Molecular Systems Biology 3; Article number 106; doi:10.1038/msb4100147
Citation: ***Molecular Systems Biology*** **3:106**

www.molecularsystemsbiology.com

molecular systems biology

Genome-wide transcriptional plasticity underlies cellular adaptation to novel challenge

Shay Stern[1], Tali Dror[1], Elad Stolovicki[2], Naama Brenner[1] and Erez Braun[2,*]

[1] Department of Chemical Engineering, Technion-Israel Institute of Technology, Haifa, Israel and [2] Department of Physics, Technion-Israel Institute of Technology, Haifa, Israel
* Corresponding author: Department of Physics, Technion-Israel Institute of Technology, Haifa 32000, Israel. Tel.: +972 48292879; Fax: +972 48295755; E-mail: erez@physics.technion.ac.il

Received 11.12.06; accepted 16.2.07

Cells adjust their transcriptional state to accommodate environmental and genetic perturbations. An open question is to what extent transcriptional response to perturbations has been specifically selected along evolution. To test the possibility that transcriptional reprogramming does not need to be 'pre-designed' to lead to an adaptive metabolic state on physiological timescales, we confronted yeast cells with a novel challenge they had not previously encountered. We rewired the genome by recruiting an essential gene, *HIS3*, from the histidine biosynthesis pathway to a foreign regulatory system, the GAL network responsible for galactose utilization. Switching medium to glucose in a chemostat caused repression of the essential gene and presented the cells with a severe challenge to which they adapted over approximately 10 generations. Using genome-wide expression arrays, we show here that a global transcriptional reprogramming (>1200 genes) underlies the adaptation. A large fraction of the responding genes is nonreproducible in repeated experiments. These results show that a nonspecific transcriptional response reflecting the natural plasticity of the regulatory network supports adaptation of cells to novel challenges.
Molecular Systems Biology 24 April 2007; doi:10.1038/msb4100147
Subject Categories: metabolic and regulatory networks; chromatin & transcription
Keywords: adaptation; cellular metabolism; expression arrays; plasticity; transcriptional response

Introduction

Recent genome-wide expression measurements have revealed that a broad range of environmental perturbations and gene deletions lead to a global transcriptional response in which a sizeable fraction of the genome responds to the perturbation by induction or repression (Gasch *et al*, 2000; Jelinsky *et al*, 2000; Causton *et al*, 2001; Ideker *et al*, 2001; Lai *et al*, 2005). The hallmark of these large-scale responses is a rapid transient followed by relaxation, within a cell generation time, to a steady state (Gasch *et al*, 2000; Koerkamp *et al*, 2002; Wu *et al*, 2004). It is not known, however, what fraction of genes within such massive transcriptional responses is essential to the specific cellular demands. Using known gene annotations, the fraction of responding genes directly relevant to the perturbation is usually found to be very small, raising the question what is the functional role of the rest. On more general grounds, one wonders whether the transcriptional reprogramming following a perturbation has been specifically selected along evolution toward this challenge, or reflects the fundamental large-scale connectivity and dynamic characteristics of the transcriptional regulatory network (Kafri *et al*, 2005). If the transcriptional response has been specifically selected, one expects to find the coexpression of genes with cofunctionality reflecting the specific demand imposed by the challenge. This requires some degree of overlapping regulatory cascades among responding genes (Chu *et al*, 1998; Spellman *et al*, 1998; Hughes *et al*, 2000; Jansen *et al*, 2002; Segal *et al*, 2003; Ihmels *et al*, 2004; Kharchenko *et al*, 2005). This regulatory mode then has been selected in evolution and is 'pre-designed' to allow transcriptional reprogramming to meet specific environmental and genetic challenges. An alternative but not mutually exclusive possibility is that the massive transcriptional response to perturbations is a universal feature of the underlying regulatory network, a necessity of its large-scale interactions across functional groups (intermodular interactions). A genetic or environmental perturbation then causes a response that is largely nonspecific toward the perturbation. This response of the genetic network in turn allows efficient plastic adaptation of cellular metabolism to a broad range of unforeseen challenges. There are indications in the literature that similar phenotypes, including similar metabolic states and growth rates in microorganisms, can be supported by different underlying expression states (Fong *et al*, 2005). The relation between metabolism and transcription is not a simple one-to-one, owing to the flexibility and robustness of the metabolic network and cells can generate multiple transcriptional states to achieve the desired metabolic condition. The question then arises to what extent cells use this transcriptional flexibility to address unforeseen challenges on physiological timescales (not through new mutations). Such freedom is crucial for a high evolvability potential (Gerhart and Kirschner, 1997; Kirschner and Gerhart, 1998).

To study the generality and plasticity of transcriptional reprogramming, we challenged yeast cells with a novel perturbation they had not encountered before along their history in evolution. This approach eliminates the possibility that the regulatory system has been specifically selected to address this perturbation. A strain of the yeast *Saccharomyces cerevisiae* was engineered to recruit the gene *HIS3*, an essential enzyme from the histidine biosynthesis pathway (Hinnebusch, 1992), to the GAL system, responsible for galactose utilization (Stolovicki *et al*, 2006). The arbitrary regulatory linkage made between these two conserved and highly specific modules represents an unforeseen perturbation for the cells. In our strain, *HIS3* is under the exclusive control of the GAL regulatory system and is entirely detached from its natural regulation. The GAL system behaves as a strong switch and is heavily repressed when switched to a glucose-containing medium (Johnston *et al*, 1994; Carlson, 1999; Braun and Brenner, 2004). Therefore, upon switching to a medium containing glucose and lacking histidine, the GAL system and with it *HIS3* are highly repressed immediately following the switch and the cells encounter a severe challenge. We have recently shown that a cell population carrying this rewired genome can adapt to grow competitively in a chemostat in a medium containing pure glucose (Stolovicki *et al*, 2006). This adaptation of the population occurred on a timescale of $\sim$10 generations during which the ability to grow competitively in glucose was stably inherited in the population. Moreover, introducing *3-amino-triazole* (*3AT*), a competitive inhibitor of HIS3p, caused a significantly larger environmental pressure and led eventually to similar adaptive response, albeit on somewhat longer timescales (Stolovicki *et al*, 2006). It was shown that the adaptation process involved the tuning of the *HIS3* and all the GAL genes transcription levels according to the amount of inhibitor in the medium, but the mechanism of this transcriptional reprogramming process remained unknown. Yeast cells had not encountered recruitment of *HIS3* to the GAL system along their evolutionary history and their genome could not possibly have been selected to specifically address glucose repression of *HIS3*. Nevertheless, we show here that the adaptation process following the switch to glucose-based medium in the chemostat involves a global transcriptional response of hundreds of genes, emerging at the onset of the medium switch and relaxing on the timescale of the population adaptation. This global transcriptional response is sensitive to the applied pressure by HIS3p inhibition and thus underlies the adaptation of the cells to the unforeseen challenge. The observed response is largely nonspecific; repeated experiments result in low reproducibility of their transcriptional states showing that a large fraction of the responding genes, although enabling the adaptation process, are nonspecific toward the challenge.

Results

Figure 1 shows the dynamics of the population's cell density (blue lines, measured by optical density (OD) and reflecting the integrated metabolic state of the population) following a medium switch from galactose to glucose in the chemostat without (A) and with (B) *3AT*. The transient population

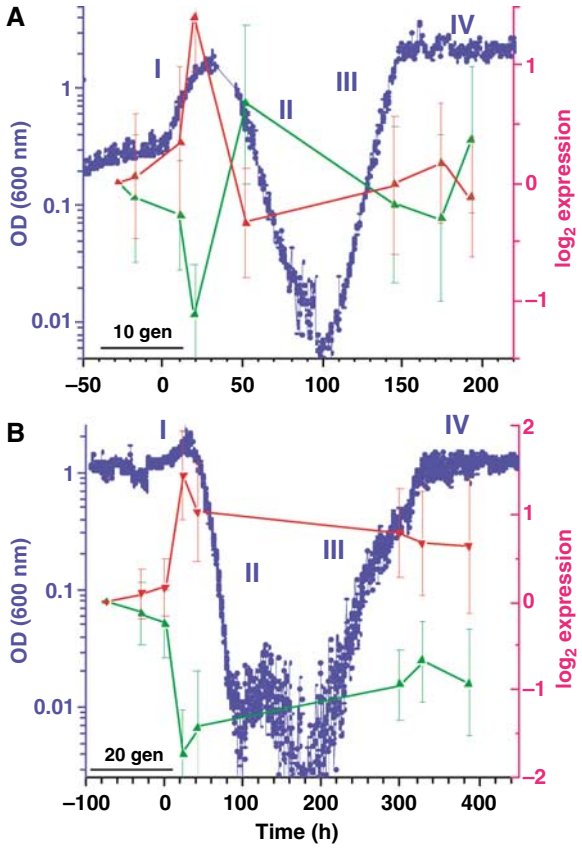

Figure 1 Adaptive population dynamics and associated global transcriptional response. Population dynamics as measured by the cell density in the chemostat (blue line) for (**A**) no *3AT* and (**B**) 40 mM *3AT*. The population in each experiment exhibited four phases (I–IV) of dynamics as depicted. Expression arrays were measured at eight time points along the course of the population adaptation following the medium switch from galactose to glucose (at t=0). A SOM clustering method (see Materials and methods and Supplementary Figure S1) led to two dominant global clusters: induced (red; a—543, b—701 genes) and repressed (green; a—692, b—998 genes). Note the symmetry between induced and repressed clusters (correlation coefficient between the two clusters' mean expression profiles, a: $-$0.92, b: $-$0.98). The error bars present the standard deviation of expression values among genes belonging to each cluster. Note the logarithmic scale. The generation time equals chemostat dilution time $\times$ ln2 $\sim$ 5 h.

responses converged within 10–20 generations to a stable steady state in glucose. There are four phases of the population dynamics (Stolovicki *et al*, 2006). In phase I, The GAL genes and *HIS3* linked to them were repressed, but the OD transiently increased following the switch into glucose metabolism, due to existing resources (e.g. histidine) in the population from the previous galactose steady state. Then, the population fitness decayed in phase II due to improper expression levels of *HIS3*, followed by a recovery after a few generations (III) to a steady-state level (IV) with a cell density similar to the peak in phase I. In phase IV, the cells are fully adapted to grow in pure glucose, tuning the expression of *HIS3* to the appropriate level according to the environmental pressure (level of *3AT*) (Stolovicki *et al*, 2006).

To study the genome-wide transcriptional response, eight samples of cells harvested from the chemostat (see Materials and methods) at time points along the different phases of the

population adaptation were analyzed by expression arrays for the two experiments, with and without *3AT*. Genome-wide measurements of the mRNA expression levels normalized by their expression in galactose steady state revealed that a sizeable fraction of the genome responded by induction or repression to the switch into glucose (Figure 2). Superimposed on the OD traces, Figure 1 shows the results of a clustering analysis (Tamayo *et al*, 1999; Shamir *et al*, 2005) of the expression of genes as measured by the arrays along time in the experiments (see the details of the clustering analysis in Supplementary Figure S1). This analysis revealed two dominant clusters, each containing hundreds of genes in each experiment. The genes of these clusters responded to the medium switch to glucose by a strong transient induction or repression followed by relaxation to steady state on the timescale of the population adaptation process, approximately 10 generations. During this relaxation period, the external conditions were kept constant in the chemostat. The two clusters in each experiment are characterized by similar but opposite dynamics. The striking symmetry between induction and repression (Gasch *et al*, 2000; Nautiyal *et al*, 2002) suggests that a global conservation principle, possibly competition for cellular resources, is involved in the dynamics of the genetic regulatory system. These results are not sensitive to the clustering method (data not shown).

We first analyzed the clusters that appeared in the transcriptional response based on known annotations. In the cluster that is transiently repressed, we found the genes of the GAL system, exhibiting the expected transient repression upon the switch to glucose followed by recovery of the expression, which is correlated with the population dynamics (see Supplementary Figure S2 in agreement with previous k-PCR results; Stolovicki *et al*, 2006). An example for transiently induced modules is provided by the glycolysis and ribosomal protein genes (Supplementary Figure S3). The glycolysis and ribosomal genes are known to be overexpressed in glucose compared to galactose (Yin *et al*, 2003). Surprisingly, following the transient induction, these groups exhibited relatively fast relaxation to a steady-state level, similar to the one in galactose medium. More globally, we characterized the biological

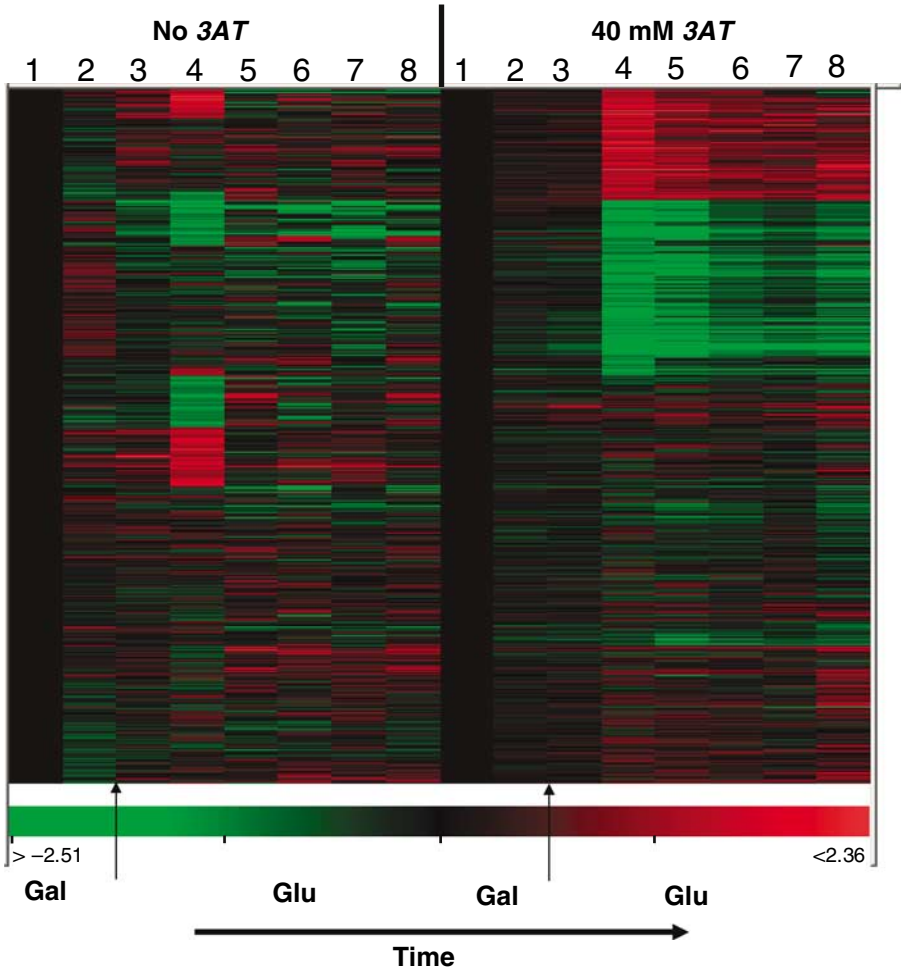

Figure 2 The genome-wide transcription pattern. The raw transcription levels at eight time points for the two experiments, (left) no *3AT*, (right) 40 mM *3AT*, in a color code: red—induced, green—repressed. There are a total of 4148 genes that passed all filters (see Materials and methods). The medium switch from galactose to glucose is marked and the numbers above the columns are the measurement points as shown in Figure 1. Note the differences between the patterns of expression for the two experiments (rows correspond to the same gene in both experiments).

functions in each of the two main clusters in Figure 1, using the Gene Ontology annotations (SGD) (Materials and methods). In both experiments, the transiently induced cluster was significantly enriched for general energetic and biosynthesis modules such as glycolysis, ribosome biogenesis, macromolecule biosynthesis and also for regulatory genes involved in the proteins translational machinery. By contrast, the transiently repressed cluster was significantly enriched for modules that are assigned to DNA replication, DNA repair and regulation of transcription. This distribution of biological functionalities between the two clusters suggests that the induced cluster corresponds mainly to cytoplasmic processes, whereas the repressed cluster to processes in the nucleus ($P<0.005$ in cell compartment enrichment analysis). The functional enrichment analysis is a statistical method that is based on the tradition of assigning well-defined functions to specific genes. This approach, however, explains only a small fraction of the global response as shown below.

One is led to ask whether the observed expression dynamics reflect a known stress response (Gasch *et al*, 2000; Causton *et al*, 2001). In particular, the repression of *HIS3* raises the possibility that the cells are under amino-acid starvation (Natarajan *et al*, 2001). We have performed a detailed comparison between the genomic transient response observed here with and without *3AT*, at different time points of the dynamics, and published large-scale results of known stress responses (Supplementary Table S1). The maximal number of induced genes overlapping between the transient response in our experiments and the stress response are 39 and 31 (out of 201) for the no *3AT* and 40 mM *3AT*, respectively. Similarly, the number of overlapping repressed genes are 85 and 48 (out of 320) for the no *3AT* and 40 mM *3AT*, respectively. This analysis shows that the transcriptional response at any time point of our experiment has a very low overlap with the universal stress response observed for a wide range of environmental stresses (Gasch *et al*, 2000; Causton *et al*, 2001). It is also not correlated with the typical response to amino-acid starvation (Natarajan *et al*, 2001) (Supplementary Table S2). In particular, the general amino-acid response (regulated by *Gcn4*) (Hinnebusch, 1992) does not operate in our experiments at any point even when a high level of *3AT* is applied. Additionally, all known specific responses to stress are characterized by transient induction or repression with relaxation to steady state within a generation time (Gasch *et al*, 2000; Koerkamp *et al*, 2002; Wu *et al*, 2004). Thus, given the different transcription patterns and the extremely long timescales of the transient response observed here, we conclude that it is not a typical stress or starvation response.

We used *3AT* as a control parameter, which enabled us to distinguish specific metabolic effects, including the switch of carbon sources from galactose to glucose, from regulatory changes induced by the increase in the environmental pressure applied directly on the rewired gene, *HIS3*. Both populations grown with and without *3AT* initiated from very similar steady-state patterns of expression in galactose (Supplementary Figure S4). Thus, *3AT* by itself does not cause a significant modification in the expression state. This is consistent with previous work showing that *3AT* does not introduce significant side effects besides direct inhibition of the HIS3p, and thus has similar effect to reducing *HIS3* effective expression levels (Marton *et al*, 1998). The gene content overlap between the transiently induced and transiently repressed clusters in both experiments (with and without *3AT*) is only partial, 15% (389 genes out of 2545 assigned to the induced or repressed clusters), but significantly larger than would expected by chance (Supplementary Figure S1c). This partial overlap contains also the genes responding to the carbon source switch from galactose to glucose. This result indicates that the switch of carbon sources caused only a small part of the response, whereas the major effect is due to the *HIS3* repression pressure.

To assess the reproducibility of the transcriptional response, we repeated the chemostat experiment with no *3AT*. The population density exhibited reproducible adaptation dynamics and the same glucose steady state, but nevertheless the transcriptional response showed significant variations. We quantified the degree of reproducibility by computing the correlation coefficient among expression patterns (Supplementary Figure S5). Within a single experiment, the correlation between time-separated glucose steady states is high (R=0.8–0.9). This high value is close to the reproducibility in duplicate arrays (R=0.9). Even for time points separated by as much as 237 h, the correlation coefficient is R=0.9, showing the stability of this pattern over many generations. By contrast, between different experiments, the correlation coefficient is significantly lower (R=0.32). The reproducibility of transient transcriptional patterns showed similar trends. The correlation coefficient between the initial transient responses following the medium switch to glucose (phase I of the dynamics in Figure 1) in repeated experiments is very low ($R<0.1$) compared to the high correlation between two transient points within the same chemostat experiment (R=0.5–0.9; Supplementary Figure S6). An additional way to assess the reproducibility is to compare the total number of genes significantly induced or repressed in each case and to compute the degree of overlap among these groups of genes. Table I shows the number of genes significantly induced (>twofold) or repressed (<twofold) in repeated experiments both in the transients and the steady states. It shows that these numbers vary significantly among the experiments, consistent with the

Table I Comparison of the total number of genes significantly induced or repressed and the degree of overlap among these groups of genes in different experiments

Total number of genes	No-*3AT*, first repeat	No-*3AT*, second repeat	40 mM *3AT*	Fraction overlap in repeats	
3344	418	830	524	7% (85 genes)	Transiently induced (>2-fold)
	514	780	960	14% (160 genes)	Transiently repressed (<2-fold)
3728	90	136	217	10% (20 genes)	Induced at steady state (>2-fold)
	262	432	464	30% (159 genes)	Repressed at steady state (<2-fold)

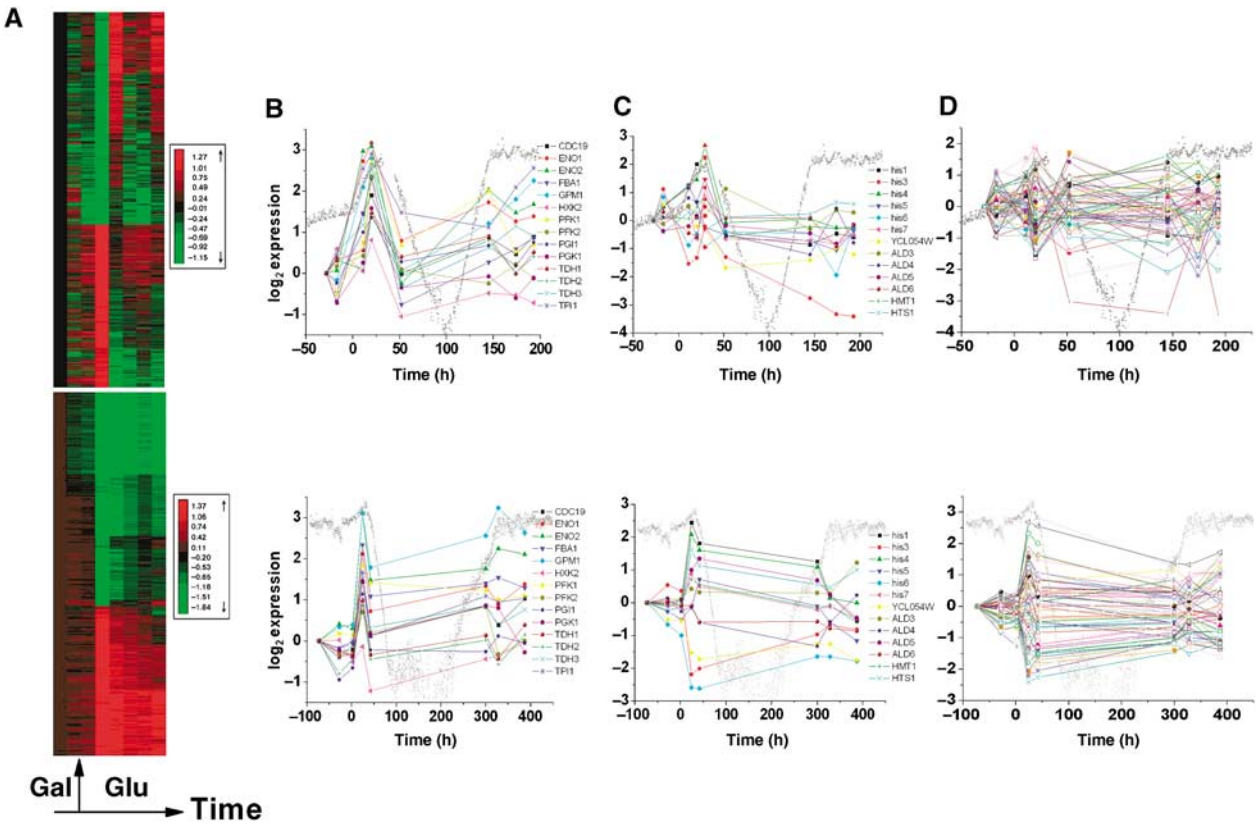

Figure 3 Environmental pressure leads to highly correlated transcriptional response. (**A**) Color-coded figure of transcriptional response for the eight time points in the two experiments, with no *3AT* (top) and with 40 mM *3AT* (bottom). The genes were ordered in each experiment according to the clusters presented in Figure 1. The significant increase in coherency of the response with the increase of environmental pressure by *3AT* is apparent in the image. Expression profiles of genes belonging to glycolysis (**B**), histidine (**C**) and purine (**D**) pathways. Note the emergence of highly correlated patterns of transcription owing to the environmental pressure in the lower panel. A given functional module simultaneously contains correlated and anti-correlated trajectories.

behavior of the correlation coefficients. The fraction of overlapping induced or repressed genes in the different experiments is small (10–30%; Table I), with no significant enrichment for biological functionality ($P>0.001$) outside of the expected induction of the glycolysis genes and repression of the GAL genes. Thus, the majority of the responding genes do not overlap at any stage in repeated experiments. We conclude that a non-negligible portion of the genes that changed their expression during the adaptation process does not have a well-defined and reproducible function in the challenging environment.

Our experiment enables us to characterize the dynamics of the transcriptional response in addition to its gene content. Upon the switch to glucose, the average dynamics of the transcriptional response in the two experiments, with and without *3AT*, seem somewhat similar (Figure 1 and Supplementary Figure S4). However, the larger pressure applied by the *3AT* results in a markedly higher correlation among the temporal patterns of the hundreds responding genes. Figure 3A compares the array data in color code for the two experiments. It is seen that the emergent pattern of transcription exhibited a higher degree of order by the introduction of high external pressure in the form of *3AT*; more than 25% of gene pairs exhibited absolute correlation coefficient larger than 0.9 in the high-pressure experiment compared to 5% in the low-pressure one (Supplementary Figure S7). Figure 4 compares the distributions of correlation coefficients among all genes for the two experiments. Note the significant peaks at the high positive and negative correlation edges for the *3AT* experiment compared with a distribution peaked on zero correlation for the experiment without *3AT*.

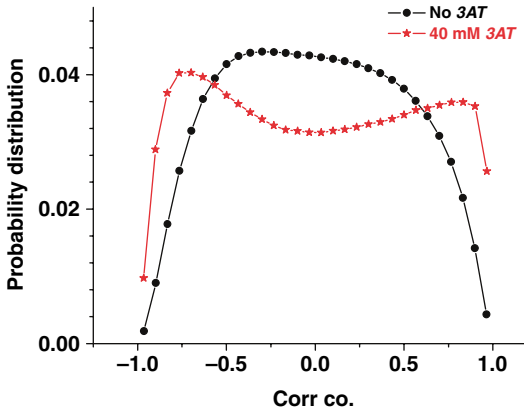

Figure 4 The correlations of transcriptional response. A Pearson correlation coefficient was computed between all pairs of genes in both experiments, with and without *3AT*. The figure shows the distributions of correlation coefficient between all possible pairs, for no *3AT* (black) and 40 mM *3AT* (red).

Observation of the transcriptional temporal trajectories for specific metabolic pathways in our experiments illustrates the different contributions to the correlated dynamics (Figures 3B–D). A general energetic module, such as glycolysis, exhibited similar patterns of induction and relaxation in both experiments (Figure 3B). This, however, was not the general behavior. We found that more than one-third of the known metabolic gene modules (30 out of 88 modules described in KEGG (Kanehisa *et al*, 2004), $P<0.05$, see Materials and methods) exhibited high correlation in expression among their genes when the environmental pressure was high (40 mM *3AT*, average absolute correlation 0.55 ± 0.06), but not when it was low (no *3AT*, average absolute correlation 0.39 ± 0.04). As an example, Figure 3C shows the histidine biosynthesis pathway and Figure 3D the purine pathway. Note the highly ordered trajectories in the lower panels (with *3AT*) compared to the disordered ones in the upper panels (no *3AT*). These pathways are directly connected to the gene under pressure, *HIS3*, but as mentioned above, these modules were not unique in exhibiting such high correlation when the environmental pressure was high (see Figure 3A). The increase in expression correlation for a sizeable fraction of the genome at higher pressure on *HIS3* indicates that a global transcriptional regulatory mechanism is in operation, rather than a local specific one. Interestingly, genes belonging to the same metabolic pathway exhibited simultaneous positively and negatively correlated dynamics. Even genes encoding for enzymes residing along a linear metabolic pathway exhibited negatively correlated dynamics (Ihmels *et al*, 2004; Kharchenko *et al*, 2005). These general features in the *3AT* experiment, highly correlated expression dynamics and simultaneous positively and negatively correlated trajectories, appeared also for genes belonging to the same protein complex (Jansen *et al*, 2002). For example, Figure 5 shows the expression trajectories for the nucleotide excision repair (NER) protein complex. The generality of these results are demonstrated by comparing the gene-pair correlations between *GAL1* and all other genes for the two experiments, with and without *3AT* (Figure 6). Note that many of the low-correlated gene pairs in the no *3AT* environment exhibited high correlation when *3AT* is present.

Taken together, the highly correlated response observed here reveals that coexpression does not necessarily imply co-functionality (Niehrs and Pollet, 1999) and can exist among genes residing in different functional modules. Indeed, the highly correlated pattern of expression spans the entire metabolic network across modules and correlations do not decay for genes residing in remote modules (Kharchenko *et al*, 2005) (Supplementary Figure S8). These results indicate that the crosstalk between functional modules plays important role in enabling the emergence of a proper metabolic state. The opposite is also true—the symmetric patterns of induced and repressed transcriptional responses observed within modules means that co-functionality does not necessarily imply coexpression and there is no simple connection between transcription and metabolism.

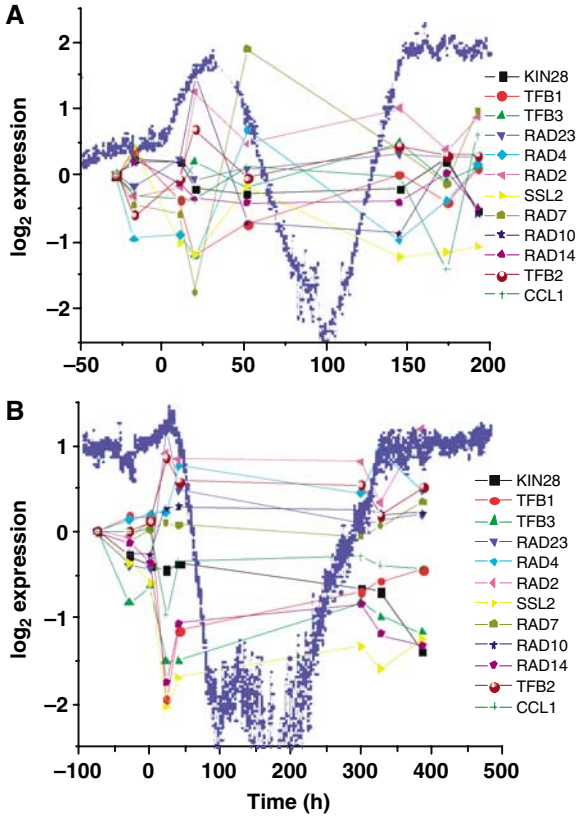

Figure 5 Expression profiles of a protein complex. Expression profiles of all genes belonging to the NER protein complex as defined in MIPS (http://mips.gsf.de) superimposed on the population density curves (blue lines), for (**A**) no-*3AT* and (**B**) 40 mM *3AT* experiments. Note the significant increase in order among the transcriptional response of the genes in the *3AT* experiment compared to the no-*3AT* experiment. Note also that genes belonging to the same protein complex exhibited anti-correlated expression patterns.

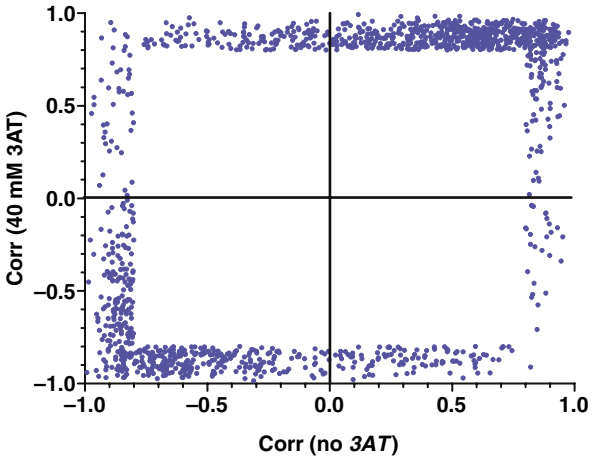

Figure 6 Correlation coefficient between *GAL1* and all other genes. The Pearson correlation coefficient was computed between the transcriptional dynamic profiles of *GAL1* and all other genes for the two experiments, with and without *3AT*. The correlation coefficient value of each pair in the *3AT* experiment was plotted against the correlation coefficient value in the no-*3AT* experiment. Only pairs with an absolute Pearson correlation above 0.8 in at least one of the experiments were plotted for clarity. There were 339 genes in the *3AT* experiment and 109 genes in the no-*3AT* experiment with absolute correlations above 0.9. Note that genes highly correlated in one experiment may exhibit low and even negative correlation in the other experiment. The *GAL1* gene served as an example here, but similar results were obtained for many other genes belonging to different functional modules.

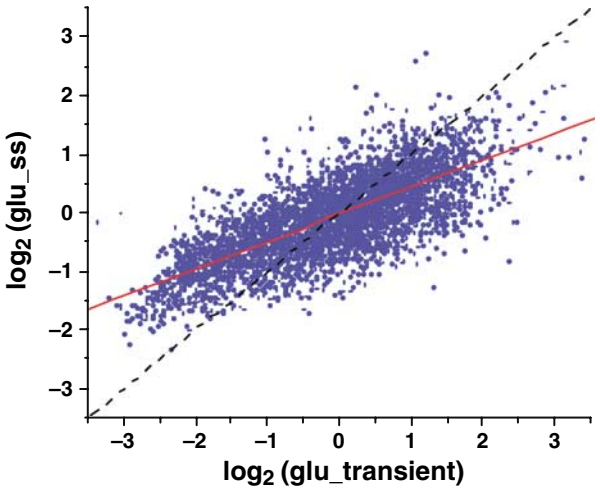

Figure 7 Steady-state transcriptional pattern is well predicted from the transient response. The steady-state expression level (average of the two measurement points in glucose steady state) versus the transient expression level (4th time point in Figure 1B) for the experiment with 40 mM *3AT*. The Pearson correlation between the transient response and steady state is 0.76. The slope deviation from the reference dashed black line represents the overshoot or undershoot in transient expression levels compared to the steady state.

The long timescale of the transcriptional response raises the question whether it is the result of a population level selection process (Ferea *et al*, 1999) or a cellular process that is faithfully transmitted along generations (Molinier *et al*, 2006). In a selection process, the initial state is an average over a mixture of a large number of subpopulations, one of which will eventually take over and determine the final state. Therefore, one expects no significant correlation between the initial and final states at the level of the population averages. Figure 7 shows that the final steady state in our experiment is highly correlated with the initial transient transcription level following the medium switch from galactose- to glucose-containing medium. The final steady state is well predicted by the initial transient response. A gene exhibiting higher (lower) steady-state transcription level in glucose relative to that in galactose exhibited an overshoot (undershoot) and then relaxed to the proper steady state on the population adaptation timescale (Figure 1B). Thus, the transcriptional reprogramming was initiated instantaneously with the medium switch from galactose to glucose, and the emergent pattern of transcription was faithfully transmitted along generations till the convergence to steady state. We conclude that the observed expression dynamics was not the result of a selection process on the population, but rather a plastic transcriptional response of the cellular regulatory network.

Discussion

We presented yeast cells with a severe perturbation they had not encountered along their evolutionary history and to which they adapted within physiological timescales. We have found that underlying this adaptation is a global transcriptional response involving a sizeable fraction of the genome, which relaxed on the timescale of the population adaptation, of the order of 10 generations. This response of the cells in the chemostat is a genuine transcriptional reprogramming process and not due to a population selection mechanism. Increasing the pressure applied on the rewired gene *HIS3* resulted in a significant increase in correlations among hundreds of genes residing in different modules. This sensitivity to the level of pressure imposed by the novel challenge shows that transcriptional response is directly involved in the cellular adaptation process. The highly correlated global transcriptional response is consistent with the massive crosstalk known to exist between functional modules (Ideker *et al*, 2001; Tong *et al*, 2001) and the dynamic properties of the regulatory network (Luscombe *et al*, 2004). The characteristics of the transcriptional reprogramming observed here support the conclusion that it is a universal feature of the transcriptional regulatory system reflecting its plasticity and transgeneration stability.

The novelty of the challenge presented to the cells excludes the possibility that the transcriptional response had been specifically selected in evolution. Indeed, analyzing the details of the transcriptional response revealed that, for a large fraction of the responding genes, there is no simple biological interpretation, connecting them to the specific cellular demands imposed by the novel challenge (e.g., amino-acid production). In particular, surprisingly we found that genes belonging to the same functional module, even those active within a linear chain of a biosynthesis pathway, might respond in opposite directions; some are highly induced whereas the others are highly repressed. Thus, an important conclusion of this work is that the global transcriptional response to an unforeseen challenge cannot be explained by simple cellular or metabolic logic. This is to be expected if the response had not been specifically selected in evolution and was not pre-designed for the challenge. Support for this conclusion is found in the fact that repeating experiments do not reproduce similar transcription patterns neither in the transient phase nor in the adapted state in glucose.

Nevertheless, our data raise the following apparent paradox to this conclusion: if the adaptation to the novel perturbation had not been pre-designed, how could the 'correct' direction of the transcriptional response (the 'correct' set of induced and repressed genes) be instantaneously generated (Figure 7)? A plausible explanation is that the direction of the transcriptional response is not in itself adaptive, but rather reflects the large-scale interactions among functional modules of the regulatory network. This possibility is supported by the symmetry between the dynamics of induced and repressed genes. The mechanism underlying the broad nonspecific transcriptional response is yet to be determined. One possibility is that a small number of master regulators are responsible for this response. Alternatively, in the absence of a specific transcriptional regulatory response shaped in evolution toward a specific demand, many parallel regulatory pathways can freely respond without initially having a constraining feedback. Global mechanisms, such as chromatin remodeling, could be involved in such a process. Once a global transcriptional response emerges, an adaptive metabolic response could be generated provided the underlying expression pattern is broad enough to support it. Metabolic requirements feeding back on transcription regulation cause the relaxation of the transcriptional response in magnitude, but cannot affect its

directionality. The transcriptional reprogramming observed here then reflects the complex interplay between transcriptional regulation and metabolism. This idea, if correct, explains the ability of cells to overcome a broad range of novel challenges and stress environments. If there is no specific local solution to a perturbation, one that was specifically shaped in evolution toward this perturbation, the cells have an alternative general mechanism. A mismatch between the metabolic requirements and the cellular state works as a driving force causing a nonspecific large-scale transcriptional response. The drastic change in the expression state of the cell opens multiple new metabolic pathways. Physiological selection works then on these multiple metabolic pathways to stabilize an adaptive state that causes relaxation of the perturbed expression pattern. This scenario, involving the creation of a library of possibilities and physiological selection over this library, is compatible with our understanding of a broad class of biological systems, placing the cellular metabolic/regulatory networks on the same footing as the neural or the immune systems (Gerhart and Kirschner, 1997).

The way organisms deal with unforeseen changes is one of the intriguing questions in biology. Recruitment of a gene to a foreign regulatory system is considered as a powerful driving force in evolution, responsible for example, for the evolution of developmental systems (Carroll *et al*, 2001; Davidson, 2001; True and Carroll, 2002; Wilkins, 2002; Carroll, 2005). Such genomic perturbation presents severe challenges to the cell; the evolvability potential of organisms (Gerhart and Kirschner, 1997; Kirschner and Gerhart, 1998) depends on their ability to cope with them. The global transcriptional plasticity described here shows how an efficient adaptive response could emerge on physiological timescales, a prerequisite for an efficient evolutionary process.

Materials and methods

Strain and chemostat growth conditions

Experiments were carried out with the haploid yeast strain YPH499 [*Mata, ura3-52, lys2-801, ade2-101, trp1-Δ63, his3Δ200, leu2Δ1*] carrying the plasmid vector pESC-LEU (Stratagene) containing the pGAL1-pGAL10 divergent promoter with *HIS3* under pGAL1 (Stolovicki *et al*, 2006). *his3Δ200* is a deletion that removed the entire *HIS3*-coding region plus the upstream promoter region, including the *Gcn4* regulatory sequence. Cells were grown in a homemade chemostat (Stolovicki *et al*, 2006) in synthetic dropout medium lacking histidine and leucine with the appropriate amino-acid supplement and 2% of either pure galactose or pure glucose as the sole carbon source. Throughout the experiments, the sugar (either galactose or glucose) was always in excess (maximal consumption of the cells is 25% of the sugar fed). The medium had the following (concentrations in g/l): 1.7 yeast nitrogen base without amino acids and ammonium sulfate, 5 ammonium sulfate, 1.4 amino-acid dropout powder (without tryptophan, histidine, leucine and uracil; Sigma), 0.01 L-tryptophan, 0.005 uracil. Growth in the chemostat was limited by the concentration of the amino-acid supplement. In the *3AT* experiment, 40 mM of the competitive inhibitor *3-amino-1,2,4-triazole* (Sigma), sterilized by filtration, was introduced into the feeding medium. An online measurement system (Stolovicki *et al*, 2006) was used to measure the OD of cells in the chemostat producing the blue curves in Figure 1. A homemade cell collector (Stolovicki *et al*, 2006) was used to automatically collect samples of cells from the chemostat at precise time points along the experiment and instantaneously freeze them. These samples were used for the microarray experiments.

Expression arrays

For each sample, 15 μg of total RNA was isolated from cells using hot phenol extraction. mRNA was reverse transcribed (superscript II, Invitrogen) and labeled indirectly with cy5/3 dyes (Amersham) using amino-allyl dUTP (Ambion). For each time point, two cDNA microarrays (yeast 6.4 k, UHN microarray center, www.microarrays.ca) containing all ~6400 yeast ORFs in duplicate (a total of four spots for each ORF) were hybridized overnight (42°C) with the sample labeled with cy5 and a reference sample labeled with cy3. Arrays were scanned using a commercial scanner and software (GenePix 4000B, Axon instruments). For each microarray, cy5/cy3 intensity ratios were normalized using the Acuty software (Axon instruments), so the ratio of medians was 1. $\log_2$(cy5/cy3) values of all spots for each gene were averaged for each time point and only genes with at least two high-quality spots in each time point and full dynamic path along the experiments were subject for further analysis. Duplicate arrays were checked to yield high correlated signal for each gene (Supplementary Figure S5e) and the data from each array were compared to real-time PCR measurements for various genes from each sample. There were a total of 4148 genes that passed all filters in both experiments. For all analyses the $\log_2$(cy5/cy3) values in each time point were normalized to the first galactose steady-state time point.

Clustering analysis

All genes with two-fold change in at least one time point were clustered using the *EXPANDER* software (Shamir *et al*, 2005). The self-organized maps (SOM) clustering method (Tamayo *et al*, 1999; Shamir *et al*, 2005) was applied to the gene profiles, with 16 clusters as a pre-defined parameter (Supplementary Figure S1). Clusters that show the same fundamental mean expression profile were joined into two large clusters presented in Figure 1. These results are not sensitive to the SOM clustering method and similar results have been obtained with different methods (data not shown). Enrichment of biological process was computed for clusters of genes using the *GO* TermFinder (SGD). All *P*-values were computed using the hypergeometric distribution.

Comparing the coherency for KEGG groups between experiments

We computed the *P*-value for mean difference between the two distributions of absolute pairwise correlation of a specific KEGG (Kanehisa *et al*, 2004) group, between the no *3AT* and 40 mM *3AT* experiment, using the Wilcoxon's test. All *P*-values were corrected for multiple hypothesis testing using false discovery rate.

Supplementary information

Supplementary information is available at the *Molecular Systems Biology* website (www.nature.com/msb).

Acknowledgements

We thank L David, E Ruppin, R Sharan, M Kupiec, K Keren and Y Soen for useful remarks on the manuscript and Y Arava for assistance with the array experiments. This research was supported by the Israeli Science Foundation (ISF to EB), the German-Israeli Fund (GIF) and the Yeshaya Horowitz Association through the Center of Complexity Science. S Stern acknowledges the support of the Sherman scholarship.

References

Braun E, Brenner N (2004) Transient responses and adaptation to steady state in eukaryotic gene regulation system. *Phys Biol* **1:** 67

Carlson M (1999) Glucose repression in yeast. *Curr Opin Microbiol* **2:** 202

Carroll SB (2005) Evolution at two levels: on genes and form. *PLoS Biol* **3:** 1159–1166

Carroll SB, Grenier JK, Weatherbee SD (2001) *From DNA to Diversity: Molecular Genetics and the Evolution of Animal Design*. Malden, MA: Blackwell Science Inc

Causton HC, Ren B, Koh SS, Harbison CT, Kanin E, Jennings EG, Lee TI, True HL, Lander ES, Young RA (2001) Remodeling of yeast genome expression in response to environmental changes. *Mol Biol Cell* **12:** 323–337

Chu S, DeRisi J, Eisen M, Mulholland J, Botstein D, Brown PO, Herskowitz I (1998) The transcriptional program of sporulation in budding yeast. *Science* **282:** 699–705

Davidson EH (2001) *Genomic Regulatory Systems, Development and Evolution*. London: Academic Press

Ferea TL, Botstein D, Brown PO, Rosenzweig RF (1999) Systematic changes in gene expression patterns following adaptive evolution in yeast. *Proc Nat Acad Sci USA* **96:** 9721

Fong SS, Joyce AR, Palsson BO (2005) Parallel adaptive evolution cultures of *Escherichia coli* lead to convergent growth phenotypes with different gene expression states. *Genome Res* **15:** 1365–1372

Gasch AP, Spellman PT, Kao CM, Carmel-Harel O, Eisen MB, Botstein D, Brown PO (2000) Genomic expression programs in the response of yeast cells to environmental changes. *Mol Biol Cell* **11:** 4241–4257

Gerhart J, Kirschner M (1997) *Cells, Embryos, and Evolution*. Malden, MA: Blackwell Science Inc

Hinnebusch AG (1992) General and pathway-specific regulatory mechanisms controlling the synthesis of amino acid biosynthesis enzymes in *Saccharomyces cerevisiae*. In *The Molecular and Cellular Biology of the Yeast Saccharomyces Gene Expression*, Jones EW, Pringle JR, Broach JR (eds). Cold Spring Harbor, Woodbury, NY: Cold Spring Harbor Laboratory Press

Hughes TR, Marton MJ, Jones AR, Roberts CJ, Stoughton R, Armour CD, Bennet HA, Coffey E, Dai H, He YD, Kidd MJ, King AM, Meyer MR, Slade D, Lum PY, Stepaniants SB, Shoemaker DD, Gachotte D, Chakraburtty K, Simon J, Bard M, Friend SH (2000) Functional discovery via a compendium of expression profiles. *Cell* **102:** 109–126

Ideker R, Thorsson V, Ranish JA, Christmas R, Buhler J, Eng JK, Bumgarner R, Goodlett DR, Aebersold R, Hood L (2001) Integrated genomic and proteomic analyses of a systematically perturbed metabolic network. *Science* **292:** 929

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

Jansen R, Greenbaum D, Gerstein M (2002) Relating whole-genome expression data with protein–protein interactions. *Genome Res* **12:** 37–46

Jelinsky SA, Estep P, Church GM, Samson LD (2000) Regulatory networks revealed transcriptional profiling of damaged *Saccharomyces cerevisiae* cell: Rpn4 links base excision repair with proteasomes. *Mol Cell Biol* **20:** 8157–8167

Johnston M, Flick JS, Pexton T (1994) Multiple mechanisms provide rapid and stringent glucose repression of GAL gene expression *Saccharomyces cerevisiae*. *Mol Cell Biol* **14:** 3834

Kafri R, Bar-Even A, Pilpel Y (2005) Transcription control reprogramming in genetic backup circuits. *Nat Genet* **37:** 295–299

Kanehisa M, Goto S, Kawashima S, Okuno Y, Hattori M (2004) The KEGG resource for deciphering the genome. *Nucleic Acids Res* **32:** D277–D280

Kharchenko P, Church GM, Vitkup D (2005) Expression dynamics of a cellular metabolic network. *Mol Syst Biol* **1:** 2005.0016

Kirschner M, Gerhart J (1998) Evolvability. *Proc Nat Acad Sci USA* **95:** 8420–8427

Koerkamp MG, Rep M, Bussemaker HJ, Hardy GPMA, Mul A, Piekarska L, Szigyarto CAK, Mattos JMTd, Tabak HF (2002) Dissection of transient oxidative stress in *Saccharomyces cerevisiae* by using DNA microarrays. *Mol Biol Cell* **13:** 2783–2794

Lai LC, Kosorukoff AL, Burke PV, Kwast KE (2005) Dynamical remodeling of the transcriptome during short-term anaerobiosis in *Saccharomyces cerevisiae*: different response and role of Msn2 and/or Msn4 and other factors in galactose and glucose media. *Mol Cell Biol* **25:** 4075–4091

Luscombe NM, Babu MM, Yu H, Snyder M, Telchmann SA, Gerstein M (2004) Genomic analysis of regulatory network dynamics reveals large topological changes. *Nature* **431:** 308–312

Marton MJ, DeRisi JL, Bennet HA, Iyer VR, Meyer MR, Roberts CJ, stoughton R, Burchard J, Slade D, Dai Jr H, DEB, Hartwell LH, Brown PO, Friend SH (1998) Drug target validation and identification of secondary drug target effects using DNA microarrays. *Nature Med* **4:** 1293–1301

Molinier J, Ries G, Zipfel C, Hohn B (2006) Transgeneration memory of stress in plants. *Nature* **442:** 1046–1049

Natarajan K, Meyer MR, Jackson BM, Slade D, Roberts C, Hinnebusch AG, Marton MJ (2001) Transcriptional profiling shows that Gcn4p is a master regulator of gene expression during amino acid starvation in yeast. *Mol Cell Biol* **21:** 4347–4368

Nautiyal S, DeRisi JL, Blackburn EH (2002) The genome-wide expression response to telomerase deletion in *Saccharomyces cerevisiae*. *Proc Nat Acad Sci USA* **99:** 9316–9321

Niehrs C, Pollet B (1999) Synexpression groups in eukaryotes. *Nature* **402:** 483–487

Segal E, Shapira M, Regev A, Pe'er D, Botstein D, Koller D, Friedman N (2003) Module networks: identifying regulatory modules and their condition-specific regulators from gene expression data. *Nat Genet* **34:** 166–176

SGD www.yeastgenome.org

Shamir R, Maron-Katz A, ATanay Linhart C, Steinfeld I, Sharan R, Shiloh Y, Elkon R (2005) EXPANDER—an integrative program suite for microarray data analysis. *BMC Bioinform* **6:** 232

Spellman PT, Sherlock G, Zhang MQ, Iyer VR, Anders K, Eisen MB, Brown PO, Botstein D, Futcher B (1998) Comprehensive identification of cell cycle-regulated genes of the yeast *Saccharomyces cerevisiae* by microarray hybridization. *Mol Biol Cell* **9:** 3273–3297

Stolovicki E, Dror T, Brenner N, Braun E (2006) Synthetic gene recruitment reveals adaptive reprogramming of gene regulation in yeast. *Genetics* **173:** 75–85

Tamayo P, Slonim D, Mesirov J, Zhu Q, Kitareewan S, Dmitrovsky E, Lander ES, Golub TR (1999) Interpreting patterns of gene expression with self-organizing maps: methods and application to hematopoietic differentiation. *Proc Nat Acad Sci USA* **96:** 2907–2912

Tong AHY, Evangelista M, Parsons AB, Xu H, Bader GD, Pag'e N, Robinson M, Raghibizadeh S, Hogue CWV, Bussey H, Andrews B, Tyers M, Boone C (2001) Systematic genetic analysis with ordered arrays of yeast deletion mutants. *Science* **294:** 2364–2368

True JR, Carroll SB (2002) Gene co-option in physiological and morphological evolution. *Annu Rev Cell Dev Biol* **18:** 53–80

Wilkins AS (2002) *The Evolution of Developmental Pathways*. Sunderland, MA: Sinauer Associates Inc

Wu J, Zhang N, Hayes A, Panoutsopoulou K, Oliver SG (2004) Global analysis of nutrient control of gene expression in *Saccharomyces cerevisiae* during growth and starvation. *Proc Nat Acad Sci USA* **101:** 3148–3153

Yin Z, Wilson S, Hauser NC, Tournu H, Hoheisel JD, Brown AJP (2003) Glucose triggers different global responses in yeast, depending on the strength of the signal, and transiently stabilizes ribosomal protein mRNAs. *Mol Microbiol* **48:** 713–724