{NADPH}	Changes in gene expression need to accommodate an increase in reducing equivalents; the flux of NADPH production is used as a proxy for this increase.	1.77	4.31 < V3 < 11.21
V4	GLC	Changes in gene expression should allow cells to avoid needless increases in the concentration of intermediates, thus minimizing possible toxic effects and the taxing of the solvent capabilities of the cell.	0.035	0.0094 < V4 < 0.080
V5	G6P	Increases in the production/uptake of Glucose-6P are needed for the upregulation of energy production.	1.01	2.48 < V5 < 19.91
V6	F16P	Depletion of Fructose-1,6,BisP needs to be tightly regulated and minimized, as this metabolite is an important bifurcation point in glycolysis that provides flux for the production of glycerol.	9.10	0.111 < V6 < 20.58
V7	PEP	Changes in gene expression should allow cells to avoid needless increases in the concentration of intermediates, thus minimizing possible toxic effects and the taxing of the solvent capabilities of the cell	0.0094	0.00019 < V7 < 0.014
V8	ATP	ATP concentration should increase to meet energy demands.	1.12	2.39 < V8 < 6.73
V9	Cost	Adaptation should be economic. We use changes in gene expression as a proxy for this variable; GEP (gene expression profiles that allow adaptation with minimal changes in gene expression should be favored.	0	8.10 < V9 < 14.09
V10	V_{Glyce}	Glycerol has a protective role in heat stress adaptation, and its production should either increase or not decrease by much.	1.93	0.18 < V10 < 2.07
V11	ψ	Changes in the activity of the enzymes TPS and PFK should be coordinately balanced after heat stress, in order to appropriately regulate the branching point in the glycolytic flux that divides material between glycolysis and trehalose production.	52.06	5.34 < V11 < 34.48

See Data S1 for a description of the dynamic behavior of these variables.
[a]See Pozo et al. (2011), Sorribas et al. (2010), Vilaprinyo et al. (2006, 2010), Voit (2003a), Voit and Radivoyevitch (2000), and references therein.

combine that model with dozens of independent experimental measurements to estimate the quantitative feasibility regions for changes in gene expression and the quantitative physiological requirements that functionally constrain those regions. We identify physiological requirements that define three distinct feasibility regions, specific for adaptation to heat stress, desiccation/rehydration, and pH, respectively. Our results also show that alternative models that focus on other parts of metabolism are required to identify physiological constraints and feasibility regions for adaptive responses to other types of stress.

RESULTS

A Feasibility Space for Physiological Adaptation of Yeast to Heat Stress

First, we focus on the adaptive response to heat stress. *S. cerevisiae* copes with this stress by mounting a transcriptional response that modulates and adapts its physiology to the temperature increase. The adaptation requires that production of energy, reducing equivalents, and protective metabolites (such as glycerol and trehalose) is upregulated, while the metabolic fluxes through glycolysis must remain balanced and coordinated. In addition, concentration of glycolytic intermediates should remain as low as possible, changes in regulatory metabolites such as F16P should be fine-tuned, and changes in gene expression should lead to an adaptive response that is as economical as possible. There are 11 specific metabolic variables defined in Table 1 that can be used as a proxy for quantifying these general physiological criteria (Sorribas et al., 2010; Vilaprinyo et al., 2006, 2010; Voit, 2003a; Voit and Radivoyevitch, 2000). More details are given in Data S1.

By using an appropriate mathematical model of metabolism, one can estimate how experimentally determined changes in gene expression during response to heat stress propagate and change the physiological variables identified in Table 1. The model we use is described in more detail in the Experimental Procedures below and in the Supplemental Experimental Procedures. The basal values for the 11 variables in Table 1 are calculated using the model, thus characterizing the basal steady state of pre-stressed yeast.

In order to characterize the boundaries within which the genes considered in the model change their expression under HS (heat stress), we selected the 9 datasets pertaining to this response and referenced in the tables of data file Data S1 and in the Supplemental Experimental Procedures. The transcriptional changes of all the genes coding for enzymes in the model (see

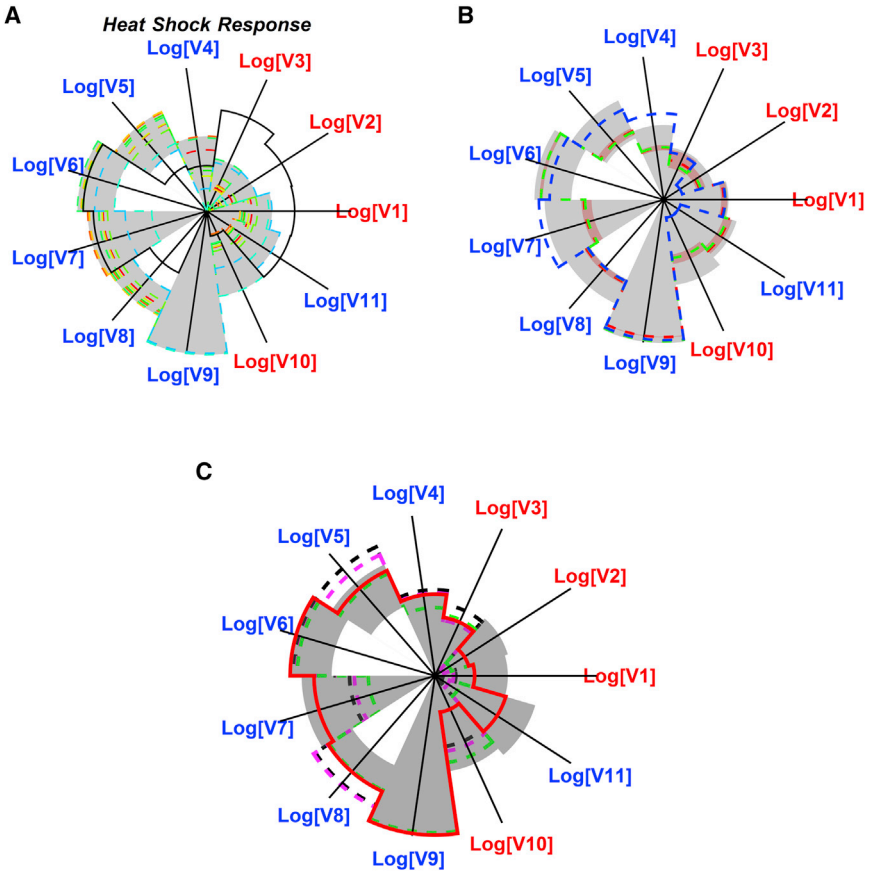

Figure 1. Spider Plot Representation of the Feasibility Range of Adaptation of the 11 Physiological Variables from Table 1 during Heat Stress Response

Each axis represents the logarithm of one of the variables. Variables in red grow toward the center of the axis. Variables in blue grow toward the outside of the axis. The gray area in all panels indicates the range of values that the eleven variables can assume in yeasts that adapt well to heat stress. The black line in all panels indicates the basal steady-state values for each variable.

(A) Determination of the feasibility range using the heat stress experiments from Data S1. Each dashed curve represents one of the databases.

(B) Validation of the feasibility range with independent experiments. The red and green curves represent the median and average (respectively) responses of our macro array experiment used to validate the feasibility range of the variables with a new yeast strain. The red area represents quantiles 0.25–0.75 around the median determined using bootstrap. The blue line represents the values for the RefSeq experiment GSE58319 used to validate the feasibility range of the variables for a newer, more accurate, measurement technique.

(C) The red line represents the values for the 11 variables from Table 1 in response to a temperature shift from 29°C–33°C (GDS36). The dashed lines represent the values for the 1 variables from Table 1 for preadapted yeast that are subjected to a stronger heat stress (GDS15, 33°C–37°C black line; GDS112, 30°C–37°C magenta line; GDS2910, 30°C–37°C green line). See Figure S1 for details about the dynamic behavior of these variables during adaptive responses. The ranges for each axis are the following: V1∈[123.35, 339.19]; V2∈[0.0076, 0.094]; V3∈[4.31, 11.21]; V4∈[0.0094, 0.080]; V5∈[2.48, 19.91]; V6∈[0.11, 20.58]; V7∈[0.00019, 0.013]; V8∈[2.39, 6.73]; V9∈[8.10, 14.09]; V10∈[0.18, 2.07]; and V11∈[5.34, 34.48].

Data S1) are then extracted from the resulting datasets and used to estimate the changes in protein activities, as described in the Experimental Procedures. Those changes in protein activity were plugged into the model, and the corresponding metabolic state under those new activities was calculated independently for each of the HS datasets. This allowed us to assess the approximate quantitative boundaries between which each of the variables from Table 1 can change to enable heat stress adaptation and survival (Figure 1A). That figure identifies a well-defined region, marked in gray, within which the physiological adaptation of yeast to heat stress occurs, according to the 11 variables being estimated from the experimental results. This region is a proxy for the feasibility space of phenotypical adaptation of yeast to heat stress. We note that the smaller the fraction of the axis within the gray region, the smaller the range within which the corresponding variable falls during the adaptive response.

The quantitative feasibility space identified in Figure 1A could be dependent on biological-environmental factors and on measurement techniques. To investigate if that space is robust to changes in biological-environmental factors we performed additional heat stress experiments with a strain of *S. cerevisiae* that is different from those used to generate the feasibility space of Figure 1A. The new experiments are described in the Experimental procedures section. The results from these experiments fall within the feasibility region defined in Figure 1A (Figure 1B).

To further investigate if the feasibility region is robust to changing experimental techniques, we searched for whole transcriptome RNA sequencing (RNA-seq) experiments in GEO that measured changes in gene expression during heat stress adaptation (GEO: GSE58319) (Swamy et al., 2014). According to our model, the changes in gene expression for these experiments lead to changes in variables V1–V3, V5–V6, and V8–V11 that fall within the feasibility region identified using array techniques (Figure 1C). Only variables V4 (glucose concentration) and V7 (phosphoenolpyruvate concentration) fall slightly outside of their feasibility ranges. These results suggest that feasibility regions could be a feature of adaptive responses that is robust to the measurement technique.

If the feasibility region from Figure 1A is a biological design principle, one should expect that the adaptive response of cells pre-adapted with a mild heat stress (phase 1 of the response) and then subjected to stronger temperature increases (phase 2) should, overall, fall within the feasibility space defined in Figure 1A for the 11 physiological variables. To test this hypothesis we used the datasets for gene expression changes under mild heat stress to calculate how the values for the eleven variables changed (phase 1). Then, we took this *preadapted steady state*

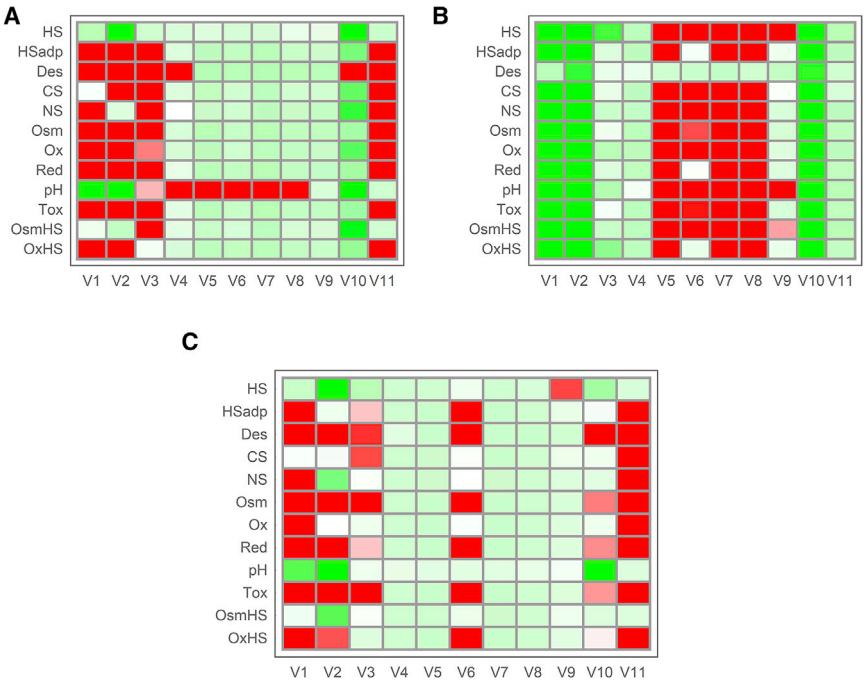

Figure 2. Specificity of Feasibility Space for Adaptation to Heat Stress, Desiccation/Rehydration, and pH Shifts

Each row represents a type of stress and each column represents one of the physiological variables. Green (red) entries indicate that the value of the variable falls within (outside of) the feasibility range for adaptation. More intense colors are further away from the feasibility boundaries. White indicates that the criteria are about the boundary value. Stresses: HS, heat stress; HSadapt, preadapted yeast subjected to stronger heat stress; CS, cold shock; OxS, oxidative; RS, reductive; Osm, osmotic; NS, nutrient; Tox, toxic; pH, pH stress; Des, desiccation; OsmHS, yeast subjected to osmotic stress followed by heat stress; OxHS, oxidative combined with heat stress.

(A) Feasibility space for heat stress. See Figure S2A for details.

(B) Feasibility space for desiccation/rehydration. See Figure S2B for details.

(C) Feasibility space for pH shifts. See Figure S2C for details.

and used other, independent, datasets for gene expression changes of preadapted yeasts subsequently subjected to stronger heat stresses (phase 2) to estimate the physiological variables of Table 1 (Figure 1C). We see that most of the 11 variables fall within the feasibility regions identified in Figure 1A. We emphasize that the consistency of the results from this two-step experiment with the feasibility space calculated in Figure 1A is remarkable, taking into account the approximations that come as a consequence of combining independent experiments from different labs. Further details about the results from Figure 1 are presented and contextualized in Data S1.

Is the Feasibility Space Specific for Physiological Adaptation to Heat Stress?

We wanted to understand if the feasibility space identified in Figure 1A is also valid for adaptive responses to other types of stress. To answer this question, we downloaded GEO gene expression datasets from experiments that exposed *S. cerevisiae* to various types of stress (see Data S1). These datasets measured changes in whole-genome gene expression during yeast adaptation to desiccation, rehydration, osmotic, oxidative, reductive, and nutrient stresses. As before, transcriptional changes of genes coding for enzymes in the model were extracted from each dataset and the mathematical model was used to calculate how those transcriptional changes affected the eleven variables. Figure 2A shows that only heat stress responses fall within the feasibility region for all 11 variables from Table 1. Figures 2B and 2C also show that none of the curves that represent the changes in gene expression during the adaptive responses to other stress conditions fall fully within the feasibility space defined in Figure 1A. Thus, the feasibility space of adaptation in Figure 1A is specific for heat stress. They also suggest that variables V1–V3 are important in separating the adaptive response of yeast to heat stress from other adaptive responses. Overall, the results indicate that the variables from Table 1 are sufficient to identify unique and specific quantitative requirements imposed on yeast by adaptation to heat stress, although they are not a complete molecular description of that adaptation.

Can the Same Physiological Variables Be Biologically Relevant in Defining Specific Feasibility Spaces for Other Adaptive Responses?

Although the feasibility space in Figure 1A is specific for HS response, fine-tuning of the same physiological variables might also be important for the natural selection of adaptive responses to other types of stress. If this is so, they could be used to identify quantitatively different feasibility spaces that are specific for the adaptive responses to each type of stress.

To investigate this possibility, for each type of stress, we established the quantitative boundaries of the physiological changes observed for the 11 physiological variables defined in Table 1, in the same way as we did for HS adaptive responses. This revealed that those variables can be used to identify two independent feasibility spaces that are specific for the adaptive responses to desiccation/rehydration, and (to a lesser extent) pH stresses, respectively (Figures 2B, 2C, S2B, and S2C). This was not true for adaptive responses to other stress types. Thus, other metabolic variables need to be identified and used in a modified mathematical model to identify feasibility spaces for the adaptive responses to the remaining types of stress (more details are given in Data S1).

Mapping Phenotype to Genotype

The feasibility space of metabolic adaptation shown in Figure 1A is obtained by mapping the changes in gene expression

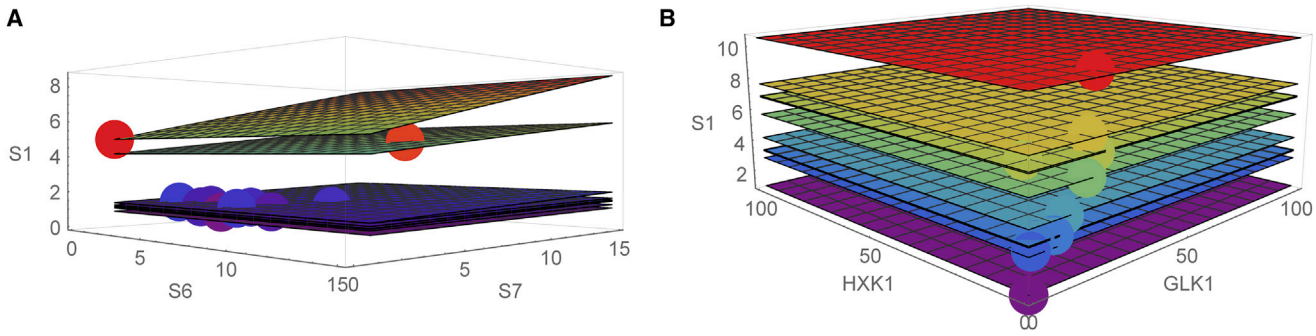

Figure 3. Evolution Can Find Different Combinations of Changes in Gene Expression and Enzyme Activities that Are Equivalent with Respect to the Changes They Cause in Variables V1–V11

Graphical representation of this situation for hexose transport activity S1. Each plane represents one of the heat stress response databases used to calculate the feasibility region shown in Figure 1A.

(A) Activity S1 as a function of activities S6 and S7. Each plane represents all possible sets of values for S1, S6, and S7 that would generate the same values for V1–V11 for the same heat stress database. The dot that falls in each plane represents the actual activity estimated from the experimental changes in gene expression data.

(B) Activity S1 as a function of two high-affinity transporters (HTX6 and HXT7) for the same heat stress responses we use as an example in (A). Each plane corresponds to one of the databases. All points falling on a plane are formally equivalent, leading to the same S1 activity. The dots in each plane represent the actual measurement for the adaptive response. See Figure S3 for details.

("genotype") to the changes in metabolism ("phenotype") using a mathematical model. This "Genotype"-to-"Phenotype" mapping is a surjective mapping, as a set of changes in gene expression uniquely generates a set of changes in the physiological variables.

The same mathematical model can be used to create an inverse mapping of the feasibility space for physiological changes to a corresponding feasibility space for changes in gene expression. This "Phenotype"-to-"Genotype" mapping is degenerate, in the sense that a set of changes in physiological variables can map to more than one set of changes in gene expression. Taking the steady state for the dependent variables, we used the ODE system to calculate the required changes in five enzyme activities as functions of that steady state and of the remaining enzyme activities (details in Data S1). The feasibility range for enzyme activities S1–S5 falls on a plane and depends on the exact value for S6 and S7 (see Figures 3 and S3). This shows that cells can function at different values for the independent enzyme activities and still survive heat stress, if the differences between the activities of the various enzymes are coordinated in such a way that the values for the physiological variables remain within their own feasibility range for survival, which is consistent with previous results from other groups (Chen et al., 2013).

A similar analysis can be done for the changes in gene expression. The enzyme activities represented by S1–S7 depend linearly on subsets of a total of 22 genes. The mapping of the changes in enzyme activity to the changes in gene expression is defined in the Experimental Procedures. Figure 3A shows an example of the gene expression change to enzyme activity change mappings for the same examples represented in Figure 3B. Again, we show that cells can use a wide range of coordinated changes in gene expression to adapt metabolism and make the value of physiological variables move to their feasibility region of adaptation.

Dynamic Physiological Adaptation of *S. cerevisiae* to Heat Stress

The transient dimension of adaptive responses is very important. In order to understand how the feasibility space of physiological variables changes over time we take all databases from Data S1 that contain time series information for gene expression changes (heat stress, osmotic stress, oxidative and reductive stress, and desiccation).

We used these databases to create interpolated time series for relevant gene expression changes. These interpolated functions were used as input for the model to simulate the transient response of the physiological variables during stress adaptation (see the Experimental Procedures for details). Snapshots of these simulations are shown at 2, 10, 20, and 60 min after stress in Data S1 and in Figure S1. During heat stress response, production of energy (ATP) reducing equivalents (NADPH) and protective molecules (trehalose plus glycerol) sharply increases until 20 min after the heat stress and tends to stabilize afterward. The resources invested by the cell in adapting metabolism to the new situation increase sharply for the first 10 min of the response, remaining approximately constant afterward. Similarly, at 10 min, the cell reaches a new balance for resources allocated to the various glycolytic flux branches. The timing at which the various variables from Table 1 reach the new steady state was similar for all types of stress we analyzed. However, the quantitative changes in energy production, NAD(P)H production, and trehalose production are always different from those observed during heat stress. In addition, the way that the glycolytic material is distributed between production of glycerol and ATP is also different between heat stress and the other stresses (details in Data S1).

DISCUSSION

Biological Design Principles in Adaptive Responses

Evolution is fundamentally constrained by basal metabolism. In spite of this, the quasi modularity of many biological circuits

enables evolution to almost independently select and optimize each functional module that performs a specific task within the network of metabolism (Afek et al., 2011; Friedlander et al., 2013; Kashtan and Alon, 2005; Kashtan et al., 2009; Ryan et al., 2012; Thompson et al., 2013). That selection may eventually find biological design principles, identifying specific circuit topologies as optimal for the function they perform and fixing them in the population (Davidson et al., 2012; Lim et al., 2013; Poyatos, 2012; Salvado et al., 2011; Savageau, 1971a, 1975, 2013; Steinacher and Soyer, 2012). Fine tuning the parameters, concentrations, and fluxes of those circuits allows evolution to further identify, select, and fix quantitative operating principles for their adaptive responses (Guillén-Gosálbez and Sorribas, 2009; Nikerel et al., 2012; Sorribas et al., 2010; Vilaprinyo, 2007; Vilaprinyo et al., 2006; Voit, 2003a, 2003b; Voit and Radivoyevitch, 2000).

Stressful and frequent environmental changes make cells evolve increasingly efficient adaptive responses that ensure an appropriate reallocation of cellular resources in order to deal with and survive the insult (Dhar et al., 2011; Kutyna et al., 2012; Lopes et al., 2008; Sulmon et al., 2015). Long-term evolutionary experiments (Dhar et al., 2011, 2013; Sucena et al., 2014; Teotónio and Rose, 2000; Teotónio et al., 2009) show that many different gene expression programs might produce equivalent adaptive phenotypes. Our results emphasize this aspect and show that evolution explores a multidimensional space of gene expression to find solutions that are equivalent with respect to the metabolic variables that yeast must modulate to survive heat stress.

Quantitative Adaptation of Yeast to Heat Stress

There is a multi-level molecular adaptation of yeast cells to temperature increases. At the genomic level, there is modulation of gene expression that induces the production of chaperones, heat shock proteins, metabolic enzymes, and antioxidant defense proteins (Boy-Marcotte et al., 1999; Vilaprinyo et al., 2006). At the proteome level, the activity of pre-existing and newly made proteins is regulated, both by temperature and by other metabolic events associated with the temperature increase (Nickells and Browder, 1988; Voit and Radivoyevitch, 2000). Finally, at the metabolomic level, the production of small molecules and metabolites is adjusted in order to allow yeast to meet the new physiological demands imposed on the cell by the temperature increase (Berovic and Herga, 2007; Berovič et al., 2007; Gibney et al., 2015; Voit, 2003a). Taken together, these events protect proteins and cellular structures, enabling recovery of the cell after stress adaptation.

The metabolic variables in Table 1 provide a set of possible descriptors to measure how yeast changes its metabolism as it adapts to the various physiological demands imposed by heat stress (Guillén-Gosálbez and Sorribas, 2009; Sorribas et al., 2010; Vilaprinyo et al., 2006; Voit and Radivoyevitch, 2000). The current study establishes the boundaries for the feasibility regions within which the physiological variables can change to create a successful phenotype of adaptation in a data driven way, by integrating information from a large number of gene expression experiments done in independent labs. These regions can be viewed as operating principles that evolution found to enable heat stress adaptation. The feasibility regions for the physiological variables map onto feasibility regions for change its gene expression and protein activities. We find that the feasibility regions are valid for a large variety of *S. cerevisiae* strains (we compared 11 different strains). Furthermore, adapting the model to compare the heat stress response between *S. cerevisiae*, *S. pombe*, *C. albicans*, and *C. glabrata* cautiously suggests that the feasibility regions for the metabolic variables might also be at least partially generalizable to other unicellular yeasts with a similar lifestyle and metabolism (details in Data S1). Our time course analysis emphasized that the bulk part of the adaptive response occurred at most 20 min after the heat stress, both at the genetic and biochemical level, which is consistent with decades of research on the subject (Morano et al., 2012; Verghese et al., 2012).

Quantitative Adaptation of Yeast to Other Stresses

We also ask if the same physiological variables can be used to define feasibility regions that are specific for other types of stress response. We find that these variables define feasibility regions specific for responses to desiccation/rehydration and pH shifts, but not to other types of stress responses.

The work presented here is a proof of principle that one can develop methodologies to identify multi-level feasibility spaces for adaptive responses. This methodology can be summarized as follows. First, the metabolites, fluxes, and other metabolic variables that are important for the response should be tentatively identified. If detailed experimental information about metabolic adaption is not available, one could for example identify which metabolic pathways globally change their expression in whole-genome gene expression measurements. Second, a model for the pathways that contribute to the changes in those variables is needed. Third, estimates of how the various activities in the model change in response to stress are required. Fourth, these estimates are used to predict how the metabolic variables change in response to stress and the metabolic changes are used to identify the feasibility space of the metabolic changes. This feasibility space for physiological adaptation can then be used, together with the model, to estimate the feasibility space for the changes in protein activity and in gene expression, thus allowing us to establish a multilevel (metabolic, proteomic, and genomic) set of feasibility spaces for adaptation to stress. In principle, with enough available data, this methodology can be applied to any organism and stress response.

EXPERIMENTAL PROCEDURES

Mathematical Model

In order to understand how the eleven variables constrain changes in gene expression during heat stress response, we created a minimal mathematical model of the parts of metabolism that affect those variables. We used the GMA (generalized mass action) mathematical formalism (see the Supplemental Experimental Procedures for details.). This model includes a simplified version of glycolysis that can be used to calculate how a specific change in a given gene will affect the physiological requirement that was identified in Table 1. The mathematical model we use is given in full detail in the Supplemental Experimental Procedures (Equations SE1–SE7).

We note that all calculations, simulations, and figures can be reproduced using Notebooks EV1–EV9, which are provided in Data S1.

Organizing Gene Expression Data According to Type of Stress

Experiments that exposed *S. cerevisiae* to stress and measured how the yeast adapts its gene expression were identified by first searching GEO (National Center for Biotechnology Information) for "stress" and "cerevisiae" and then manually going through the list and identifying all experiments where classical stress challenges where given to any strain of *S. cerevisiae*. All such databases of micro array data were downloaded and stored locally. 38 databases, containing 81 different independent experiments (see Data S1) were analyzed and organized into 13 classes of stressful challenges (Section 1.5 in the Supplemental Experimental Procedures).

Estimating Changes in Gene Expression

All entries for the 22 different genes considered in the model were extracted from each database. For each database, we eliminated missing values for the relevant gene and then used the average of the remaining entries as the representative measure for the change in gene expression. Details regarding how this was done are given at the end of Section 1.5 in the Supplemental Experimental Procedures.

Estimating Changes in Enzyme Activity

All genes coding for proteins directly involved in the enzyme activities of the model were considered. These were: S1 – hexose transporters (HXT) genes: *HXT1*, *HXT2*, *HXT3*, *HXT4*, *HXT6*, *HXT8*; S2 – glucokinase/hexokinase (GLK) genes: *GLK1*, *HXK1*, *HXK2*; S3 – phosphofructokinase (PFK) genes: *PFK1*, *PFK2*; S4 – glyceraldehyde-3-phosphate dehydrogenase (TDH) genes: *TDH1*, *TDH2*, *TDH3*; S4 – pyruvate kinase (PYK) genes: *PYK1*, PYK2; S6 – trehalose synthase complex (TPS) genes: *TPS1*, *TPS2*, *TPS3*; and S7 – glucose-6-phosphate dehydrogenase (GD6PDH) gene: *ZWF1* (Voit and Radivoyevitch, 2000).

We could not find direct measurements for the changes in all enzyme activities of the model under heat stress. Nevertheless, it is well-documented that changes in enzyme activity and gene expression are highly correlated in glycolysis (Ihmels et al., 2004). Because of this we assumed that the fold-change in gene expression directly translates into a similar fold-change in the activity of the corresponding enzymes, whenever a single gene coded for that enzyme activity. This fold change was further weighted considering the basal abundance of the protein, its specific activity, and whether or not more than one isoform contribute to the activity (details in Section 1.6 of the Supplemental Experimental Procedures and Data S1).

Finding Orthologs in Other Yeast Species

Orthologs for the 22 *S. cerevisiae* genes in *Schizosaccharomyces pombe*, *Kluyveromyces lactis*, *Candida glabrata*, and *Candida albicans* were identified using UniProt (The UniProt Consortium), searching for the species name combined with the words: heat shock. These orthologs are given in Data S1.

Microarray Experiments

S. cerevisiae wild-type strain W303-1A was employed for the determination of mRNA levels upon heat stress. Cells were grown exponentially in YPD medium at 25°C, at time 0 they were quickly shifted to 37°C by dilution with 3 vol of pre-warmed fresh medium at 41°C and then maintained in a 37°C water bath for subsequent recovery of samples at different time points. Four independent experiments were carried out, and for each experiment two samples were processed for each time point (eight replicates per time point). Total RNA isolation and labeling, and determination of mRNA levels were done as described in Molina-Navarro et al. (2008) at 0, 3, 6, 9, 12, 15, 18, 21, 25, 30, 45, and 60 min after heat stress. Values at each time point after the beginning of the experiment were normalized by those at time 0.

Bootstrapping was used to determine confidence intervals for the changes in gene expression at each time point in the following way. Four replicates were randomly selected from the eight experiments 100 times. The average time series for each set of replicates was estimated. Then, we calculated quantiles 0.025 and 0.975 of the bootstrapped datasets to estimate the 95% confidence interval for the changes in gene expression at each time point.

Model Calculations

The steady state for each database was calculated by setting Equations SE1–SE5 (see the Supplemental Experimental Procedures) to 0 and numerically calculating their roots using Mathematica's (Wolfram, 1996) *FindRoot* function. Using 1,000 random initial conditions for the dependent variables followed by numerically calculation of the adapted steady state always led to the same steady-state values for the same set of gene expression changes. Time course simulations were done using the *NDSolve* function and using the basal concentrations for initial values.

Steady-State Robustness

Biological systems must be able to adapt to and survive in an ever-changing environment, without being overly sensitive to small changes that are spurious. To achieve this, most biological systems have low sensitivity to fluctuation in parameters (e.g., enzyme activity or *Km*) and such fluctuation will not greatly affect its steady state or homeostasis (Kitano, 2002; Konopka, 2006; Savageau, 1969). This is called robustness of the steady state and it can be measured using sensitivity analysis (Heinrich and Rapoport, 1974; Kacser and Burns, 1973; Kitano, 2002; Konopka, 2006; Savageau, 1969, 1971b). In this work we evaluated the local robustness of the model by analyzing the differential relative sensitivities of each variable with respect to each parameter (see Section 1.3 of the Supplemental Experimental Procedures for details). In approximate terms, if $\overline{S}en(V_i, S_j) = 0.5$ (or -0.5), this means that when the value of S_j changes by 100%, the value of V_i is expected to increase (or decrease) by ~50%.

Steady-State Stability

Return to homeostasis after a perturbation is an important property of biological systems whose mathematical equivalent is steady-state stability. We performed stability analysis of each steady state as described in Section 1.4 of the Supplemental Experimental Procedures.

Principal Component Analysis

Principal component analysis (PCA) is a method to reduce the dimensionality of a dataset and identify which orthogonal linear combinations of variables contribute more strongly to the quantitative variation in the data. *Varimax* PCA of the correlation matrix containing the eleven metabolic variables for each stress experiments was done by calculating the *eigenvalues* and *eigenvectors* of the matrix (Glenn and Myatt, 2009).

Feature Analysis

While PCA provides a way to determine how many dimensions one needs to describe the variability in the data at various degrees of accuracy, often the PC themselves are difficult to interpret. Because of that we also performed feature analysis. Feature analysis was done using two methods: relief-based feature selection (RFS) and correlation-based feature selection (CFS) RFS works by randomly sampling an instance from the data and then locating its nearest neighbor from the same and opposite class. The values of the attributes of the nearest neighbors are compared to the sampled instance and used to update relevance scores for each attribute (Lee et al., 2011). CFS works by evaluating the subsets of attributes rather than individual attributes, and takes into account the usefulness of individual features for predicting the class along with the level of inter-correlation among them (Hall and Holmes, 2003).

Analysis of the Transient Response

The temporal dynamics of the model was studied in all cases where time series were available for the gene expression data (databases GDS16, GDS20, GDS30, GDS31, GDS34, GDS36, GDS108, GDS112, GDS113, GDS2712, GDS2713, GDS2715, GDS2910, GDS3030, GDS3035 and GSE38478 from Data S1). We simulated the adaptation of yeast to stresses from 0 to 60 min after stress as described in Section 1.7 of the Supplemental Experimental Procedures.

DATA AND SOFTWARE AVAILABILITY

The accession numbers for the data used in this work are GEO: GDS15, GDS16, GDS17, GDS18, GDS19, GDS20, GDS21, GDS30, GDS31, GDS34, GDS35, GDS36, GDS108, GDS111, GDS112, GDS113, GDS115, GDS1711,

GDS2196, GDS2338, GDS2343, GDS2522, GDS2712, GDS2713, GDS2715, GDS2716, GDS2910, GDS2925, GDS3030, GDS3035, GDS3137, GDS3438, GDS3591, GDS3332, GDS3866, GSE58319, and GSE38478. All new accession numbers are provided in Data S1. Additional information and quantitative data are also provided in the additional data notebooks EV1–EV9.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and one data file and can be found with this article online at https://doi.org/10.1016/j.celrep.2018.02.020.

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AUTHOR CONTRIBUTIONS

Conceptualization, E.V., A.S., and R.A.; Methodology & Software, T.P., E.V., and R.A.; Formal Analysis, T.P., E.V., R.A., B.S., G.A., and A.S.; Investigation, G.B., E.H., T.P., E.V., and A.S.; Resources, E.H., E.V., G.B., R.A., and A.S.; Data Curation, T.P., E.V., G.A., and A.S.; Writing – Original Draft, T.P. and R.A.; Writing – Review & Editing, all authors; Visualization, T.P. and R.A.; Supervision, R.A.; Project Administration, R.A., E.V., and A.S.; Funding Acquisition, R.A. and A.S.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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REFERENCES

Afek, Y., Alon, N., Barad, O., Hornstein, E., Barkai, N., and Bar-Joseph, Z. (2011). A biological solution to a fundamental distributed computing problem. Science *331*, 183–185.

Berovic, M., and Herga, M. (2007). Heat shock on Saccharomyces cerevisiae inoculum increases glycerol production in wine fermentation. Biotechnol. Lett. *29*, 891–894.

Berovič, M., Pivec, A., Košmerl, T., Wondra, M., and Čelan, S. (2007). Influence of heat shock on glycerol production in alcohol fermentation. J. Biosci. Bioeng. *103*, 135–139.

Berry, D.B., and Gasch, A.P. (2008). Stress-activated genomic expression changes serve a preparative role for impending stress in yeast. Mol. Biol. Cell *19*, 4580–4587.

Bidle, K.D. (2015). The molecular ecophysiology of programmed cell death in marine phytoplankton. Annu. Rev. Mar. Sci. *7*, 341–375.

Boy-Marcotte, E., Lagniel, G., Perrot, M., Bussereau, F., Boudsocq, A., Jacquet, M., and Labarre, J. (1999). The heat shock response in yeast: differential regulations and contributions of the Msn2p/Msn4p and Hsf1p regulons. Mol. Microbiol. *33*, 274–283.

Castells-Roca, L., Mühlenhoff, U., Lill, R., Herrero, E., and Bellí, G. (2011). The oxidative stress response in yeast cells involves changes in the stability of Aft1 regulon mRNAs. Mol. Microbiol. *81*, 232–248.

Causton, H.C., Ren, B., Koh, S.S., Harbison, C.T., Kanin, E., Jennings, E.G., Lee, T.I., True, H.L., Lander, E.S., and Young, R.A. (2001). Remodeling of yeast genome expression in response to environmental changes. Mol. Biol. Cell *12*, 323–337.

Chen, P.-W., Fonseca, L.L., Hannun, Y.A., and Voit, E.O. (2013). Coordination of rapid sphingolipid responses to heat stress in yeast. PLoS Comput. Biol. *9*, e1003078.

Chen, P.-W., Fonseca, L.L., Hannun, Y.A., and Voit, E.O. (2015). Dynamics of the heat stress response of ceramides with different fatty-acyl chain lengths in baker's yeast. PLoS Comput. Biol. *11*, e1004373.

Coelho, P.M.B.M., Salvador, A., and Savageau, M.A. (2010). Relating mutant genotype to phenotype via quantitative behavior of the NADPH redox cycle in human erythrocytes. PLoS ONE *5*, e13031.

Curto, R., Sorribas, A., and Cascante, M. (1995). Comparative characterization of the fermentation pathway of Saccharomyces cerevisiae using biochemical systems theory and metabolic control analysis: model definition and nomenclature. Math. Biosci. *130*, 25–50.

Cushman, J.C., and Bohnert, H.J. (2000). Genomic approaches to plant stress tolerance. Curr. Opin. Plant Biol. *3*, 117–124.

Darwin, C. (1859). The Origin of Species by Means of Natural Selection; or the Preservation of Favoured Races in the Struggle for Life (John Murray).

Davidson, E.A., Windram, O.P.F., and Bayer, T.S. (2012). Building synthetic systems to learn nature's design principles. Adv. Exp. Med. Biol. *751*, 411–429.

Dhar, R., Sägesser, R., Weikert, C., Yuan, J., and Wagner, A. (2011). Adaptation of Saccharomyces cerevisiae to saline stress through laboratory evolution. J. Evol. Biol. *24*, 1135–1153.

Dhar, R., Sägesser, R., Weikert, C., and Wagner, A. (2013). Yeast adapts to a changing stressful environment by evolving cross-protection and anticipatory gene regulation. Mol. Biol. Evol. *30*, 573–588.

Diezmann, S., and Dietrich, F.S. (2011). Oxidative stress survival in a clinical Saccharomyces cerevisiae isolate is influenced by a major quantitative trait nucleotide. Genetics *188*, 709–722.

Eisen, M.B., Spellman, P.T., Brown, P.O., and Botstein, D. (1998). Cluster analysis and display of genome-wide expression patterns. Proc. Natl. Acad. Sci. USA *95*, 14863–14868.

Friedlander, T., Mayo, A.E., Tlusty, T., and Alon, U. (2013). Mutation rules and the evolution of sparseness and modularity in biological systems. PLoS ONE *8*, e70444.

Gasch, A.P. (2007). Comparative genomics of the environmental stress response in ascomycete fungi. Yeast *24*, 961–976.

Gasch, A.P., Spellman, P.T., Kao, C.M., Carmel-Harel, O., Eisen, M.B., Storz, G., Botstein, D., and Brown, P.O. (2000). Genomic expression programs in the response of yeast cells to environmental changes. Mol. Biol. Cell *11*, 4241–4257.

Gibney, P.A., Lu, C., Caudy, A.A., Hess, D.C., and Botstein, D. (2013). Yeast metabolic and signaling genes are required for heat-shock survival and have little overlap with the heat-induced genes. Proc. Natl. Acad. Sci. USA *110*, E4393–E4402.

Gibney, P.A., Schieler, A., Chen, J.C., Rabinowitz, J.D., and Botstein, D. (2015). Characterizing the in vivo role of trehalose in Saccharomyces cerevisiae using the AGT1 transporter. Proc. Natl. Acad. Sci. USA *112*, 6116–6121.

Gjuvsland, A.B., Vik, J.O., Woolliams, J.A., and Omholt, S.W. (2011). Order-preserving principles underlying genotype-phenotype maps ensure high additive proportions of genetic variance. J. Evol. Biol. *24*, 2269–2279.

Gjuvsland, A.B., Vik, J.O., Beard, D.A., Hunter, P.J., and Omholt, S.W. (2013). Bridging the genotype-phenotype gap: what does it take? J. Physiol. *591*, 2055–2066.

Glenn, J., and Myatt, W.P.J. (2009). Principal component analysis. Making Sense of Data II: A Practical Guide to Data Visualization, Advanced Data Mining Methods, and Applications (John Wiley & Sons), pp. 126–130.

Guillén-Gosálbez, G., and Sorribas, A. (2009). Identifying quantitative operation principles in metabolic pathways: a systematic method for searching feasible enzyme activity patterns leading to cellular adaptive responses. BMC Bioinformatics *10*, 386.

Hall, M.A., and Holmes, G. (2003). Benchmarking attribute selection techniques for discrete class data mining. IEEE Trans. Knowl. Data Eng. *15*, 1437–1447.

Heinrich, R., and Rapoport, T.A. (1974). A linear steady-state treatment of enzymatic chains. General properties, control and effector strength. Eur. J. Biochem. *42*, 89–95.

Ihmels, J., Levy, R., and Barkai, N. (2004). Principles of transcriptional control in the metabolic network of Saccharomyces cerevisiae. Nat. Biotechnol. *22*, 86–92.

Jayaraman, R. (2011). Hypermutation and stress adaptation in bacteria. J. Genet. *90*, 383–391.

Jenkins, G.M., Richards, A., Wahl, T., Mao, C., Obeid, L., and Hannun, Y. (1997). Involvement of yeast sphingolipids in the heat stress response of Saccharomyces cerevisiae. J. Biol. Chem. *272*, 32566–32572.

Kacser, H., and Burns, J.A. (1973). The control of flux. Symp. Soc. Exp. Biol. *27*, 65–104.

Kashtan, N., and Alon, U. (2005). Spontaneous evolution of modularity and network motifs. Proc. Natl. Acad. Sci. USA *102*, 13773–13778.

Kashtan, N., Mayo, A.E., Kalisky, T., and Alon, U. (2009). An analytically solvable model for rapid evolution of modular structure. PLoS Comput. Biol. *5*, e1000355.

Kitano, H. (2002). Computational systems biology. Nature *420*, 206–210.

Konopka, A.K. (2006). Systems Biology: Principles, Methods, and Concepts (CRC Press).

Kutyna, D.R., Varela, C., Stanley, G.A., Borneman, A.R., Henschke, P.A., and Chambers, P.J. (2012). Adaptive evolution of Saccharomyces cerevisiae to generate strains with enhanced glycerol production. Appl. Microbiol. Biotechnol. *93*, 1175–1184.

Lee, I.-H., Lushington, G.H., and Visvanathan, M. (2011). A filter-based feature selection approach for identifying potential biomarkers for lung cancer. J. Clin. Bioinforma. *1*, 11.

Lim, W.A., Lee, C.M., and Tang, C. (2013). Design principles of regulatory networks: searching for the molecular algorithms of the cell. Mol. Cell *49*, 202–212.

Lopes, P.C., Sucena, E., Santos, M.E., and Magalhães, S. (2008). Rapid experimental evolution of pesticide resistance in C. elegans entails no costs and affects the mating system. PLoS ONE *3*, e3741.

Malinovska, L., Kroschwald, S., Munder, M.C., Richter, D., and Alberti, S. (2012). Molecular chaperones and stress-inducible protein-sorting factors coordinate the spatiotemporal distribution of protein aggregates. Mol. Biol. Cell *23*, 3041–3056.

Molina-Navarro, M.M., Castells-Roca, L., Bellí, G., García-Martínez, J., Marín-Navarro, J., Moreno, J., Pérez-Ortín, J.E., and Herrero, E. (2008). Comprehensive transcriptional analysis of the oxidative response in yeast. J. Biol. Chem. *283*, 17908–17918.

Morano, K.A., Grant, C.M., and Moye-Rowley, W.S. (2012). The response to heat shock and oxidative stress in Saccharomyces cerevisiae. Genetics *190*, 1157–1195.

National Center for Biotechnology Information. Gene expression omnibus (GEO). https://www.ncbi.nlm.nih.gov/geo/.

Nevo, E. (2001). Evolution of genome-phenome diversity under environmental stress. Proc. Natl. Acad. Sci. USA *98*, 6233–6240.

Nickells, R.W., and Browder, L.W. (1988). A role for glyceraldehyde-3-phosphate dehydrogenase in the development of thermotolerance in Xenopus laevis embryos. J. Cell Biol. *107*, 1901–1909.

Nikerel, E., Berkhout, J., Hu, F., Teusink, B., Reinders, M.J.T., and de Ridder, D. (2012). Understanding regulation of metabolism through feasibility analysis. PLoS ONE *7*, e39396.

Poyatos, J.F. (2012). On the search for design principles in biological systems. Adv. Exp. Med. Biol. *751*, 183–193.

Pozo, C., Marín-Sanguino, A., Alves, R., Guillén-Gosálbez, G., Jiménez, L., and Sorribas, A. (2011). Steady-state global optimization of metabolic non-linear dynamic models through recasting into power-law canonical models. BMC Syst. Biol. *5*, 137.

Reusch, T.B.H., and Wood, T.E. (2007). Molecular ecology of global change. Mol. Ecol. *16*, 3973–3992.

Ryan, C.J., Roguev, A., Patrick, K., Xu, J., Jahari, H., Tong, Z., Beltrao, P., Shales, M., Qu, H., Collins, S.R., et al. (2012). Hierarchical modularity and the evolution of genetic interactomes across species. Mol. Cell *46*, 691–704.

Salvado, B., Karathia, H., Chimenos, A.U., Vilaprinyo, E., Omholt, S., Sorribas, A., and Alves, R. (2011). Methods for and results from the study of design principles in molecular systems. Math. Biosci. *231*, 3–18.

Savageau, M.A. (1969). Biochemical systems analysis. II. The steady-state solutions for an n-pool system using a power-law approximation. J. Theor. Biol. *25*, 370–379.

Savageau, M.A. (1971a). Concepts relating the behavior of biochemical systems to their underlying molecular properties. Arch. Biochem. Biophys. *145*, 612–621.

Savageau, M.A. (1971b). Parameter sensitivity as a criterion for evaluating and comparing the performance of biochemical systems. Nature *229*, 542–544.

Savageau, M.A. (1975). Optimal design of feedback control by inhibition: dynamic considerations. J. Mol. Evol. *5*, 199–222.

Savageau, M.A. (2013). System design principles. In Quantitative Biology, M.E. Wall, ed. (CRC Press), pp. 23–50.

Savageau, M.A., Coelho, P.M.B.M., Fasani, R.A., Tolla, D.A., and Salvador, A. (2009). Phenotypes and tolerances in the design space of biochemical systems. Proc. Natl. Acad. Sci. USA *106*, 6435–6440.

Seo, Y.-S., Chern, M., Bartley, L.E., Han, M., Jung, K.-H., Lee, I., Walia, H., Richter, T., Xu, X., Cao, P., et al. (2011). Towards establishment of a rice stress response interactome. PLoS Genet. *7*, e1002020.

Serra-Cardona, A., Canadell, D., and Ariño, J. (2015). Coordinate responses to alkaline pH stress in budding yeast. Microb. Cell *2*, 182–196.

Sorribas, A., Curto, R., and Cascante, M. (1995). Comparative characterization of the fermentation pathway of Saccharomyces cerevisiae using biochemical systems theory and metabolic control analysis: model validation and dynamic behavior. Math. Biosci. *130*, 71–84.

Sorribas, A., Pozo, C., Vilaprinyo, E., Guillén-Gosálbez, G., Jiménez, L., and Alves, R. (2010). Optimization and evolution in metabolic pathways: global optimization techniques in Generalized Mass Action models. J. Biotechnol. *149*, 141–153.

Steinacher, A., and Soyer, O.S. (2012). Evolutionary principles underlying structure and response dynamics of cellular networks. Adv. Exp. Med. Biol. *751*, 225–247.

Sucena, É., Vanderberghe, K., Zhurov, V., and Grbić, M. (2014). Reversion of developmental mode in insects: evolution from long germband to short germband in the polyembrionic wasp *Macrocentrus cingulum* Brischke. Evol. Dev. *16*, 233–246.

Sulmon, C., van Baaren, J., Cabello-Hurtado, F., Gouesbet, G., Hennion, F., Mony, C., Renault, D., Bormans, M., El Amrani, A., Wiegand, C., and Gérard, C. (2015). Abiotic stressors and stress responses: What commonalities appear between species across biological organization levels? Environ. Pollut. *202*, 66–77.

Swamy, K.B.S., Lin, C.-H., Yen, M.-R., Wang, C.-Y., and Wang, D. (2014). Examining the condition-specific antisense transcription in S. cerevisiae and S. paradoxus. BMC Genomics *15*, 521.

Teotónio, H., and Rose, M.R. (2000). Variation in the reversibility of evolution. Nature *408*, 463–466.

Teotónio, H., Chelo, I.M., Bradić, M., Rose, M.R., and Long, A.D. (2009). Experimental evolution reveals natural selection on standing genetic variation. Nat. Genet. *41*, 251–257.

Thompson, D.A., Roy, S., Chan, M., Styczynsky, M.P., Pfiffner, J., French, C., Socha, A., Thielke, A., Napolitano, S., Muller, P., et al. (2013). Evolutionary principles of modular gene regulation in yeasts. eLife *2013*, 1–37.

Tirosh, I., Wong, K.H., Barkai, N., and Struhl, K. (2011). Extensive divergence of yeast stress responses through transitions between induced and constitutive activation. Proc. Natl. Acad. Sci. USA *108*, 16693–16698.

The UniProt Consortium. The universal protein resource (UniProt). https://www.uniprot.org/.

Verghese, J., Abrams, J., Wang, Y., and Morano, K.A. (2012). Biology of the heat shock response and protein chaperones: budding yeast (Saccharomyces cerevisiae) as a model system. Microbiol. Mol. Biol. Rev. *76*, 115–158.

Vilaprinyo, E. (2007). Principles of design and operation in genetic and biochemical systems: Adaptive responses of yeast to stress. PhD thesis (University of Lleida).

Vilaprinyo, E., Alves, R., and Sorribas, A. (2006). Use of physiological constraints to identify quantitative design principles for gene expression in yeast adaptation to heat shock. BMC Bioinformatics *7*, 184.

Vilaprinyo, E., Alves, R., and Sorribas, A. (2010). Minimization of biosynthetic costs in adaptive gene expression responses of yeast to environmental changes. PLoS Comput. Biol. *6*, e1000674.

Voit, E.O. (2003a). Biochemical and genomic regulation of the trehalose cycle in yeast: review of observations and canonical model analysis. J. Theor. Biol. *223*, 55–78.

Voit, E.O. (2003b). Design principles and operating principles: the yin and yang of optimal functioning. Math. Biosci. *182*, 81–92.

Voit, E.O., and Radivoyevitch, T. (2000). Biochemical systems analysis of genome-wide expression data. Bioinformatics *16*, 1023–1037.

Wang, Y., Gjuvsland, A.B., Vik, J.O., Smith, N.P., Hunter, P.J., and Omholt, S.W. (2012). Parameters in dynamic models of complex traits are containers of missing heritability. PLoS Comput. Biol. *8*, e1002459.

Wolfram, S. (1996). The Mathematica Book (Cambridge University Press).

Zackrisson, M., Hallin, J., Ottosson, L.-G., Dahl, P., Fernandez-Parada, E., Ländström, E., Fernandez-Ricaud, L., Kaferle, P., Skyman, A., Stenberg, S., et al. (2016). Scan-o-matic: high-resolution microbial phenomics at a massive scale. G3 (Bethesda) *6*, 3003–3014.