

Decoupling nutrient signaling from growth rate causes aerobic glycolysis and deregulation of cell size and gene expression

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ABSTRACT To survive and proliferate, cells need to coordinate their metabolism, gene expression, and cell division. To understand this coordination and the consequences of its failure, we uncoupled biomass synthesis from nutrient signaling by growing, in chemostats, yeast auxotrophs for histidine, lysine, or uracil in excess of natural nutrients (i.e., sources of carbon, nitrogen, sulfur, and phosphorus), such that their growth rates (GRs) were regulated by the availability of their auxotrophic requirements. The physiological and transcriptional responses to GR changes of these cultures differed markedly from the respective responses of prototrophs whose growth-rate is regulated by the availability of natural nutrients. The data for all auxotrophs at all GRs recapitulated the features of aerobic glycolysis, fermentation despite high oxygen levels in the growth media. In addition, we discovered wide bimodal distributions of cell sizes, indicating a decoupling between the cell division cycle (CDC) and biomass production. The aerobic glycolysis was reflected in a general signature of anaerobic growth, including substantial reduction in the expression levels of mitochondrial and tricarboxylic acid genes. We also found that the magnitude of the transcriptional growth-rate response (GRR) in the auxotrophs is only 40–50% of the magnitude in prototrophs. Furthermore, the auxotrophic cultures express autophagy genes at substantially lower levels, which likely contributes to their lower viability. Our observations suggest that a GR signal, which is a function of the abundance of essential natural nutrients, regulates fermentation/respiration, the GRR, and the CDC.

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INTRODUCTION

Regulating growth in diverse and fluctuating environments is essential for the survival of any organism. For a microorganism, the primary factors determining growth are natural nutrients that provide essential chemical elements and energy (Johnston *et al.*, 1977; Hedbacker and Carlson, 2008; Broach, 2012). Yeast has evolved to

detect the concentrations of such essential natural nutrients and transduce them into an appropriate growth-rate response (GRR). This GRR involves a systems-level coordination of metabolism, gene expression, and cell division that is similar for different nutrient limitations and sources of carbon and energy (Brauer *et al.*, 2008; Slavov and Botstein, 2010, 2011). To better understand this coordination and the physiological consequences of its failure, we studied yeast cultures whose growth is limited by an auxotrophic requirement, a nutrient made necessary by a mutation.

Brauer *et al.* (2008) discovered that leucine and uracil auxotrophs, whose growth is limited by their respective auxotrophic requirements (leucine or uracil), catabolize glucose through glycolysis to ethanol even in well-oxygenated media (aerobic glycolysis) and fail to arrest their cell division cycle (CDC). Building on this discovery, Boer *et al.* (2008) extended the known differences between auxotrophic and natural limitations by measuring a five-times-faster decline in viability of auxotrophs starved for their auxotrophic requirements compared with prototrophs starved for natural nutrients.

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Abbreviations used: CDC, cell division cycle; ERG, ergosterol biosynthesis genes; GR, growth rate; GRR, growth-rate response; GRS, growth-rate signal; PAU, seripauperin; TCA, tricarboxylic acid; TOR, target of rapamycin; UGRR, universal growth-rate response.

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Furthermore, Boer *et al.* (2008) found that inactivating mutations in the target of rapamycin (TOR) network mitigate substantially the phenotypes of glucose wasting and decreased viability exhibited by auxotrophs limited on their auxotrophic requirement. These findings suggest a hypothesis, namely that the growth-rate signal (GRS) mediated by the TOR network, which normally signals nutrient sufficiency, is likely misregulated when cellular growth is limited only by an auxotrophic requirement and not by natural nutrients, such as the sources of carbon, nitrogen, sulfur, or phosphorus. In other words, the cells are misled by the output of the TOR network and attempt to grow faster than allowed by the limiting concentration of the auxotrophic requirement.

Such misregulation by the TOR-mediated GRS raises the possibility that auxotrophs limited for their auxotrophic requirements might not be able to induce a wild-type universal growth-rate response (UGRR) as prototrophs do when cell growth is limited by a natural nutrient (Slavov and Botstein, 2011). In particular, auxotrophs may fail to induce genes specific to slow growth because they sense high concentrations of natural nutrients even at the slowest dilution rates when their growth is limited by an auxotrophic requirement. One may expect to see such failure in the leucine- and uracil-limited cultures grown by Brauer *et al.* (2008). Because of the glucose wasting, however, those auxotrophic cultures were also at least partially limited on glucose as indicated by the low residual concentration of glucose (below the limit of detection) in the culture media (Brauer *et al.*, 2008). To measure the GRR in auxotrophs independent of glucose depletion, we conducted a series of GR experiments with histidine, lysine, and uracil auxotrophs fed with media containing 2% glucose, four times more glucose than in the Brauer *et al.* (2008) experiments. Like Brauer *et al.* (2008), we observed glucose wasting in our experiments; however, the residual glucose remained high in all cultures and at all growth rates (GRs), such that the cultures were limited only by their auxotrophic requirement and glucose remained in excess.

The results of these experiments showed that the cell size varies substantially in auxotrophs limited for their auxotrophic requirements, indicating a disconnect between biomass increase and cell division. We also found that, in auxotrophs, the magnitude of induction of GRR genes is only 40–50% of the magnitude in prototrophs, signaling dysregulation of the GRR. Furthermore, we found that genes known to be specific to aerobic growth (ter Linde *et al.*, 1999) are expressed at low levels in auxotrophs limited for their auxotrophic requirements. Such genes include most mitochondrial genes (especially the ones coding for mitochondrial ribosomal proteins), all Krebs and glyoxylate cycle genes, isocitrate and alcohol dehydrogenases (*IDP2*, *IDH1*, *IDH2*, *ADH1*, and *ADH2*), and regulatory metabolic enzymes (*ICL1*, *CIT1*, and *FOX2*). Similarly, gene sets up-regulated in anaerobic cultures were also up-regulated in auxotrophs limited for their auxotrophic requirements, including the citrate synthase *CIT2* and seripauperin (*PAU*) genes. These findings help characterize the physiological and transcriptional mechanisms regulating the CDC, GR, and respiration.

RESULTS

Experimental design

To explore how lysine and histidine auxotrophs adapt or fail to adapt their growth and metabolism to changes in the doubling time that are controlled by the levels of their auxotrophic requirements, we grew and characterized continuous cultures of *his3* and *lys2* non-reverting mutants. The amino acids histidine and lysine were chosen to expand the scope of profiled auxotrophs limited for their auxotrophic requirements. Also, histidine limitation was chosen as a common condition with previous work (Boer *et al.*, 2008). To avoid

complications from glucose wasting, the chemostat feed media had 20 g/l glucose to ensure excess residual glucose in the cultures even when the cultures waste glucose. The media were supplemented with the limiting concentrations of the auxotrophic requirements (amino acids); see *Materials and Methods*. The GRs were the same as in the experiments by Brauer *et al.* (2008), $\mu = 0.05, 0.10, 0.15, 0.20, 0.25, 0.30 \text{ h}^{-1}$ with corresponding doubling times of the cultures, $\ln(2)/\mu = 13.8, 6.9, 4.6, 3.5, 2.8, 2.3 \text{ h}$. For each steady-state culture, we measured cell density, distribution of cell sizes, residual glucose, generated ethanol, and gene expression.

Glucose consumption and ethanol production

At slow dilution rates, the rate of influx of fresh media is low and the cells spend more time in the reaction vessels. In addition, the concentration of the limiting auxotrophic requirement in the chemostat vessel is low, resulting in stringent limitation, which is likely to cause more glucose wasting. All of these three factors suggest that the concentration of residual glucose should be inversely correlated to the GR of the cultures, which is in fact what was experimentally observed (Figure 1A). The cultures have large specific consumption of glucose because they ferment it to ethanol (Figure 1B). However, the residual glucose concentration is high even at the slowest dilution rate ($\mu = 0.05 \text{ h}^{-1}$), and thus the growth of the cultures is not likely to be limited by glucose. The same three factors that result in low concentration of glucose at slow GR suggest that the concentration of generated ethanol in the culture media should be high at slow growth and decrease as GR increases. In agreement with this expectation, we measured monotonically decreasing ethanol concentrations with increase in GR (Figure 1B).

In contrast, Brauer *et al.* (2008) measured increasing ethanol concentrations with increase in the GR of the cultures limited by leucine or uracil. This difference likely arises from glucose depletion in the leucine and uracil cultures grown by Brauer *et al.* (2008), especially at the slowest GRs. To test whether this is indeed the case, we measured ethanol in the uracil- and leucine-limited cultures grown by Boer *et al.* (2010) with 22 g/l glucose in the feed media. The results (Figure 1C) indicate the expected trend: ethanol concentrations decrease monotonically with GR.

Failure to coordinate biomass increase and cell division

The steady-state cell densities of all auxotrophs limited for their auxotrophic requirements (Figure 1D) decrease with increasing GR, similar to the results for prototrophic cultures whose growth is limited by natural nutrients (Brauer *et al.*, 2008) and consistent with theoretical expectations (Slavov and Botstein, 2010, 2011). In contrast to cell density, the cell-size distributions for all three studied auxotrophs (Figure 2, A–C) differ markedly from the cell-size distributions for prototrophic cultures whose growth is limited by natural nutrients; for comparison, Figure 2D shows the results for a glucose-limited prototroph, and more data across more conditions can be found in our previous publications (Brauer *et al.*, 2008; Slavov and Botstein, 2011). The cell-size distributions for all auxotrophs and for most GRs are distinctively bimodal and broader than the distributions for the natural limitations (Figure 2). For all auxotrophs, the median cell sizes and the bimodality increase with the increase in steady-state GRs. These GR trends likely reflect two factors. First, the mean residence time for a cell in the chemostat vessel is inversely proportional to the GR. Thus the slower the GR, the longer the cells are exposed to abundant natural nutrients without adequate biomass synthesis and the more likely they are to divide without having reached a normal size. Second, increasing the GR of the cultures increases the residual concentrations of the limiting auxotrophic

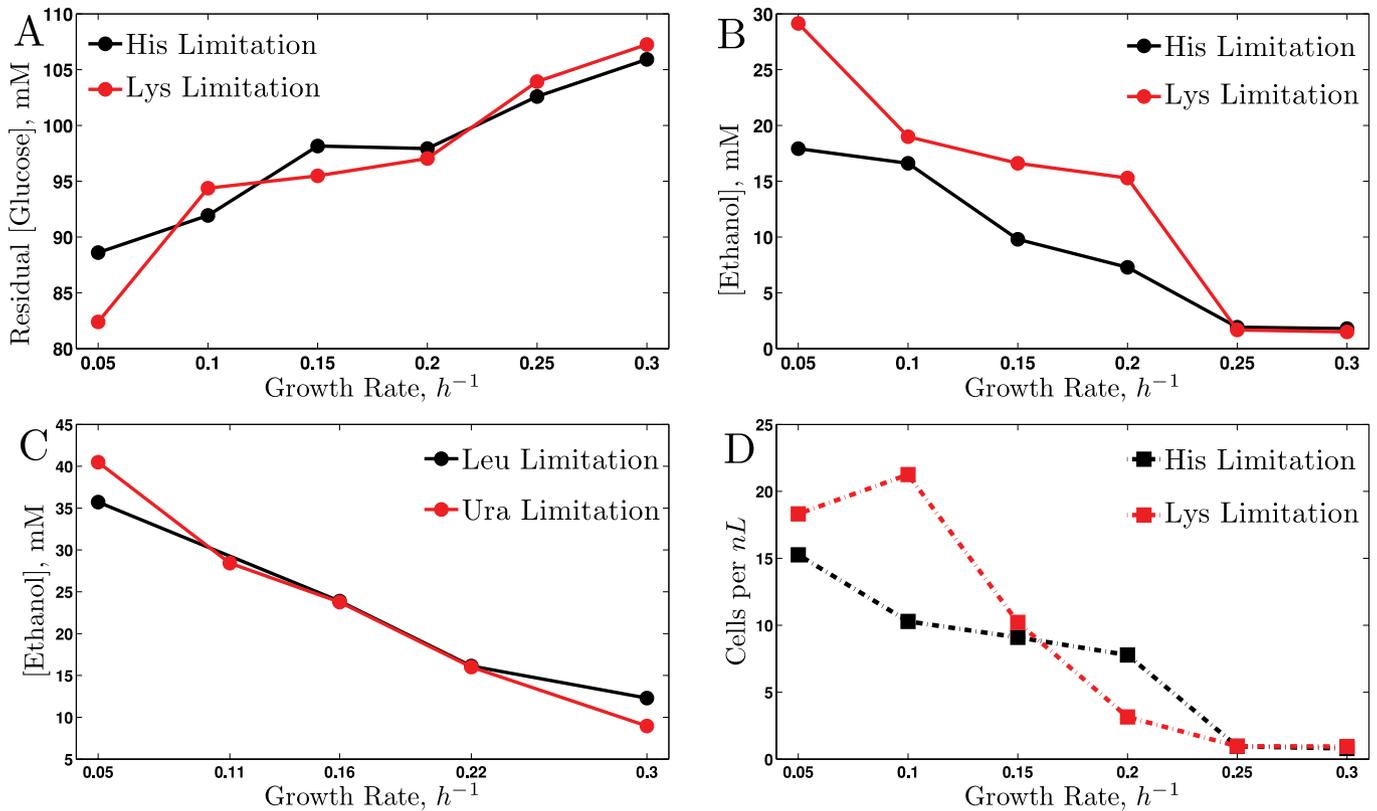


FIGURE 1: Physiological data during steady-state growth. (A) Glucose and (B) ethanol concentrations in the chemostat vessels of *his3* and *lys2* auxotrophs fermenting glucose to ethanol. (C) Ethanol concentrations in *leu* and *ura* auxotrophs grown by Boer *et al.* (2010). (D) Cell density of *his3* and *lys2* auxotrophs.

requirements and thus decreases the severity of the limitation. Conversely, the lower the GR, the more severe the limitation and the larger the fraction of small cells.

Next we sought to test whether the failure to coordinate cell growth and division that we observed in continuous cultures, manifested by decreasing cell sizes by increased severity of the auxotrophic limitation, is also present in batch cultures of auxotrophs limited for their auxotrophic requirements and whether this failure is general to strains with different genetic background (Figure 3). For batch growth, decreasing the GR and increasing the severity of the limitation correspond to increased time spent in the batch cultures. Given the low viability of starving auxotrophs measured by Boer *et al.* (2008), we limited the time courses of batch growth to 4 d because a large fraction of the cells are likely to have lost viability in longer starvation experiments. The earlier time points correspond to the higher GRs in the continuous cultures, and the later time points (days 3 and 4) correspond to the low dilution rates in the continuous cultures. The trend in these batch experiments (Figure 3), for both genetic backgrounds tested, is consistent with the trend from the chemostat experiments: as time progresses, the severity of the starvation increases, and so does the fraction of small cells. We conclude that, both in batch and in continuous cultures, sensing extracellular natural nutrients is essential for the coordination of cell growth and division (Johnston *et al.*, 1977; Jorgensen *et al.*, 2002) and this coordination is substantially perturbed when GR signaling is misregulated and decoupled from actual increase in biomass.

Transcriptional data

The gene expression data for the histidine- and lysine-limited cultures are clustered hierarchically together with the gene expression

data from prototrophs whose growth is limited by glucose or ammonium (Figure 4). The clustering pattern indicates that auxotrophs and prototrophs differ both in the mean expression levels of sets of genes and in the GR trends. The prototrophic limitations show a pronounced GR trend as described by Brauer *et al.* (2008) and Slavov and Botstein (2011). Strikingly, this trend is not as pronounced in the auxotrophic limitations. This observation is consistent with our hypothesis that the signaling networks mediating the GRRs are activated by natural nutrients but may not be activated to the same extent by nutrients made necessary by mutations.

Although the expression levels of most genes are similar between the two auxotrophs, there are significant differences in the mean expression levels of genes between the auxotrophic and prototrophic limitations (Figure 4). The first cluster of such genes, expressed more highly in the prototrophs, is strongly enriched for mitochondrial genes, redox functions, and respiration. The repression of those genes in auxotrophs likely reflects the shift from respiration to fermentation (Brauer *et al.*, 2008). The second cluster of differentially expressed genes, expressed more highly in the auxotrophs, is strongly enriched for genes from amino acid metabolism, biosynthesis, and generation of nucleotides and precursor metabolites. Amino acid-related genes tend to be expressed most highly in the slowest growing auxotrophic cultures, which likely reflects the lower concentration of the limiting amino acids (higher severity of the limitation) at low dilution rates. The third large cluster of differentially expressed genes, expressed more highly in the prototrophs, is strongly enriched for autophagy and vacuolar genes (Figure 4). This observation suggests that auxotrophs fail to induce the expression of autophagy genes to wild-type levels even at slow growth, perhaps because they sense high concentrations

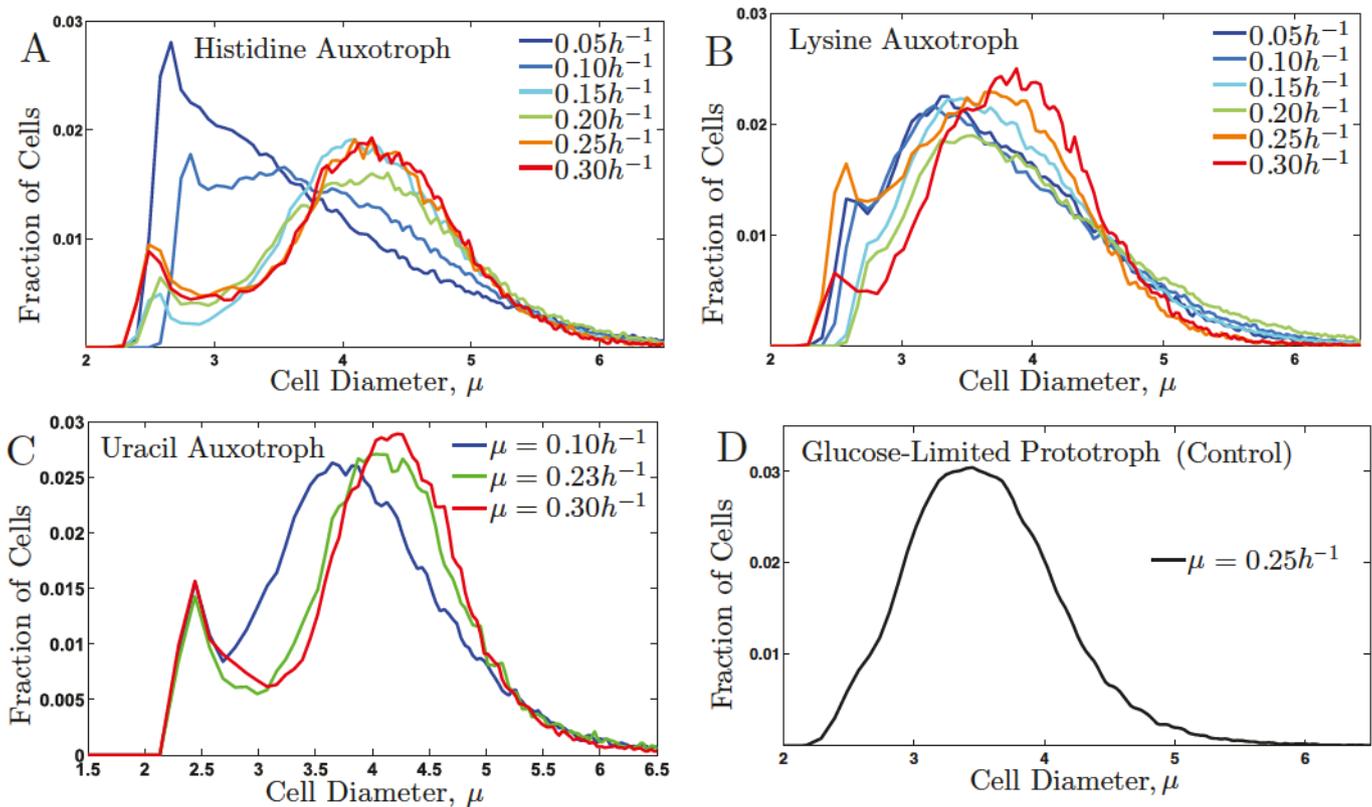


FIGURE 2: Cell size distributions of continuous chemostat cultures. (A) *his3* auxotroph at GRs: $\mu = \{0.05, 0.10, 0.15, 0.20, 0.25, 0.30\} h^{-1}$; (B) *lys2* auxotroph at GRs: $\mu = \{0.05, 0.10, 0.15, 0.20, 0.25, 0.30\} h^{-1}$; (C) *ura* auxotroph at GRs: $\mu = \{0.10, 0.23, 0.30\} h^{-1}$; (D) prototroph (DBY10085, isogenic to the uracil auxotroph except for the *ura* mutation) growing at $\mu = 0.25 h^{-1}$.

of all the natural nutrients and are unable to transduce the low concentrations of auxotrophic requirements into GR regulatory responses.

Quantifying the GRR

To identify and quantify monotonic GR trends in gene expression, we regressed gene expression levels on GR and computed GR slopes (Brauer *et al.*, 2008; Airolidi *et al.*, 2009; Slavov and Botstein, 2011); see *Materials and Methods* for details. Applying this analysis to the GRRs of prototrophic cultures whose growth is limited by

natural nutrients, we previously identified a core set of 1500 genes that either increase or decrease with GR, at steady state, in a similar way across all studied nutrient limitations and sources of carbon and energy (Slavov and Botstein, 2011). We termed this condition-independent response to changes in the steady-state GR “universal growth-rate response (UGRR).” Next we explore the extent to which the auxotrophic limitations induce the UGRR by comparing the distributions of GR slopes for UGRR genes between cultures whose growth is limited either by auxotrophic requirements or by natural nutrients.

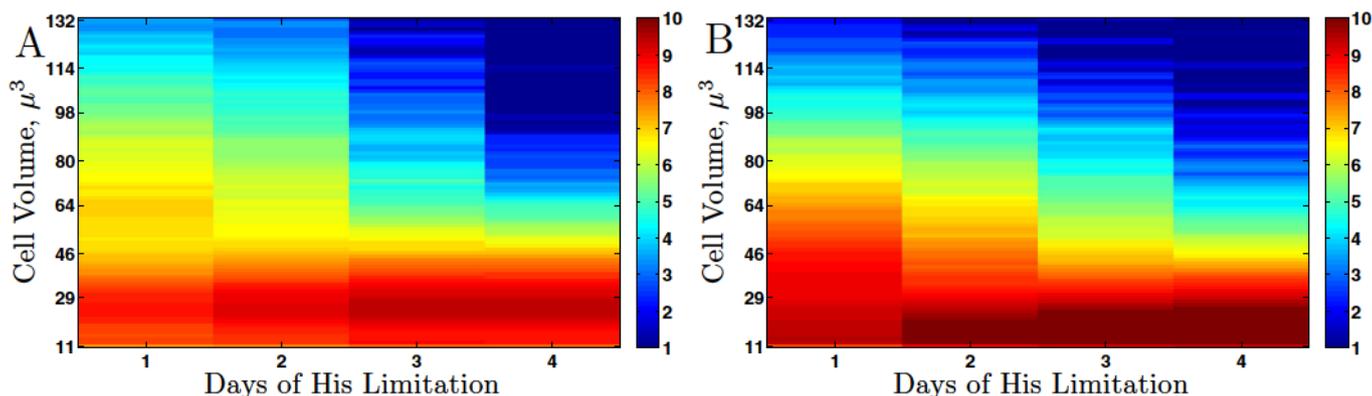


FIGURE 3: Cell size distributions of batch cultures. Cell size distributions in batch cultures of *his3* auxotrophs with *CEN.PK* background (DBY9496) (A) and with *S288c* background (DBY12029) (B). To display the full dynamical range, the data are shown on \log_2 scale so that bright red 10 corresponds to $2^{10} = 1024$ cells and deep blue 2 corresponds to $2^2 = 4$ cells.

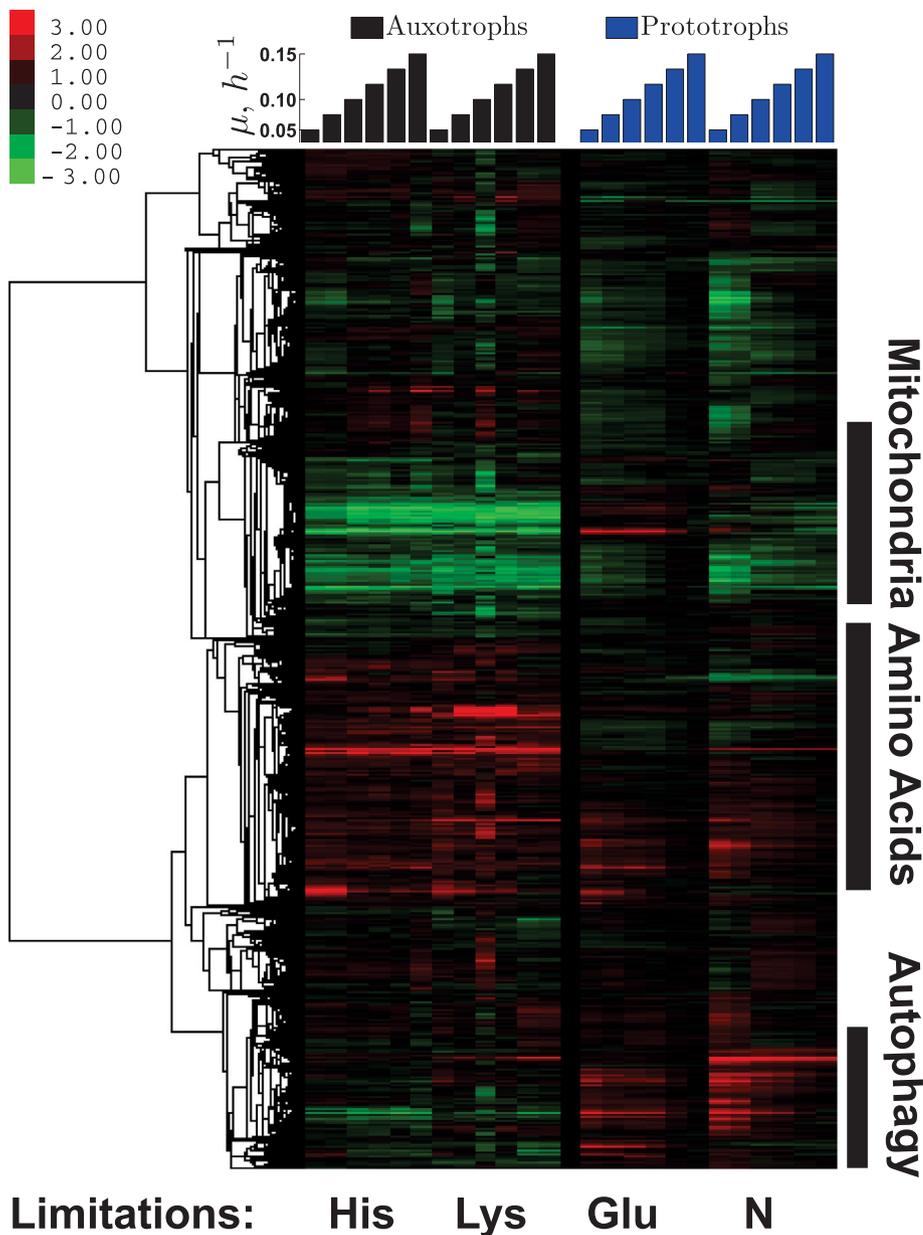


FIGURE 4: Transcriptional response to changes in growth rate and nutrient limitations. Hierarchically clustered gene expression data from our continuous cultures of *his3* limited for His and *lys2* limited for Lys (left set of columns) and from a prototroph limited either for glucose (Glu) or for ammonium (N; right set of columns) (Brauer *et al.*, 2008). The similarity metric used for clustering (noncentered, variance normalized correlations) is computed using all data shown in the clustergram. The data are displayed as fold changes, relative to the reference (a glucose-limited culture growing at $\mu = 0.25 \text{ h}^{-1}$), on a \log_2 scale.

Weaker induction of the UGRR

Our hypothesis is that auxotrophs forced to grow slowly because of the shortage of a nutrient made necessary by a mutation may not be able to induce sufficiently the genes that are highly expressed when growth is limited by natural nutrients, as observed in the UGRR. To test this hypothesis, we quantified the magnitude of the UGRR. The distributions of GR slopes for the prototrophic limitations (glucose and ammonium) are shown in Figure 5A, with genes having positive (red distribution with mean slope 4.5) and negative (green distribution with mean slope -5.0) UGRR. The corresponding distributions of slopes for the histidine and lysine auxotrophs show similar qualitative trends but with significantly smaller magnitudes (Figure 5B). Some of

the genes with positive UGRR have negative GR slopes, and the mean GR slope is only 1.9. Similarly, genes with negative UGRR have weaker induction in the auxotrophs, with a mean GR slope of -1.9 . The ratios of the mean GR slopes for the genes with positive and negative UGRR in auxotrophs and prototrophs (1.9/4.5 and $-1.9/-5.0$) indicate that on average the induction of the UGRR in auxotrophs is more than twofold weaker than the induction in prototrophs.

Autophagy and ribosomal genes are among the most significantly overrepresented functional groups among the genes with UGRR (Slavov and Botstein, 2011). However, not all ribosomal and autophagy genes have UGRR. To test the possibility that auxotrophs respond to GR changes by inducing a different set of ribosomal and autophagy genes, we compare the distributions of GR slopes for all genes annotated as autophagy or ribosomes by the gene ontology (Figure 5, C–E). The comparison indicates that the weaker induction of the UGRR is general to all autophagy and ribosomal genes. Furthermore, the induction of the UGRR (as quantified by the distributions of GR slopes of ribosomal and autophagy genes) is also weaker for the leucine and uracil limitations grown by Brauer *et al.* (2008), suggesting that weaker induction of the UGRR may be general to auxotrophic limitations. The difference between the GR slope distributions of histidine- and lysine-limited cultures growing in 2% glucose and the corresponding distributions for leucine and uracil cultures growing in 0.5% glucose media is likely due to the fact that the leucine and uracil cultures were also in part limited on a natural nutrient, glucose.

These differences in the GR slopes between different types of growth conditions raise the question whether the GR slopes are similar within each type of growth conditions. In other words, is the magnitude of the UGRR (as measured by GR slopes) characteristic of the type of growth conditions (auxotrophic vs. prototrophic limitations), or is it specific to each limiting nutrient (*His* vs. *Lys*)? To answer this question, we focused on autophagy

genes with negative UGRR and two types of growth conditions: 1) auxotrophic limitations for His and Lys in 2% glucose and 2) prototrophic limitations for glucose and ammonium. Plotting the slope distributions of autophagy genes for the two types of growth conditions (Figure 6A) recapitulated the results from Figure 5. Consistent with the up-regulated growth signal from the TOR network, autophagy genes have significantly smaller GR in auxotrophs (Figure 6A). The same difference in induction of autophagy genes at slow growth can be represented as a plot of rank-ordered (sorted) slopes (Figure 6B). We then compared the distribution of slopes within each type of growth condition (Figure 6C). The distributions of slopes for the His and the Lys limitations are statistically indistinguishable from

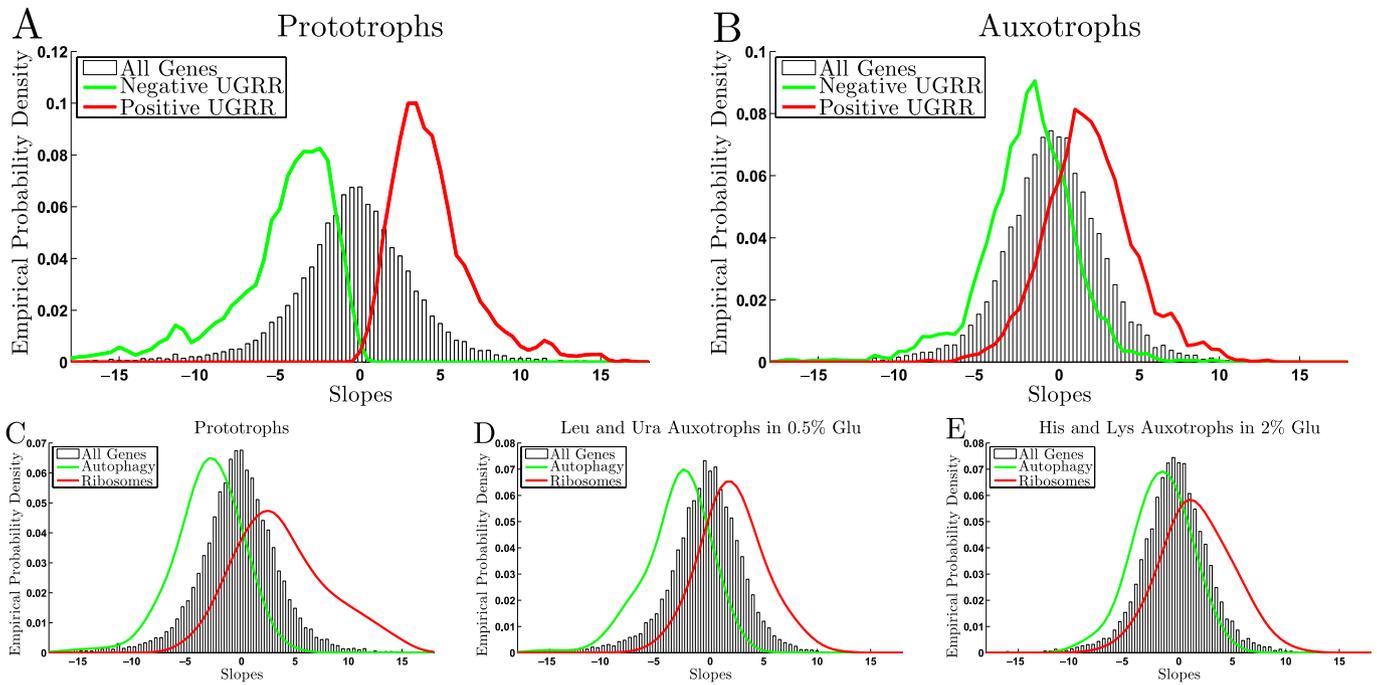


FIGURE 5: Weaker GRR of auxotrophs compared to prototrophs. GR slopes of the genes with UGRR in glucose and nitrogen limitations (A); histidine and lysine limitations (B). GR slopes of autophagy (green) and ribosomal (red) genes in glucose and nitrogen limitations (C); leucine and uracil limitations (D); histidine and lysine limitations (E);

one another, as indicated by the large probability (computed from a rank sum test) that they come from the same distribution. Similarly, the distributions of slopes for the glucose and the ammonium limitation are statistically indistinguishable from one another, reinforcing the conclusion that the magnitude of the GRR is the same within each type of growth condition. In contrast, any of the auxotrophic limitations has significantly weaker GRR compared with any of the prototrophic limitations. The *within*-group similarity and *between*-group difference indicate that the magnitude of the UGRR is characteristic of the nature of the limitations, natural nutrient, or auxotrophic requirement. The difference between the GRR of autophagy genes may be a reflection of the weaker induction of the autophagy genes at slow growth (Figure 6D).

Next we applied the same type of GR slope comparison to the peroxisomal genes, one of the functional groups of genes with common negative GRR in glucose carbon source and positive GRR in ethanol carbon source (Brauer *et al.*, 2008; Slavov and Botstein, 2011). The results (Figure 7A) indicate the same trend of reduced GR induction in the auxotrophs. Similar to the autophagy genes, the GRR of peroxisomal genes is characteristic of the nature of the limitation (Figure 7A) and may be attributed at least in part to reduced induction of peroxisomal genes in slowly growing ($\mu = 0.05 \text{ h}^{-1}$) auxotrophs (Figure 7B).

Anaerobic transcriptional response

Despite high oxygen levels (85–95% of the saturation level) in the growth media, the auxotrophic cultures fermented glucose to ethanol (Figure 1), a phenomenon known as aerobic glycolysis. This aerobic glycolysis is also reflected in the transcriptional response as exemplified by the lower expression levels of mitochondrial genes found from the cluster analysis of the hierarchically clustered gene expression data (Figure 4). We sought to expand this anaerobic transcriptional signature by identifying and quantifying the expression of sets of genes whose transcriptional responses reflect the aerobic glycolysis. To quantify the difference in the expression of

mitochondrial genes, we plotted the distributions of fold changes for 1) auxotrophs (our cultures limited for His and Lys) fed by media containing 2% glucose as a sole source of carbon and energy, for 2) prototrophs (the cultures limited for glucose and ammonium) growing on glucose as a sole source of carbon and energy, and for 3) prototrophs (the cultures limited for ethanol and ammonium) growing on ethanol as a sole source of carbon and energy (Figure 8, A and B). All sets of mitochondrial genes turned out to have the highest expression in ethanol carbon source, lower expression in the prototrophs growing in glucose carbon source, and the lowest expression in the auxotrophs. These differences in expression were greatest for the mitochondrial ribosomal proteins (Figure 8A) that have UGRR (Slavov and Botstein, 2011) and were also found for other mitochondrial genes as exemplified in Figure 8A with the mitochondrial envelope genes.

We explored the expression of other sets of genes for which Ter Linde *et al.* (1999) reported differential regulation between aerobic and anaerobic steady-state chemostat cultures. Genes which are expressed at higher levels in anaerobic cultures, such as the seripaperin (PAU) genes (Rachidi *et al.*, 2002; Hickman *et al.*, 2011), were also expressed at higher levels in the auxotrophs relative to the prototrophs (Figure 9A). The PAU genes constitute a large family of genes with highly homologous sequences. These genes are induced in anaerobic conditions and during alcoholic fermentation. Although the microarrays are optimized to minimize cross-hybridization, it could contribute to the detected signal. Figure 9A shows that all PAU genes detected unambiguously by the microarrays (*PAU1*, *PAU2*, *PAU3*, *PAU4*, *PAU5*, *PAU6*, and *PAU7*) are induced in the auxotrophs relative to the prototrophs.

Conversely, genes which are expressed at lower levels in anaerobic cultures are also expressed at lower levels in the auxotrophs relative to the prototrophs. Such genes include metabolic enzymes and the *HAP* transcription factors (Figure 9B). We also found that all genes from the tricarboxylic acid (TCA) cycle were strongly down-regulated in the auxotrophs (Figure 9C). This down-regulation is

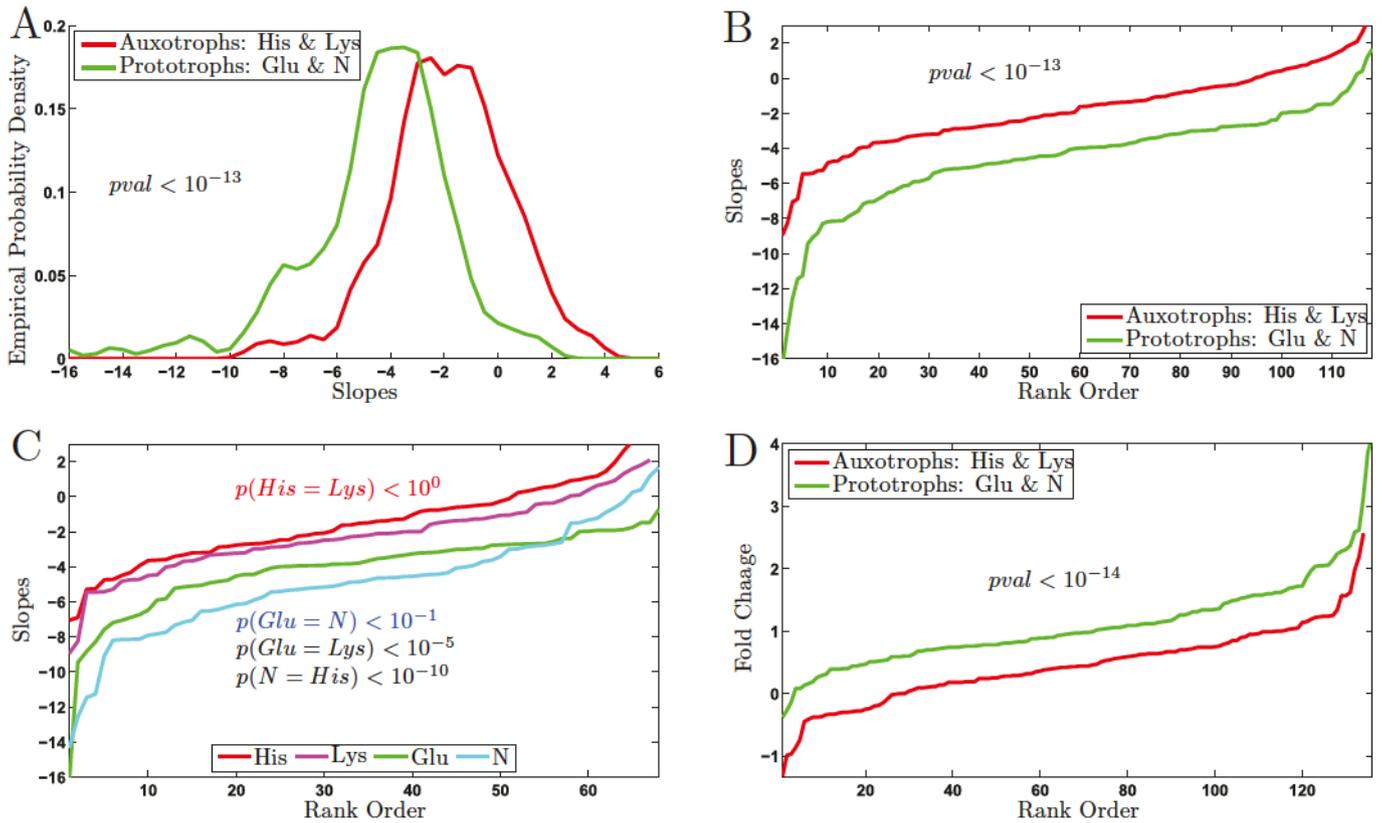


FIGURE 6: The GR slopes and fold changes of autophagy genes differ between auxotrophs and prototrophs. (A) Distributions of slopes for autophagy genes in auxotrophs and in prototrophs. (B) Rank orders of GR slopes for autophagy genes in auxotrophs and in prototrophs. (C) The data from panel B are presented separately for each growth condition and strain, demonstrating that GRR are similar within and different between auxotrophs and prototrophs. (D) Rank orders of fold changes for autophagy genes in auxotrophs and in prototrophs. The expression data for all strains are shown only for the slowest growth rate $\mu = 0.05 \text{ h}^{-1}$. The statistical significance of the difference between the distributions is quantified by a nonparametric rank sum test.

reminiscent of the down-regulation of these genes in the first (fermentative) phase of the diauxic shift (Brauer *et al.*, 2005). The down-regulation of TCA genes likely reflects a major shift toward fermentative metabolism in the auxotrophs, which was not seen in the prototrophs growing at the same GRs (Figure 9C). ter Linde *et al.* (1999) found that the peroxisomal citrate synthase *CIT2* is up-regulated in anaerobic conditions, and we find that this gene is

also up-regulated in the auxotrophic limitations (Figure 9C). Thus, in addition to mitochondrial genes, we find that many other genes known to be regulated differently between aerobic and anaerobic cultures (ter Linde *et al.*, 1999) are also expressed differentially between the auxotrophic and the prototrophic limitations, forming an extensive expression signature characteristic of anaerobic/fermentative growth and metabolism (Figure 9). This expression signature is

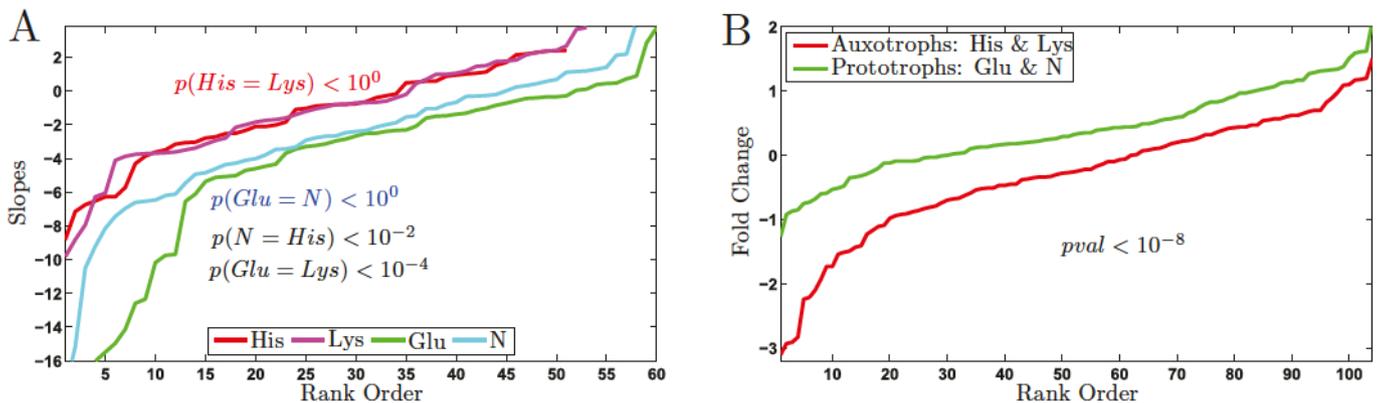


FIGURE 7: Peroxisomal genes. (A) Rank orders of GR slopes for peroxisomal genes in the auxotrophs and in the prototrophs. (B) Rank orders of fold changes for peroxisomal genes in the auxotrophs and in the prototrophs. The expression data for all strains are shown only for the slowest GR $\mu = 0.05 \text{ h}^{-1}$. The statistical significance of the difference between the distributions on both panels is quantified by a nonparametric rank sum test.

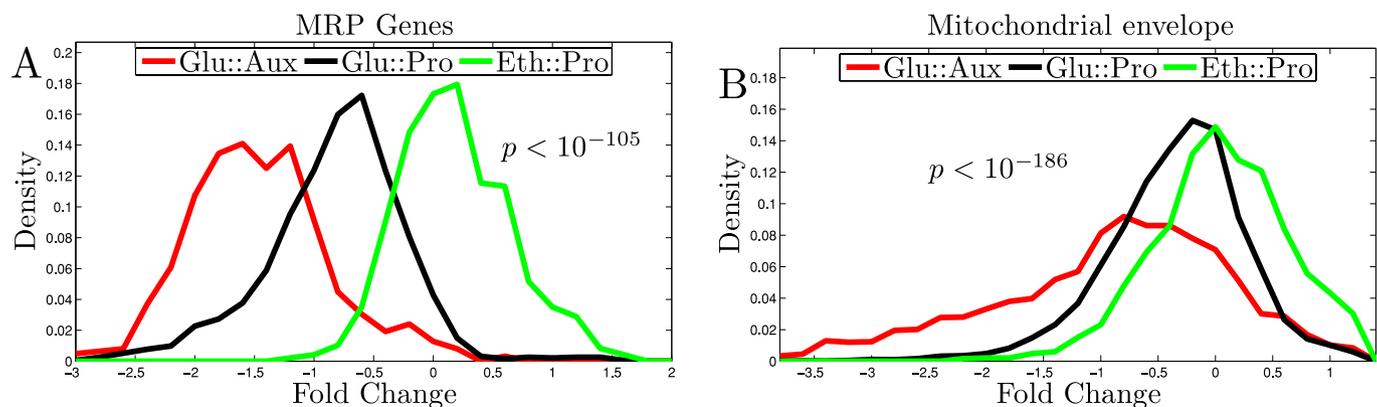


FIGURE 8: Down-regulation of mitochondrial genes in the auxotrophs. Distributions of \log_2 fold changes for mitochondrial ribosomal protein (MRP) genes (A) and for mitochondrial envelope genes (B) in auxotroph cultures grown on glucose carbon source (Glu::Aux), prototroph cultures grown on glucose carbon source (Glu::Pro), and prototroph cultures grown on ethanol carbon source (Eth::Pro). The statistical significance of the difference between the distributions on both panels is quantified by a rank sum test.

consistent with the report by Petti *et al.* (2011) that stationary cultures of auxotrophs consume less oxygen than do stationary cultures of prototrophs; it is harder to reconcile with the report by Basso *et al.* (2010) that uracil auxotroph whose growth-rate ($\mu = 0.1 \text{ h}^{-1}$) is limited by uracil consumes two times more oxygen than the prototrophic strain whose GR is the same and limited by the carbon source.

Despite the many transcriptional similarities between the auxotrophs limited for their auxotrophic requirements and anaerobic cultures, there are some differences. For example, as shown in Figure 9A, most ergosterol biosynthesis genes (ERGs) are induced in the auxotrophs (especially *ERG1*, *ERG2*, *ERG3*, *ERG7*, *ERG8*, *ERG11*, *ERG24*, *ERG25*, *ERG27*) but some of these genes (such as *ERG2* and *ERG11*) are down-regulated in anaerobic conditions by Hap1-mediated repression (Hickman and Winston, 2007). This difference between the expression of ERG genes during aerobic and anaerobic glycolysis most likely reflects the effect of oxygen on Hap1 (Hickman and Winston, 2007) and the fact that oxygen is required for the biosynthesis of ergosterol. Thus at least some regulatory mechanisms differ between aerobic and anaerobic glycolysis whereas many transcriptional responses are highly similar.

DISCUSSION

Decoupling the GRS from the GR

The evidence described strongly supports our hypothesis that growth under the limitation for an auxotrophic requirement decouples the GRS, which in yeast is a function of the concentrations of natural nutrients present in the growth medium, from the actual biomass production, which is fundamentally limited by the auxotrophic requirement. Under these growth conditions, we found that the induction of the UGRR (Slavov and Botstein, 2011) is incomplete. This finding suggests that the GRR is at least partially a consequence of nutrient sensing integrated into a common GRS that ultimately results in the induction of the UGRR. The integration of the nutrient signals into a GRS—the signal inducing the GRR—likely involves the TOR because mutations inactivating TOR signaling mitigate phenotypes (glucose wasting and reduced viability) resulting from the decoupling of growth and GR signaling (Boer *et al.*, 2008).

The decoupling of the GR from the concentrations of natural nutrients allows us to distinguish between two possible mechanisms of transcriptional and GR regulation: 1) the sensing of extracellular nutrients and 2) the sensing of the availability of intracellular metabolites and energy. Levy *et al.* (2007) and Levy and Barkai (2009) attempted

to distinguish between these two mechanisms by using either the temporal sequence of adaptations to perturbations or a deletion mutant of *ADH1* to decouple GR from nutrient sensing. On the basis of the results, Levy *et al.* (2007) concluded that the gene expression is regulated by sensing external stimuli, in other words, mechanism 1. In contrast, Boer *et al.* (2010) measured hundreds of metabolites in continuous steady-state cultures limited on different nutrients and growing over a range of growth rates and used the data to identify intracellular metabolites limiting the GR for each condition, in other words, mechanism 2. Our data are consistent with and support both mechanisms for regulation of gene expression and GR. The differences in the physiological behavior (fermentation vs. respiration) and the gene expression patterns (including the substantially weaker GRR in auxotrophs) indicate that sensing extracellular natural nutrients (mechanism 1) plays an important role in setting the GR and the appropriate gene expression response. Such sensing of glucose is extensively studied (Youk and Van Oudenaarden, 2009; Broach, 2012), and our observations that diverse natural nutrients and carbon sources induce a similar GRR (Slavov and Botstein, 2011) suggest a general role for the sensing of a variety of natural nutrients in setting the appropriate GR and gene levels. At the same time, the finding that most GR-responsive genes in the auxotrophs limited for their auxotrophic requirements have qualitatively the same type of GRR as they do in prototrophs, even if quantitatively much weaker, supports mechanism 2; internal regulatory feedback loops that sense intracellular metabolites, such as amino acid sensing by the TOR, also play a role in regulating gene expression and the GR.

Viability and the induction of autophagy at slow growth

It has long been known that mutants deficient in autophagy have lower viability, and the induction of autophagy is required for preserving viability at slow growth (Tsukada and Ohsumi, 1993; Takeda *et al.*, 2010). Recently Gresham *et al.* (2010) conducted a genome-wide, systems-level experiment demonstrating that the deletion of autophagy genes results in reduced viability during starvation. We found here that auxotrophs limited for their auxotrophic requirements express autophagy genes at significantly lower levels than prototrophs with the same doubling period, and this decrease likely contributes to the lower viability of auxotrophs limited for their auxotrophic requirements (Boer *et al.*, 2008). More recently, Petti *et al.* (2011) also found that survival during starvation is correlated with expression of autophagy genes. A possibility, reconciling all data, is

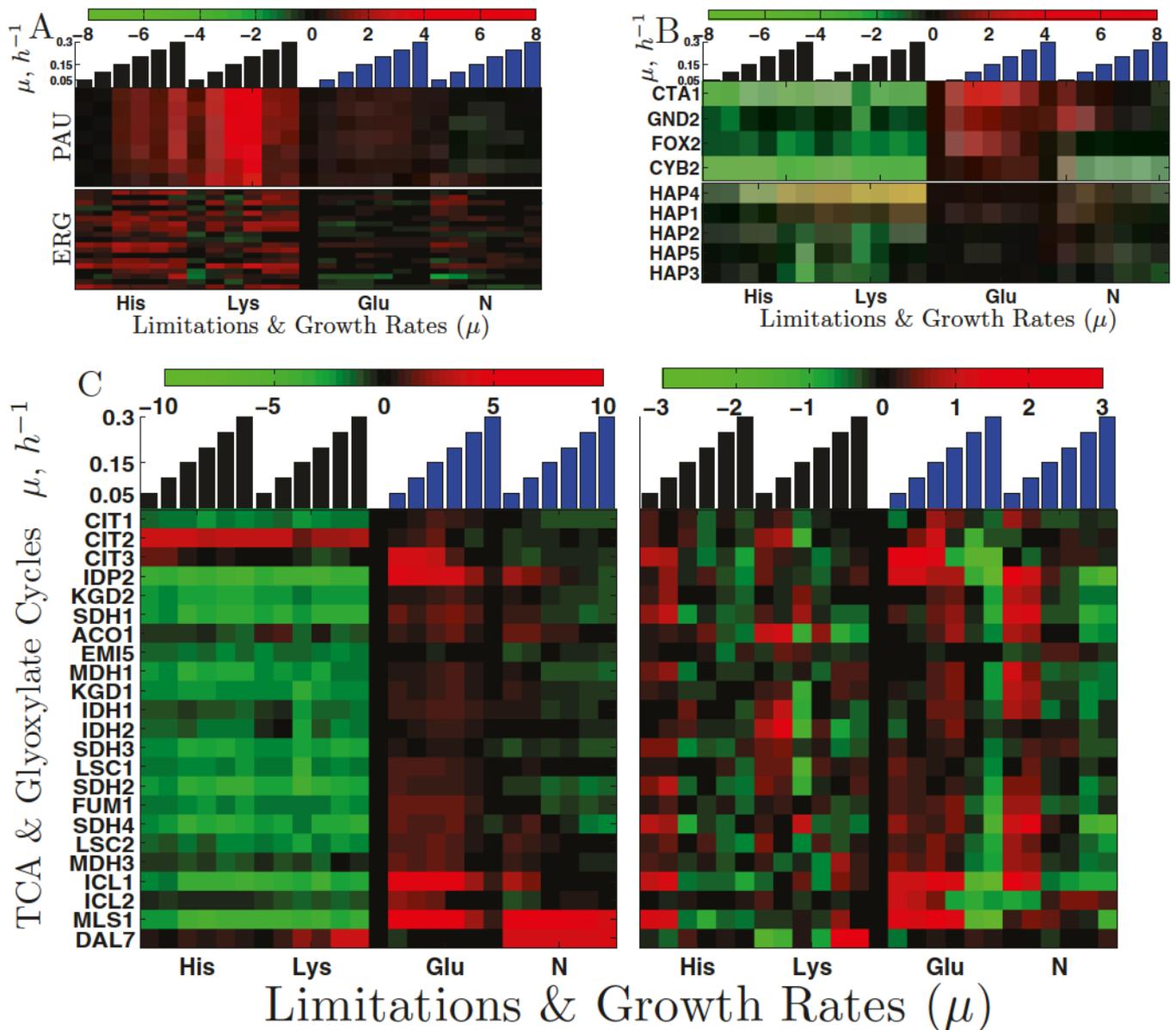


FIGURE 9: Expression signature of fermentative growth and metabolism in auxotrophs. (A) PAU and ergosterol biosynthesis genes. (B) Genes down-regulated in anaerobic conditions. The data are displayed as fold changes, relative to the reference (a glucose-limited culture growing at $\mu = 0.25 \text{ h}^{-1}$), on a \log_2 scale. (C) Enzymes participating in the Krebs and glyoxylate cycles. The left panel shows the fold changes relative to the reference, and the right panel shows zero-centered fold changes to enhance the visibility of the GR trends.

that slowly growing auxotrophs fail to transmit a signal (likely the growth signal involving TOR) for nutrient shortage and thus fail to induce normal autophagy. The mediation of such a signal by the TOR signaling pathway may be one of the reasons why mutations in TOR signaling mitigate the loss of viability in starving auxotrophs (Boer *et al.*, 2008).

The GRS regulates respiration and fermentation

The decoupling of the GRS from growth (biomass production) helps clarify the regulatory role of the GRS in the balance between respiratory and fermentative growth. Brauer *et al.* (2008) discovered that prototrophic cultures growing slowly because of the shortage of a natural nutrient respire even when the residual glucose in the growth media is high. In stark contrast, auxotrophs limited for their auxotrophic requirements ferment at high rates at all GRs, in the

experiments reported both here and elsewhere (Boer *et al.*, 2008; Brauer *et al.*, 2008; Gresham *et al.*, 2010). These results show that, in all studied cases when the GRS is high (because of abundant natural nutrients), yeast catabolizes glucose primarily by fermentation. Conversely, as the GRS is reduced (because of the shortage of a natural nutrient), yeast catabolizes glucose increasingly by oxidative phosphorylation. In addition to confirming the physiological phenotypes associated with strong GRS in auxotrophs, we find a strong and clear transcriptional signal for anaerobic metabolism.

The reduced expression of mitochondrial and TCA genes is an expression signature reminiscent of the first phase of diauxic growth (Brauer *et al.*, 2005) and a clear signal for transcriptional regulation favoring fermentation and not respiration. This conclusion is further reinforced by an extensive anaerobic transcriptional signature; genes expressed highly in anaerobic cultures are also expressed

highly in the auxotrophs limited for their auxotrophic requirements. Conversely, genes expressed at low levels in anaerobic cultures are also expressed at low levels in the auxotrophs limited for their auxotrophic requirements (Figures 8 and 9).

We can generalize this regulatory link between the GRS and respiration/fermentation to higher eukaryotes by taking into account that in higher eukaryotes the GRS depends on growth factors. At low GRS, eukaryotic cells tend to primarily respire, with examples including regulated slow growth in late-stage embryos and adult organisms. In contrast, at high GRS, eukaryotic cells tend to primarily ferment, with examples including early embryonic development, cell cultures growing in media containing an excess of growth factors, and cancer cells with up-regulated proliferative signals. The data in this paper and in the literature (Brand and Hermf sse, 1997b; Heiden *et al.*, 2001, 2009; Elstrom *et al.*, 2004; Boer *et al.*, 2008; Brauer *et al.*, 2008; Wise *et al.*, 2008; Slavov and Dawson, 2009; Levine and PuzioKuter, 2010; Cairns *et al.*, 2011; Dang, 2012) are all consistent with the possibility that the cellular regulatory networks are hardwired so that the strength of the GRS regulates the transition between respiratory and fermentative metabolisms, resulting in the observed correlation between aerobic glycolysis and rapid proliferation.

Importantly, however, the correlation between fast growth and aerobic glycolysis does not indicate that respiration, in principle, cannot sustain fast cell growth. To the contrary, some eukaryotes, including Crabtree-negative yeasts, achieve fast cell growth without resorting to aerobic glycolysis (De Deken, 1966). Even budding yeast growing in glucose-limited chemostatic conditions can evolve a substantial reduction in the rate of aerobic glycolysis (Ferea *et al.*, 1999; Gresham *et al.*, 2008; Wenger *et al.*, 2011). Rather, we suggest that the inefficient energy production per glucose molecule during aerobic glycolysis offers advantages to rapidly growing cells. The identity of such trade-offs has remained rather controversial for a century (Warburg *et al.*, 1924; Warburg, 1962; Crabtree, 1929; Newsholme *et al.*, 1985; Brand and Hermf sse, 1997a; Pfeiffer *et al.*, 2001; Heiden *et al.*, 2009; Levine and Puzio-Kuter, 2010; Vazquez *et al.*, 2010; Shlomi *et al.*, 2011; Dang, 2012). Our data support the hypothesis that the induction of aerobic glycolysis is regulated by the GRS and underscores the necessity for further experiments designed to *directly* identify the advantages of fermentation for fast-growing cells, and auxotrophs limited for their auxotrophic requirements may be a good model system for such experiments. Similarly, further work is necessary to investigate the mechanisms behind the striking similarity between the gene expression patterns of our auxotrophic cultures and cultures growing in anaerobic conditions. Whereas the transcriptional responses are similar, the mechanisms of gene regulation in the presence and absence of oxygen may differ (Hickman and Winston, 2007; Hickman *et al.*, 2011). Combining some of the transcriptional differences, such as the expression of ergosterol biosynthesis genes with network inference and other computational algorithms (Tagkopoulos *et al.*, 2005; Slavov, 2010; Petti *et al.*, 2011), can help identify regulators that differ between aerobic and anaerobic glycolysis. Similarly, it would be interesting to investigate whether the regulatory mechanisms involve chromatin remodeling (Rando and Winston, 2012).

Decoupling of cell division and cell growth

The large dispersion (variance) and bimodality in the distributions of cell sizes observed in all studied auxotrophs limited for their auxotrophic requirements suggest a decoupling between biomass production and cell division (Figures 2 and 3). This result likely reflects decoupling between the CDC and the growth cycle of biomass

production (Silverman *et al.*, 2010; Slavov *et al.*, 2011, 2012), possibly resulting from the combination of a strong GRS and insufficient supply of the auxotrophic requirement. This decoupling appears to be a general phenomenon as we observed it in strains having different genetic backgrounds and growing under different auxotrophic limitations. The GR trend in the cell size distributions in Figure 2, as well as in the time courses in Figure 3, suggests that auxotrophs limited for their auxotrophic requirements not only continue budding as discovered by Saldanha *et al.* (2004) and Brauer *et al.* (2008), but may also divide when starved for an auxotrophic requirement, thus circumventing the CDC cell-size control (Nurse, 1975; Johnston *et al.*, 1977, 1979; Jorgensen *et al.*, 2002) and resulting in cells with small sizes. Although the strong GRS sent by abundant nutrients may play a role in this decoupling too, the mechanistic connections are not clear. Furthermore, it is unclear whether the small cells (corresponding to the left mode of the cell-size distribution) are as viable as the large cells (right mode). This dysregulation of cell growth and cell division may be another factor contributing to the lower viability of auxotrophs limited for their auxotrophic requirements.

MATERIALS AND METHODS

Cultures and physiological measurements

For the GR experiments, we used nonreverting mutants *DBY9496* (MATa *his3-1*, MAL2-8c with CEN.PK113 background), *DBY919/GRF80* (MATα *lys2-80*), and *DBY9492* (MATa *ura3-52*, MAL2-8c with CEN.PK113 background). Consistent with the genotypes of the strains, the expression levels measured for *HIS3* in the histidine limitation and for *LYS2* in the lysine limitation are either missing (no transcript detected) or low (more than 30-fold down-regulated relative to the reference). All nonlimiting nutrient concentrations were the same as the ones used by Saldanha *et al.* (2004) and Brauer *et al.* (2005, 2008), with the exception of the glucose, the concentration of which was 20 g/l. All limiting concentrations were first determined as limiting for final biomass in batch growth and then confirmed to be limiting and within the linear response range in continuous cultures. The histidine-limited medium contained [*His*] = 4 mg/l, the lysine-limited medium contained [*Lys*] = 6 mg/l, and the uracil-limited medium contained [*Ura*] = 5 mg/l. Chemostats were established in 500-ml fermenter vessels (Sixfors; Infors AG, Bottmingen, Switzerland) containing 250 ml of culture volume, stirred at 400 rpm, and aerated with humidified and filtered air. Chemostat cultures were inoculated, monitored, and grown to steady state as described previously (Brauer *et al.*, 2005). All cultures were monitored for changes in cell density and dissolved oxygen and grown until these parameters remained steady for at least 24 h. The cell density, cell sizes, and ethanol concentrations were measured as previously described by Slavov and Botstein (2011).

Measuring mRNA levels

To measure RNA levels, we sampled 10–30 ml of steady-state culture and vacuum filtered the cells followed by immediate freezing in liquid nitrogen and then in a freezer at –80°C. RNA for microarray analysis was extracted by the acid-phenol-chloroform method. RNA was amplified and labeled using the Agilent Low RNA Input Fluorescent Linear Amplification Kit (P/N 5184-3523; Agilent Technologies, Palo Alto, CA). This method involves initial synthesis of cDNA by using a poly(T) primer attached to a T7 promoter. Labeled cRNA is subsequently synthesized using T7 RNA polymerase and either Cy3 or Cy5 uridine triphosphate.

Each Cy5-labeled experimental cRNA sample was mixed with the Cy3-labeled reference cRNA and hybridized for 17 h at 65°C to custom Agilent Yeast oligomicroarrays (8 × 15k) having eight microarrays

per glass slide. Microarrays were washed and then scanned with an Agilent DNA microarray scanner (Agilent Technologies). The resulting TIF files were processed using Agilent Feature Extraction Software version 7.5. Resulting microarray intensity data were submitted to the PUMA Database for archiving. The reference for the microarrays was also the same as the one used by Slavov and Botstein (2011): mRNA from a glucose-limited culture of *DBY10085* growing at a dilution rate of $\mu = 0.25 \text{ h}^{-1}$. To download and explore the data interactively, visit http://genomics-pubs.princeton.edu/Aerobic_glycolysis/.

MODELS

Similar to Brauer *et al.* (2008) and Slavov and Botstein (2011), we use an exponential phenomenological model to quantify the dependence between the mRNA level and the GR, $mRNA_i = b_i e^{a_i \mu}$. In semi-log space, such exponential model “appears” linear: $\log(mRNA_i) = \log(b_i) + a_i \mu$. Assuming Gaussian noise, the GR slope can be inferred by least-squares linear regression. All slopes throughout this article are computed based on regression models minimizing the sum of squared residuals. As a measure of goodness of fit, we use the fraction of variance explained by the model, which for the *j*th gene is quantified by R_j^2 :

$$R_j^2 = 1 - \frac{\sum_{i \in \alpha} (y_{ij} - f_{ij})^2}{\sum_{i \in \alpha} (y_{ij} - \bar{y}_j)^2} \quad (1)$$

In Equation 1, \mathbf{y}_j is a vector of expression levels of the *j*th gene (*j*th column in \mathbf{Y}), \bar{y}_j is its mean expression level, *i* is index enumerating the set of conditions α used in the model, and f_{ij} is the model prediction for the *i*th condition and *j*th gene. To assess statistical significance, we use bootstrapped *p* values equal to the fraction of randomly permuted data sets having R^2 equal or greater than the R^2 of the data.

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REFERENCES

Airoldi EM, Huttenhower C, Gresham D, Lu C, Caudy AA, Dunham MJ, Braoch JR, Botstein D, Troyanskaya OG (2009). Predicting cellular growth from gene expression signatures. *PLoS Comput Biol* 5, e1000257.

Basso TO, Dario MG, Tonso A, Stambuk BU, Gombert AK (2010). Insufficient uracil supply in fully aerobic chemostat cultures of *Saccharomyces cerevisiae* leads to respiro-fermentative metabolism and double nutrient-limitation. *Biotechnol Lett* 32, 973–977.

Boer VM, Amini S, Botstein D (2008). Influence of genotype and nutrition on survival and metabolism of starving yeast. *Proc Natl Acad Sci USA* 105, 6930–6935.

Boer VM, Crutchfield CA, Bradley PH, Botstein D, Rabinowitz JD (2010). Growth-limiting intracellular metabolites in yeast growing under diverse nutrient limitations. *Mol Biol Cell* 21, 198–211.

Brand K, Hermsse U (1997a). Aerobic glycolysis by proliferating cells: a protective strategy against reactive oxygen species. *FASEB J* 11, 388–395.

Brand KA, Hermsse U (1997b). Aerobic glycolysis by proliferating cells: a protective strategy against reactive oxygen species. *FASEB J Off Public Federat Am Soc Exp Biol* 11, 388–395.

Brauer MJ, Huttenhower C, Airoldi EM, Rosenstein R, Matese JC, Gresham D, Boer VM, Troyanskaya OG, Botstein D (2008). Coordination of growth

rate, cell cycle, stress response, and metabolic activity in yeast. *Mol Biol Cell* 19, 352–367.

Brauer MJ, Saldanha AJ, Dolinski K, Botstein D (2005). Homeostatic adjustment and metabolic remodeling in glucose-limited yeast cultures. *Mol Biol Cell* 16, 2503–2517.

Braoch J (2012). Nutritional control of growth and development in yeast. *Genetics* 192, 73–105.

Cairns RA, Harris IS, Mak TW (2011). Regulation of cancer cell metabolism. *Nat Rev Cancer* 11, 85–95.

Crabtree H (1929). Observations on the carbohydrate metabolism of tumours. *Biochem J* 23, 536–545.

Dang C (2012). Links between metabolism and cancer. *Genes Dev* 26, 877–890.

De Deken R (1966). The Crabtree effect: a regulatory system in yeast. *J General Microbiol* 44, 149–156.

Elstrom R *et al.* (2004). Akt stimulates aerobic glycolysis in cancer cells. *Cancer Res* 64, 3892–3899.

Ferea T, Botstein D, Brown P, Rosenzweig R (1999). Systematic changes in gene expression patterns following adaptive evolution in yeast. *Proc Natl Acad Sci USA* 96, 9721.

Gresham D, Boer V, Caudy A, Ziv N, Brandt NJ, Storey JD, Botstein D (2010). System-level analysis of genes and functions affecting survival during nutrient starvation in *Saccharomyces cerevisiae*. *Genetics* 187, 299–317.

Gresham D, Desai M, Tucker C, Jenq H, Pai D, Ward A, DeSevo C, Botstein D, Dunham M (2008). The repertoire and dynamics of evolutionary adaptations to controlled nutrient-limited environments in yeast. *PLoS Genetics* 4, e1000303.

Hedbacker K, Carlson M (2008). SNF1/AMPK pathways in yeast. *Front Biosci J Virt Libr* 13, 2408.

Heiden MG, Cantley LC, Thompson CB (2009). Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* 324, 1029–1033.

Heiden MG, Plas DR, Rathmell JC, Fox CJ, Harris MH, Thompson CB (2001). Growth factors can influence cell growth and survival through effects on glucose metabolism. *Mol Cell Biol* 21, 5899–5912.

Hickman M, Spatt D, Winston F (2011). The Hog1 mitogen-activated protein kinase mediates a hypoxic response in *Saccharomyces cerevisiae*. *Genetics* 188, 325–338.

Hickman M, Winston F (2007). Heme levels switch the function of Hap1 of *Saccharomyces cerevisiae* between transcriptional activator and transcriptional repressor. *Mol Cell Biol* 27, 7414–7424.

Johnston G, Ehrhardt C, Lorincz A, Carter B (1979). Regulation of cell size in the yeast *Saccharomyces cerevisiae*. *J Bacteriol* 137, 1–5.

Johnston G, Pringle J, Hartwell L (1977). Coordination of growth with cell division in the yeast *Saccharomyces cerevisiae*. *Exp Cell Res* 105, 79–98.

Jorgensen P, Nishikawa J, Breitkreutz B, Tyers M (2002). Systematic identification of pathways that couple cell growth and division in yeast. *Sciences STKE* 297, 395–400.

Levine A, Puzio-Kuter A (2010). The control of the metabolic switch in cancers by oncogenes and tumor suppressor genes. *Science* 330, 1340.

Levy S, Barkai N (2009). Coordination of gene expression with growth rate: a feedback or a feed-forward strategy? *FEBS Lett* 583, 3974–3978.

Levy S, Ihmels J, Carmi M, Weinberger A, Friedlander G, Barkai N (2007). Strategy of transcription regulation in the budding yeast. *PLoS ONE* 2, e250.

Newsholme E, Crabtree B, Ardawi M (1985). The role of high rates of glycolysis and glutamine utilization in rapidly dividing cells. *Biosci Reports* 5, 393–400.

Nurse P (1975). Genetic control of cell size at cell division in yeast. *Nature* 256, 547–551.

Petti AA, Crutchfield CA, Rabinowitz JD, Botstein D (2011). Survival of starving yeast is correlated with oxidative stress response and nonrespiratory mitochondrial function. *Proc Natl Acad Sci USA* 108, E1089–E1098.

Pfeiffer T, Schuster S, Bonhoeffer S (2001). Cooperation and competition in the evolution of ATP-producing pathways. *Science* 292, 504–507.

Rachidi N, Martinez M, Barre P, Blondin B (2002). *Saccharomyces cerevisiae* PAU genes are induced by anaerobiosis. *Mol Microbiol* 35, 1421–1430.

Rando O, Winston F (2012). Chromatin and transcription in yeast. *Genetics* 190, 351–387.

Saldanha AJ, Brauer MJ, Botstein D (2004). Nutritional homeostasis in batch and steady-state culture of yeast. *Mol Biol Cell* 15, 4089–4104.

Shlomi T, Benyamini T, Gottlieb E, Sharan R, Ruppin E (2011). Genome-scale metabolic modeling elucidates the role of proliferative adaptation in causing the Warburg effect. *PLoS Comput Biol* 7, e1002018.

- Silverman SJ *et al.* (2010). Metabolic cycling in single yeast cells from unsynchronized steady-state populations limited on glucose or phosphate. *Proc Natl Acad Sci USA* 107, 6946–6951.
- Slavov N (2010). Inference of sparse networks with unobserved variables. Application to gene regulatory networks. *JMLR WCP* 9, 757–764.
- Slavov N, Airoidi EM, van Oudenaarden A, Botstein D (2012). A conserved cell growth cycle can account for the environmental stress responses of divergent eukaryotes. *Mol Biol Cell* 23, 1986–1997.
- Slavov N (2010). Universality, Specificity and Regulation of *S. cerevisiae* Growth Rate Response in Different Carbon Sources and Nutrient Limitations. Ph.D. Thesis. Princeton, NJ: Princeton University.
- Slavov N, Botstein D (2011). Coupling among growth rate response, metabolic cycle, and cell division cycle in yeast. *Mol Biol Cell* 22, 1997–2009.
- Slavov N, Dawson KA (2009). Correlation signature of the macroscopic states of the gene regulatory network in cancer. *Proc Natl Acad Sci USA* 106, 4079–4084.
- Slavov N, Macinskas J, Caudy A, Botstein D (2011). Metabolic cycling without cell division cycling in respiring yeast. *Proc Natl Acad Sci USA* 108, 19090–19095.
- Tagkopoulou I, Slavov N, Kung S (2005). Multi-class biclustering and classification based on modeling of gene regulatory networks. Fifth IEEE Symposium on Bioinformatics and Bioengineering (BIBE 2005). IEEE.
- Takeda K, Yoshida T, Kikuchi S, Nagao K, Kokubu A, Pluskal T, Villar-Briones A, Nakamura T, Yanagida M (2010). Synergistic roles of the proteasome and autophagy for mitochondrial maintenance and chronological lifespan in fission yeast. *Proc Natl Acad Sci USA* 107, 3540–3545.
- ter Linde JJM, Liang H, Davis RW, Steensma HY, van Dijken JP, Pronk JT (1999). Genome-wide transcriptional analysis of aerobic and anaerobic chemostat cultures of *Saccharomyces cerevisiae*. *J Bacteriol* 181, 7409–7413.
- Tsukada M, Ohsumi Y (1993). Isolation and characterization of autophagy-defective mutants of *Saccharomyces cerevisiae*. *FEBS Lett* 333, 169–174.
- Vazquez A, Liu J, Zhou Y, Oltvai ZN (2010). Catabolic efficiency of aerobic glycolysis: the Warburg effect revisited. *BMC Syst Biol* 4, 58.
- Warburg O (1962). *New Methods of Cell Physiology: Applied to Cancer, Photosynthesis, and Mechanism of X-Ray Action*, New York: John Wiley & Sons: Interscience Publishers.
- Warburg O, Posener K, Negelein E (1924). Ueber den Stoffwechsel der Tumoren. *Biochemische Zeitschrift* 152, 319–344.
- Wenger J, Piotrowski J, Nagarajan S, Chiotti K, Sherlock G, Rosenzweig F (2011). Hunger artists: yeast adapted to carbon limitation show trade-offs under carbon sufficiency. *PLoS Genetics* 7, e1002202.
- Wise DR *et al.* (2008). Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction. *Proc Natl Acad Sci USA* 105, 18782–18787.
- Youk H, Van Oudenaarden A (2009). Growth landscape formed by perception and import of glucose in yeast. *Nature* 462, 875–879.