Proteome allocations change linearly with specific growth rate of *Saccharomyces cerevisiae* under glucose-limitation

Jianye Xia

East China University of Science and Technology

Benjamin Sánchez

Chalmers University of Technology

Yu Chen

Chalmers University of Technology <https://orcid.org/0000-0003-3326-9068>

Kate Campbell

Chalmers University of Technology

Sergo Kasvandik

University of Tartu <https://orcid.org/0000-0003-0218-2410>

Jens Nielsen (✉ nielsenj@chalmers.se)

Chalmers University of Technology <https://orcid.org/0000-0002-9955-6003>

Article

Keywords: *Saccharomyces cerevisiae*, Multiomics integration, Protein phosphorylation, Allosteric regulation, Enzyme saturation, Systems biology, Quantitative omics, Metabolism regulation

Posted Date: May 18th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-464207/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Version of Record: A version of this preprint was published at Nature Communications on May 20th, 2022. See the published version at <https://doi.org/10.1038/s41467-022-30513-2>.

Abstract

Saccharomyces cerevisiae is widely used as a cell factory and it is therefore important to understand how it organizes key functional parts when cultured under different conditions. Here we performed a multi-omics analysis of *S. cerevisiae* by culturing the strain under a wide range of specific growth rates using glucose as the sole limited nutrient. At these different conditions we measured the absolute transcriptome, the absolute proteome, the phosphoproteome, and the metabolome. Most functional protein groups showed linear dependence on the cell specific growth rate. Proteins engaged with translation showed a perfect linear increase with the specific growth rate, while glycolysis and chaperone proteins showed a linear decrease at respiratory conditions. Glycolytic enzymes and chaperones, however, show decreased phosphorylation with increasing specific growth rates, resulting in an overall increased activity that is associated with increased flux through these pathways. Further analysis showed that proteome allocation was primarily determined at the transcriptome level. Finally, using enzyme constraint genome scale modeling we found that enzyme usage play an important role for controlling flux in amino acid biosynthesis.

Introduction

Saccharomyces cerevisiae is a widely used cell factory due to its robustness under harsh industrial conditions¹. It has therefore been engineered for production of a range of different chemicals such as isoprenoids², free fatty acids³, biopharmaceutical proteins⁴ and many precursors of high value added product⁵. As one of the most studied eukaryal cells, *S. cerevisiae* is also extensively studied as a model organism for deciphering molecular mechanisms in cellular and molecular biology^{6, 7}. However, we still lack insight into how yeast cells coordinate its resources when grown at different growth rates, especially on the allocation of the functional parts of the life process, i.e. its proteome. In addition, what determines protein resources distribution across different functional groups is also not clear⁸.

Cellular proteins are responsible for all key cellular functions, i.e. transcription (RNA polymerases), catalyzing reactions (enzymes), transporting molecules across membranes (transporters), translation (ribosome), folding and assembling of proteins (chaperons), signal transduction (kinase or phosphatase) and many other functions⁹. Cellular phenotypes at different conditions are therefore determined by fine tuning of proteome fractions across different functional groups. Understanding the underlying principles for proteome allocation at different cell growth rates is therefore import for bioprocess optimization and strain improvement for industrial strains modification¹⁰. Metzel-Raz et al⁸ investigated the proteome allocation across a wide range of specific growth rates under different conditions for *S. cerevisiae* and found condition-dependent proteome profiling, and a strong positive linear relationship between the fraction of translational proteins and specific growth rate. Similar results had been first confirmed in *E. coli*¹¹ and an elegant equation called the bacterial growth law describing the relation between specific growth rate and ribosome plus non-ribosome fractions was proposed¹². *S. cerevisiae* showed varying proteome allocation patterns when cultured under different conditions¹³, but a thorough investigation of proteome allocation of *S. cerevisiae* under carbon limitation conditions is lacking.

It is well known that the *S. cerevisiae* shows distinct phenotypes when it is cultured aerobically under limited or excess glucose, firstly confirmed by De Deken¹⁴ and named after the English biochemist Herbert Grace Crabtree, who first observed the phenomena in tumor cell¹⁵. Using proteome constraint flux balance analysis it was recently shown that the Crabtree effect can be explained by a trade-off between fermentative pathway enzymes and oxidative phosphorylation enzymes¹⁶, as it was found that glycolytic enzymes have more efficient ATP synthesis capacity per enzyme mass compared to respiration.

Here, we carried out a multi-omics study of *S. cerevisiae* in glucose-limited chemostats across a wide range of specific growth rate ranging from 0.025 h⁻¹ to 0.4 h⁻¹. To understand the proteome allocation pattern when the yeast cell increases its specific growth rate and the underlying principles that regulate the process, we performed absolute proteome analysis and integrated both transcriptome and phosphoproteome data to interpret protein resources allocation patterns at different specific growth rates. Finally, a quantitative proteome constraint genome scale metabolic model was used to investigate the enzyme usage across the whole metabolic network of *S. cerevisiae* under a wide range of specific growth rate conditions, and together with metabolome data of amino acid biosynthesis pathways the model gave a detailed interpretation of how flux is controlled through these pathways for increasing specific growth rates.

Results

1. Linear relationships between functional categories of proteome and cell specific growth rate

S. cerevisiae was cultured at 9 different dilution rates (from 0.025 h⁻¹ to 0.4 h⁻¹, Fig.1A) in biological triplicates and all major exchange fluxes were quantified (Fig.1B). It was clearly observed that two distinct phenotypes were shown across the studied dilution rate range. Although the dissolved oxygen (DO) level was kept above 40% of saturation, ethanol was produced under aerobic conditions when the specific growth rate increased above 0.28 h⁻¹ (Fig.1B), a well-known phenomenon referred to as the *Crabtree effect*^{17, 18}. This also resulted in an increasing respiratory quotient, i.e. carbon dioxide production relative to oxygen consumption (Fig.1B). The *S. cerevisiae* cell relies on oxidative phosphorylation as the dominant route for ATP production when the dilution rate was below 0.28 h⁻¹, known as purely respiratory condition. For dilution rates above 0.28 h⁻¹ the cells alter their metabolism to increasingly use substrate level phosphorylation for production of ATP, known as respiro-fermentative condition.

To investigate how *S. cerevisiae* allocates its proteome at different specific growth rates, absolute proteome measurements under the 9 glucose-limited chemostats were performed and this resulted in quantitative measurements of 1787 identified proteins (Supplementary data file Supp_data1_proteome.xlsx, and supplementary text for more details about the proteins identified under different conditions) that were grouped into 11 categories according to their physiological functions (adapted and modified accordingly from Metzl-Raz et al.⁸, details can be found in supplementary data file: Supp_data2_11groups.xlsx). We found that proteome allocation varied with specific growth rates. As protein occupies a majority of the dry cell weight (ranging from 0.3 to 0.5 in *S. cerevisiae*¹⁹), protein synthesis (translation process by ribosome) rate will determine the cell specific growth rate²⁰. Our proteome data results confirmed this, by correlating the fraction

of proteins engaged in translation with cell specific growth rates, we found a perfect linear relationship ($R^2=1$) (Fig.1C). The correlated relationship is shown in Eqn (1).

$$f_r = 0.35\mu + 0.13 \quad (1)$$

with denotes ribosome protein fraction and the specific growth rate. The same relationship has been found in both yeast⁸ and *E. coli*²¹, and has been referred to as the bacterial growth law in prokaryotes¹². From our data we calculated the average translation rate of the ribosomes in our strain to be ~10 AA/ribosome/s (details of the calculation can be found in supplementary text), which agree well with what has been reported earlier for *S. cerevisiae*^{22, 23}.

Two other groups (amino acids biosynthesis and mitochondrial proteins) also showed increasing tendency along with the specific growth rate (Fig.1C). However, unlike the fraction of the translation proteins, these two groups had a profile that was metabolism depending (Fig.1C). Under respiratory conditions, the proteome fractions for these two groups increased indeed linearly, while it changed when the metabolism turned to respiro-fermentative conditions ($\mu > 0.28\text{h}^{-1}$). The proteome fraction for mitochondrial proteins decreased with a slop of -0.14 under respiro-fermentative condition. This was consistent with the Crabtree effect caused by trade-off of enzyme mass invested into the two ATP supplying pathways¹⁶: substrate level phosphorylation (mainly rely on the glycolysis to supply ATP) and oxidative phosphorylation (mainly rely on the electron transfer chain that locates in the mitochondria). In contrast, the proteome fraction for amino acid biosynthesis reached a constant level (13% of the total proteome) when metabolism shift from purely respiratory to respiro-fermentative metabolism.

To accommodate an increased allocation of proteome mass for amino acid biosynthesis and protein translation, the cell needs to decrease proteome allocation to other proteome categories. These were also found to be highly metabolism state-dependent. Two distinct conditions are shown that coincide with the cell's shift in metabolism. Under respiratory condition, the cell decreased its proteome fractions for chaperones and glycolysis together to support the increasing need of proteome for translation, amino acid biosynthesis and mitochondrial proteins (Fig.1D), while under respiro-fermentative condition, these two parts reached their minimum fraction and kept a constant level. The fraction of proteins engaged in lipid metabolism were at a constant level during respiratory metabolism, but this fraction decreased when the cells shifted to respiro-fermentative metabolism.

2. The dynamic proteome allocation pattern with cell growth rate are primarily determined at the mRNA level

In order to understand the changes in proteome allocation of *S. cerevisiae* observed, we performed absolute transcriptome analysis at the same conditions. The number of mRNA species identified was much larger than that of the proteins, with 5,401 transcripts and 2,821 proteins identified in total at all conditions, respectively. However, the overall mRNA abundance level was much lower than that of the overall protein abundance (Fig. S1), as the most abundant (from 10% to 90% percentile) mRNA level ranged from 2 to 25 copies per cell compared to the most abundant (from percentile 10% to 90%) protein level ranged from 504 to 67,440 molecules per cell. The corresponding protein over its coding mRNA ratio covers a wide range, i.e. from 0.7 to

27,424 proteins per mRNA molecules. Detail abundance information for all mRNAs across the 9 dilution rates can be found in supplementary data (Supplementary data file Supp_data3_transcriptome.xlsx).

Even though a poor correlation between the mRNAs and proteins was obtained for all data points (in Fig. S2, with linear regression $R^2=0.52$), which is consistent with previous publications^{24, 25}, we found stronger linear correlation among protein and mRNA abundance for individual genes across all studied conditions (Fig. 2A). Thus, we found the abundance of 1,229 proteins to be linearly correlated (with regression coefficient $R^2 > 0.8$) with their corresponding mRNAs (Fig. 2B), which together with earlier findings²⁶ confirms that mRNA abundance is a key determinant of protein abundance. It should be noted that slopes of correlation lines between protein and mRNA vary among different transcripts (Fig. S3), which indicates variations of translation rate of individual transcripts (similar results also found in Sharma et al.²⁷). By using *in vivo* visualization of single mRNA molecule translation, Yan et al.²⁸ reported heterogeneity in the translation of individual mRNAs in the same strain. GO terms enrichment analysis (Fig. S4) revealed that for ribosome related genes, the mRNA level and protein level significantly correlated (p value < 0.01) with each other.

Based on these findings, we expected a similar allocation pattern for the transcriptome as found for the proteome with respect to functional groups. We therefore divided the transcriptome into the same 11 functional groups, and changes of mRNA fraction of each functional group with specific growth rate showed almost the same pattern as found for the proteome (Fig. 2C & 2D), except for lipid metabolism that showed a different pattern at low specific growth rates. The result suggests that *S. cerevisiae* to a large degree shapes its proteome resources allocation at the transcriptional level according to corresponding gene functions under glucose-limited steady-state conditions. One notable difference between mRNA allocation and proteome allocation, however, is the fraction of mitochondrial genes which was much larger among the transcriptome than in the proteome.

The overall consistent allocation pattern for both the transcriptome and proteome reveals that the dynamic proteome allocation pattern is mainly determined at the mRNA level, which means that resources are regulated mainly at the transcription level when *S. cerevisiae* is cultured at glucose-limited, steady state conditions.

3. Protein Phosphorylation showed negative effect on majority of glycolysis and chaperon protein activities

Next, we analyzed correlations between reaction flux and mRNA or protein abundance. We used the genome-scale metabolic model Yeast7.6 together with the measured exchange fluxes, i.e. q_{Gluc} , $q_{Ethanol}$, q_{O_2} , q_{CO_2} , μ , for estimation of all 2302 fluxes in the metabolic network at the 9 different dilution rates. A total of 318 fluxes could be paired with both a transcript and a protein level, and these pairings were used for correlation analysis across the 9 dilution rates. Scatter plot of the Pearson correlation coefficient for both mRNA vs flux and protein vs flux is shown in Fig. 3A. At the same time correlation coefficient distribution for both pairs are plotted at the margin of the main plot Fig. 3A. We filtered out significant (p value < 0.05) positive (red points) and negative correlations (blue points) with other points as non-significantly correlated for both mRNA-flux and protein-flux pairs. We then mapped all reactions that significantly correlated with either mRNA or protein levels on the reaction network of the yeast cell using the iPath3 tool²⁹. From this analysis we found that reactions in the TCA cycle and engaged in nucleotide biosynthesis showed positive correlation, while EMP pathway reactions mainly shown negative correlations (Fig. 3B).

To replenish proteins for translation, mitochondria and amino acid biosynthesis when the cell grows faster, the cell decreases their investments into proteins involved with glycolysis and lipid biosynthesis as well as chaperones. This observation holds not only in terms of relative abundance, but also in terms of absolute abundance levels of both mRNA and proteins in each function group (Fig. S5). It is interesting that both glycolytic enzymes and chaperones abundance decrease as the cell grow faster under respiratory conditions. This is surprising as the glycolytic flux increases for increasing growth rate (Fig. 3C). This means that the specific activity of the pathway enzymes or the usage of the enzymes are increasing with growth rate. The same for chaperones, where there is an increased need for their activity at faster growth where there is higher protein synthesis level. Thus, also for chaperones there must be a an increased specific activity. We were therefore interested to see if there was a common mechanism explaining the increased specific activity of chaperones and glycolytic enzymes.

Phosphorylation of proteins is a reversible post-translational modification of amino acid residues (serine, threonine, or tyrosine) by introducing a covalently bound phosphate group to the protein molecule, and it alters the structural conformation of the protein, causing it to become activated, deactivated, or having a modified function³⁰. In yeast, more than half of the ~900 metabolic enzymes have phosphorylation sites^{31, 32}. In our phosphoproteomics data, 5,971 phospho-peptides associated with 1,450 proteins were identified, and among these 1,761 phospho-peptides associated with 782 proteins showed increase or decrease trends along with the specific growth rate. We did enrichment analysis of these proteins with respect to GO terms or KEGG pathway (Fig. S6).

Many phosphorylation sites were found in glycolysis enzymes, i.e. phosphorylation site(s) were detected among all enzymes except Pgi1p and Cdc19p. It was found that seven among the 10-step enzymes of this pathway showed linear decrease of phosphorylation level as the cell grew faster at respiratory condition. Using an algorithm for proposing inferred functional phosphorylation events (FPE)³¹, we found that phosphorylation of all these seven enzymes showed inhibition effects on their activities. Previously functional phosphorylation events on Pfk2, Fba1 and Gpm1 have been reported by Oliveira et al.³³, as found by our analysis, but our analysis further inferred that phosphorylation of Glk1, Pfk1, Pfk1, Tdh3, Eno1 and Eno2 exert flux regulation.

For chaperones, the algorithm proposed for inferring FPEs could not be used as it was not possible to obtain reaction flux information for these proteins. We therefore analyzed the phosphorylation level for all chaperones, and among the 48 chaperones identified in yeast, 17 showed 43 detected phosphorylated peptides, and 30 of the 43 phosphorylated peptides (69.8%) showed decreased phosphorylation level for increasing specific growth rate. Even though only 35% of the chaperones showed a decreased phosphorylation level, these chaperones accounted for more than 80% of the mass fraction of the chaperones at the lowest specific growth rate (0.025h^{-1}) and 58% at the highest specific growth rate of 0.38h^{-1} (Fig. S7).

We therefore conclude that phosphorylation of both glycolytic enzymes and chaperones seems likely to reduce their activities.

4. Enzyme saturation play an important role fulfil amino acid biosynthesis demand as yeast cell grows faster

Hierarchical regulation analysis can be used to decipher at which level a reaction is regulated under both steady state or dynamic condition³⁴. Flux regulation contributions of enzyme, metabolites, transcripts, or post translation modifications can be determined by the hierarchical regulation coefficients, α_i , with i here stands for e (enzyme regulation coefficient), m (metabolite regulation coefficient), t (transcript regulation coefficient), or p (protein phosphorylation regulation coefficient). According to the analysis by Yu et al.³¹, when $0.5 < \alpha_i < 1.5$, it means the reaction flux is mainly regulated by enzyme abundance. Pairwise hierarchical analysis, taking the slowest specific growth rate 0.027h^{-1} as reference, on based on proteome and fluxome data was carried out with both the central carbon metabolism and amino acid biosynthesis pathway (Fig. S8). The results show scarce protein abundance regulation on the reaction fluxes of these pathways. Combined results of protein phosphorylation analysis and hierarchical regulation analysis for the EMP pathway indicates that activities of majority enzymes of this pathway are under regulation through phosphorylation modification, and dephosphorylating activates enzymes when the enzyme abundance is low at high specific growth rates.

However, we generally did not find many protein phosphorylation events related to the biosynthesis of amino acids and nucleotides (Fig. S6), and it is unclear what regulates flux through these pathways. To analyze this, we used a Genome-scale metabolic model with Enzymatic Constraints using Kinetic and Omics data (GECKO)³⁵, which uses the catalytic capacity constraint of each individual enzyme (k_{cat} multiplied by the enzyme concentration) together with the stoichiometric constraints normally used in flux-balance analysis. GECKO allows improved calculation of fluxes through the metabolic network of yeast compared with regular flux balance analysis, and it further allows for calculation of the enzyme requirements for each reaction. The latter were used to calculate enzyme usage, i.e. the model calculated the enzyme requirement divided by the corresponding measured enzyme concentration (molecules per cell). As there were few phosphorylation events of biosynthetic enzymes, it is a fair assumption to use enzyme usage as a proxy for enzyme saturation. Using this method, we calculated non-zero usages of 321 enzymes, and found 58% of them to have less than 10% usage on average, but with 11% of them having >90% usage on average (Fig. S9). Furthermore, for 180 of the enzymes (56%), their usage showed a positive correlation with the specific growth rate ($R^2 > 0.8$) (Fig. 4A). The top ten enzymes having the strongest correlation ($R^2 > 0.99$) between enzyme usage and specific growth rate were all enzymes involved in synthesizing main building blocks of the cell (mostly amino acids).

All 180 enzymes showing a strong positive correlation of usage with specific growth rate were analyzed for KEGG pathway overrepresentation (Fig. 4B). It can be seen that translation (in the form of aminoacyl-tRNA biosynthesis) and synthesis of building blocks (mainly amino acid) show consistent trends of high correlation (Fisher Exact Test P-value < 0.01), indicating that there is increased saturation of enzymes involved in biosynthesis at increasing specific growth rates. We also found that increased enzyme usage for glycolysis occurs above the critical dilution rate (0.28 h^{-1}), i.e. only at respiro-fermentative metabolism (Fig. 4C).

To evaluate further if the metabolomics data support the model prediction of an increased enzyme saturation with growth rate in amino acid biosynthesis, we checked the relative abundance of the intermediates in the amino acid biosynthetic pathways. According to the SGD database, there are 59 intermediates for all amino acid biosynthetic pathways, out of which 20 were measured by our metabolome analysis. Fourteen of these 20 intermediates showed positive correlation ($R^2 > 0.8$) with the specific growth rate (Fig. 4D). Specifically, we found four out of the five measured intermediates of the arginine pathway and four out of the seven measured

intermediates of the lysine pathway to have positive correlation with growth rate (this is consistent with enzyme usage results for the lysine biosynthesis pathway, Fig. 4B). Arginine and lysine are both derived from 2-oxoglutarate, with their biosynthesis characterized by having low-levels of metabolite/feed-back regulation³⁶. For another six amino acids (Cys, Trp, Val, Met, Leu, His), only one biosynthetic intermediate had concentration strongly correlating with specific growth rate.

Fourteen amino acids (Ala, Arg, Asn, Cys, Gln, Gly, His, Ile, Leu, Met, Phe, Pro, Trp, Tyr), however, themselves showed negative correlation with specific growth rate under purely respiratory conditions (Fig. S10). Except for Ala, Asn, Cys and His the biosynthesis of these amino acids is characterized by extensive metabolite regulation and strong feed-back inhibition by the amino acids level. The decreasing concentration of these amino acids seems to be contradicting the increasing enzyme usage for aminoacyl tRNA biosynthesis (Fig. 4B). However, the abundance of tRNAs has been reported previously to increase with specific growth rate^{37,38}, which can explain the increased enzyme usage of these reactions. Furthermore, the K_m values for amino acids in aminoacyl tRNA biosynthesis are reported to be very low (in the mM range)³⁹ compared with the intracellular amino acid concentrations (in the mM range). Flux towards amino acids in aminoacyl tRNA biosynthesis would therefore be relatively insensitive to changes in the intracellular amino acid concentration. The decreasing concentration of amino acids with specific growth rate therefore seems to occur in order to relieve feedback inhibition and hereby allow for increased flux through the amino acid biosynthetic pathways. Increased enzyme concentration and enzyme saturation in amino acid metabolism further supports this flux increase for increasing growth rates.

Discussion

Based on transcriptome, proteome, and metabolome at a series of glucose-limitation chemostat steady states covering both respiratory and respiro-fermentative metabolism conditions we studied how yeast regulates its proteome allocation at different specific growth rate. Simple linear relationships were observed between several functional protein groups with specific growth rate. The group of translation related proteins showed perfect linear increase with cell specific growth rate in the whole studied range, which has been referred to as growth law for bacteria¹² first found in *E. coli*, and a similar relationship has been confirmed for *S. cerevisiae*⁸. Here we added to this and found linear relationships also for other functional protein groups.

Our transcriptome and proteome data showed that the resource allocation pattern at the protein level was primarily determined by the mRNA level. Even though there is poor linear relationship between mRNA and proteins in general, much due to a vast range of protein/mRNA ratios among different genes, the mRNA levels positively correlated with protein levels within the same functional group. This indicates that some underlying mechanisms regulate expression and translation of functional groups as a whole regardless of the translation efficiency of individual gene.

Interestingly we found a linear decrease in the abundance of both glycolytic enzymes and chaperones along with cell specific growth rate under respiratory metabolism conditions, despite the need for increased activity by these proteins. Both hierarchical regulation analysis and FPE inferring confirmed function inactivation due to phosphorylation of seven of the glycolysis enzymes. Also, more than 80% of the chaperones (in weight)

have decreased phosphorylation for increasing growth rate, again pointing to activation of these proteins through de-phosphorylation.

We also utilized metabolic modeling to quantitatively investigate the protein usage for all enzymes in the metabolic network, and the result showed that both enzyme abundance and saturation in amino acid biosynthesis pathways increased to fulfil the increasing demand for building blocks, in particular amino acids, for increasing specific growth rates. This analysis was confirmed by analysis of pathway intermediates that showed increasing trends with growth rate. Interestingly though was that the levels of proteogenic amino acids decreased with specific growth rate, most likely due to a requirement for reduced feed-back inhibition of pathway enzymes.

Based on our analysis we mapped the multilayer regulation structure in protein allocation for *S. cerevisiae*. Our results revealed the underlying principles how yeast coordinate its proteome resources, which primarily is determined by the transcriptome, whereas post-translation modification, enzyme saturation and allosteric regulation plays important roles in controlling metabolic fluxes. We are confident that these findings will besides providing basic insight into regulation of metabolism in the eukaryal model organism *S. cerevisiae* it will also assist in future engineering of yeast metabolism with the objective of producing valuable chemicals.

Methods

Strains used in this study.

Unless otherwise stated, the yeast *Saccharomyces cerevisiae* CEN.PK 113-7D (MAT α , MAL2-8c, SUC2) was used. For quantitative proteome analysis, *S. cerevisiae* CEN.PK113-7D *lys1::kanMX* strain was used.

Media and culturing methods

Minimal mineral medium was used, which contained 10 g of glucose, 5 g of (NH₄)₂SO₄, 3 g of KH₂PO₄ and 0.5 g of MgSO₄ per liter, with 1 mL of trace metal solution and 1 mL of vitamin solution. The trace metal solution contained per liter: FeSO₄·7H₂O, 3 g; ZnSO₄·7H₂O, 4.5 g; CaCl₂·2H₂O, 4.5 g; MnCl₂·4H₂O, 1 g; CoCl₂·6H₂O, 300 mg; CuSO₄·5H₂O, 300 mg; Na₂MoO₄·2H₂O, 400 mg; H₃BO₃, 1 g; KI, 100 mg; Na₂EDTA·2H₂O, 19 g (pH=4). The vitamin solution contained per liter: d-biotin, 50 mg; 4-aminobenzoic acid, 0.2 g; Ca pantothenate, 1 g; pyridoxine-HCl, 1 g; thiamine-HCl, 1 g; nicotinic acid, 1 g; and myoinositol, 25 g (pH=6.5). The chemostat feeding medium was the same as the minimal mineral medium except for glucose, wherein 7.5 g/L was used instead of 10 g/L.

To generate the internal standard for quantitative proteomics, the lysine auxotrophic strain (CEN.PK113-7D *lys1::kanMX*) was cultured with heavy labeled ¹⁵N, ¹³C-lysine (Cambridge Isotope Laboratories). Fully labeled biomass (> 95% incorporation) was produced and harvested under four different stages of the culture process: Fed-batch cultures of the auxotrophic strain were carried out in three 1L bioreactors with three exponential feeding rates : 0.1 h⁻¹, 0.2 h⁻¹, 0.35 h⁻¹, and the feeding continued for at least one dilution volume before cells harvest. The harvest biomass samples were mixed together thus comprised of cells in the batch phase (Sample S1) and in each of the three exponential feeding phases (Sample S2 stands for sample of 0.1 h⁻¹,

Sample S3 for 0.2 h^{-1} and Sample S4 for 0.35 h^{-1}). This was performed in order to collect biomass with varying proteome compositions, which would enable a broad spectrum of heavy labeled proteins so that we can get as more quantifiable proteins as possible.

All remaining cultures were carried out under glucose limited chemostat conditions with nine different dilution rates, ranging from 0.025 h^{-1} to $\sim 0.4 \text{ h}^{-1}$, covering both respiratory ($<0.28 \text{ h}^{-1}$) and respiro-fermentative metabolism ($>0.28 \text{ h}^{-1}$). Experiments were carried out in 1 L bioreactors (Dasgip, Jülich, Germany) equipped with an on-line off-gas analysis system alongside pH, temperature and dissolved oxygen sensors. An initial batch culture was carried out with inoculation of 10 % seed cultures. The chemostat cultures were performed in 1 L bioreactors with a working volume of 0.5 L under aerobic conditions ($\text{DO} > 40\%$) at 30°C and pH 4.5. The continuous operation of chemostat cultures were performed at the end of the batch culture. To ensure cells were growing at a steady-state, chemostat cultures were run for at least five residence times before sampling.

Sampling

For extracellular metabolome measurements, broth was sampled and filtered immediately into 1.5 mL Eppendorf tubes and stored at -20°C until high-performance liquid chromatography (HPLC) analysis was performed.

For the transcriptome sample collection, 10 mL broth was sampled and injected into a 50 mL falcon tube filled with $\sim 3/4$ ice. Cells were pelleted by centrifugation (4000 rpm, 5 min, Centrifuge 5702R, Eppendorf, Germany), and biomass pellets were snap frozen in liquid nitrogen (N_2), and then transferred to a 1.5 mL Eppendorf tube and stored at -80°C until further analysis.

For proteome sample collection, ~ 5 mL culture broth was injected into a 50 mL pre-weighed falcon tube (prechilled on ice), and the tube was reweighed after sampling to determine exact amount of broth collected. Samples were then pelleted by centrifugation (14000 rpm, 20 seconds, 4°C , Refrigerated Centrifuge 4K15, Sigma, Germany), and biomass pellet was washed in 1 mL chilled PBS followed by recentrifugation. Pellets were snap frozen in liquid N_2 , transferred to a 1.5 mL Eppendorf tube then stored at -80°C until further analysis.

For the intracellular metabolome sample collection, ~ 7 mL culture broth was injected into a pre-weighed 50 mL falcon tube containing 35 mL -40°C 100% methanol. Tube was then reweighed after sampling to determine exact amount of broth collected. Cells were pelleted in a precooled centrifuge (4000 g, 3 min, -20°C , Refrigerated Centrifuge 4K15, Sigma, Germany), supernatant was discarded and samples stored immediately at -80°C until further analysis.

mRNA sequencing

Total RNA was extracted and purified using a Qiagen RNeasy Mini Kit, according to the user manual, with DNase step included (Qiagen, Hilden, Germany). RNA integrity was verified using a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, USA) and its concentration determined using a NanoDrop 2000 (Thermo Scientific, Wilmington, USA).

To prepare RNA for sequencing, the Illumina TruSeq sample preparation kit v2 was used with poly-A selection. cDNA libraries were then loaded onto a high-output flow cell and sequenced on a NextSeq 500 platform (Illumina Inc., San Diego) with paired-end 2×75 nt length reads .

Raw data of reads generated by NextSeq 500 was processed using TopHat version 2.1.1 ⁴⁰ to map paired end reads to the CEN.PK 113-7D reference genome (<http://cenpk.tudelft.nl/cgi-bin/gbrowse/cenpk/>). 8-15 million reads were mapped to the reference genome with an average map rate of 95%. Cufflinks version 2.1.1 ⁴¹ was then used to calculate the FPKM values for each sample. Mapped read counts were generated from SAM files using bedtools version 2.26.0 ⁴². Differential expression analysis was performed with the Bioconductor R package DESeq2 ⁴³.

Absolute mRNA quantification

To quantify RNA Seq read counts, 18 mRNAs were selected with FPKM values ranging $3.4 \times 10^1 - 1.4 \times 10^4$, covering 80 % of the dynamic range of mRNA expression at reference conditions ($D = 0.1 \text{ h}^{-1}$). Absolute concentrations for these 18 mRNAs were then measured using QuantiGene assay (Affymetrix, Santa Clara, CA, United States). Further details of this measurement can be found in our previous publication ²⁴. A positive linear correlation with a Pearson R^2 value of 0.8 was achieved among these 18 selected mRNA absolute concentrations and their corresponding FPKM values (Table S2). The same correlation was then applied to all remaining mRNAs generated from RNA Sequencing, to quantify their respective absolute mRNA levels. Absolute values of mRNA under other dilution rates were then calculated based on the fold change obtained from differential expression analysis relative to the reference chemostat ($D = 0.1 \text{ h}^{-1}$). Assume the weight of yeast cells does not change under different specific growth rates, so a cell weight of 13 pg measured under reference condition ($D = 0.1 \text{ h}^{-1}$) was applied to all other chemostat conditions. The same assumption with a constant cell weight was applied for proteome absolute quantification. The calculated absolute mRNA concentrations were subsequently presented in the unit of [molecules/cell].

Total and phospho-proteome sample preparation

Cell pellets were resuspended in 10 volumes (relative to cell pellet) of 6 M guanidine HCl, 100 mM Tris-HCl pH 8.0, 20 mM DTT, heated at 95°C for 10 min and sonicated with Bioruptor (Diagenode, Denville, NJ, United States) sonication (15 min, “High” setting). Samples were further processed with FastPrep24 (MP Biomedicals, Santa Ana, CA, United States) twice at 4 m/s for 30 s with cooling between cycles. After removal of beads, the samples were precleared with centrifugation at 17,000 g for 10 min at 4°C. After protein concentration measurement with Micro-BCA assay (Thermo Fisher Scientific, Wilmington, USA) samples were spiked at 1:1 ratio with the heavy lysine labelled standard. For absolute quantification, 6 µg of heavy standard was spiked separately with 1.1 µg of UPS2 protein mix (Sigma Aldrich). Overall, 50 µg of protein was precipitated with a 2:1:3 (v/v/v) methanol:chloroform:water extraction. The precipitates were suspended in 7:2 M urea:thiourea and 100 mM ammonium bicarbonate. After disulfide reduction with 2.5 mM DTT and alkylation with 5 mM iodoacetamide, proteins were digested with 1:50 (enzyme to protein) Lys-C (Wako Pure Chemical Industries, Osaka, Japan) overnight at room temperature. The peptides were desalted using C18 material (3M Empore) tips and reconstituted in 0.5% trifluoroacetic acid (TFA).

For the phosphoproteome analysis cells were lysed as described above, except samples were not mixed with the heavy standard and proteins were digested with dimethylated porcine trypsin (Sigma Aldrich, St. Louis, MO, United States) instead of Lys-C. Sample preparation was carried out as described by the EasyPhos protocol ⁴⁴. 500 µg of cellular protein was used as input for the phosphopeptide enrichment. Final samples were reconstituted in 0.5% TFA.

Nano-LC/MS/MS analysis for protein quantification

2 µg of peptides (for phosphoenriched samples, the entire sample) were injected to an Ultimate 3000 RSLC nano system (Dionex, Sunnyvale, CA, United States) using a C18 cartridge trap-column in a backflush configuration and an in-house packed (3 µm C18 particles, Dr Maisch, Ammerbuch, Germany) analytical 50 cm × 75 µm emitter-column (New Objective, Woburn, MA, United States). Peptides were separated at 200 nL/min (for phosphopeptides: 250 nL/min) with a 5-40% B 240 and 480 min gradient for spiked and heavy standard samples, respectively. For phosphopeptides a 90 min two-step separating gradient was used, consisting of 5-15% B 60 min and 15-30% B 30 min. Buffer B was 80% acetonitrile + 0.1% formic acid and buffer A was 0.1% formic acid in water. Eluted peptides were sprayed to a quadrupole-orbitrap Q Exactive Plus (Thermo Fisher Scientific, Waltham, MA, United States) tandem mass spectrometer (MS) using a nano-electrospray ionization source and a spray voltage of 2.5 kV (liquid junction connection). The MS instrument was operated with a top-10 data dependent MS/MS acquisition strategy. One 350-1400 m/z MS scan (at a resolution setting of 70,000 at 200 m/z) was followed by MS/MS (R=17,500 at 200 m/z) of the 10 most-intense ions using higher-energy collisional dissociation fragmentation (normalized collision energies of 26 and 27 for regular and phosphopeptides, respectively). For total proteome analysis, the MS and MS/MS ion target and injection time values were 3×10^6 (50 ms) and 5×10^4 (50 ms), respectively. For phosphopeptides, the MS and MS/MS ion target and injection time values were 1×10^6 (60 ms) and 2×10^4 (60 ms), respectively. Dynamic exclusion time was limited to 45 s, 70 s and 110 s for phosphopeptide, spiked samples and heavy standard runs, respectively. Only charge states +2 to +6 were subjected to MS/MS, and, for phosphopeptides fixed first mass was set to 95 m/z. The heavy standard was analyzed with three technical replicates, all other samples with a single technical replicate.

Mass spectrometric raw data analysis and proteome quantification

Raw data were identified and quantified with the MaxQuant 1.4.0.8 software package ⁴⁵. For heavy-spiked samples the labelling state (multiplicity) was set to 2, and Lys8 was defined as the heavy label. Methionine oxidation, asparagine/glutamine deamidation and protein N-terminal acetylation were set as variable modifications, cysteine carbamidomethylation was defined as a fixed modification. For phospho-analysis serine/threonine phosphorylation was used as an additional variable modification. Search was performed against the UniProt (www.uniprot.org) *S. cerevisiae* S288C reference proteome database (version from July 2016) using the LysC/P (trypsin/P for phosphoproteomics) digestion rule. Only protein identifications with a minimum of 1 peptide with 7 amino acids long were accepted, and transfer of peptide identifications between runs was enabled. Peptide-spectrum match and protein false discovery rate (FDR) was kept below 1% using a target-decoy approach with reversed sequences as decoys.

In heavy-spiked samples, to account for any H/L 1:1 mixing deviations normalized H/L ratios (by shifting median peptide log H/L ratio to zero) were used in all down-stream quantitative analyses. Protein H/L values themselves were derived by using the median of a protein's peptide H/L ratios, and requiring at least one peptide ratio measurement for reporting quantitative values. Signal integration of missing label channels was enabled. For enriched phosphoproteome samples, an in-house written R script based on median phosphopeptide intensity was used to normalize the phosphopeptide intensities.

The heavy spike-in standard used for deriving the copy numbers was quantified using the iBAQ method as described by Scwanhauser et al ⁴⁶. Essentially, UPS2 protein intensities were divided by the number of theoretically observable peptides, log-transformed and plotted against log-transformed known protein amounts of the UPS2 proteins. This regression was then applied to derive all other protein absolute quantities using each protein's iBAQ intensity. The relative ratios of individual proteins to total protein were then converted to protein concentration in cell by multiplying the total protein content in cell for each condition. The total protein content in cell under each condition was measured using the modified Lowery method.

Phosphorylation regulation analysis

A recently developed method⁴⁷ for functional phosphorylation event identification was used for phosphorylation regulation analysis in this work. For details of the model and method the readers are recommended to the original publication. Briefly, it has been shown that correlation between changes in fluxes and phosphorylation levels suggests the contribution of the phosphorylation events to the fluxes. A phosphorylation event is inferred to activate enzyme activity if the correlation is positive while to inhibit enzyme activity if negative. Therefore, correlation analysis was performed in this study for the fold-change values of fluxes and phosphopeptide intensities by comparing with a reference dilution rate.

Relative metabolome quantification⁴⁸

For intracellular metabolomic analysis, frozen biomass pellets were delivered to Metabolon, Inc. (Durham, NC, USA) wherein non-targeted MS was performed. Briefly, metabolites were identified by matching their ion chromatographic retention index and mass spectral fragmentation signatures to the Metabolon reference library of chemical standards. Relative quantification of metabolite concentrations was then performed via peak area integration.

Metabolite quantification and data normalization. Peaks were quantified using area-under-the-curve. For studies spanning multiple days, a data normalization step was performed to correct variation resulting from instrument inter-day tuning differences. Essentially, each compound was corrected in run-day blocks by registering the medians to equal one (1.00) and normalizing each data point proportionately (termed the “block correction”). For studies that did not require more than one day of analysis, no normalization is necessary, other than for purposes of data visualization.

GECKO modelling details

The enzyme-constrained model ecYeast7, version 1.4, was used from release 1.1.1 of GECKO: <https://github.com/SysBioChalmers/GECKO/releases/tag/v1.1.1>. A fraction of metabolic enzymes of $f =$

0.4461 g/g was assumed based on Pax-DB data, and an average saturation of $\sigma = 0.49$ was assumed for any non-measured enzyme. Measured protein content was rescaled to be proportional to previous measurements of 0.46 g/gDW at 0.1h⁻¹. Manual curation was performed to some k_{cat} values, exchange fluxes of pyruvate, acetaldehyde and (R,R)-2,3-butanediol were blocked, and a non-growth associated maintenance (NGAM) of 0.7 was assumed for all growth conditions.

For each condition, the corresponding rescaled proteomic data was overlaid as constraints to any protein that had a match, taking out zero values. An additional standard deviation was added for each protein to avoid over-constrained models, and all 4 complexes from Ox.Pho. were scaled to be proportional to the average measured subunit. For all undetected enzymes, an overall “pool” constraint was used, equal to the difference between the protein content and the sum of all measured proteins, multiplied by the previously mentioned f and σ . Additional details plus the full implementation of this process is available in the script *limitModel.m*.

For each condition, the previously resulting model was used to fit the chemostat data by modifying the growth-associated maintenance and optimizing for biomass growth. The implementation of this is available in *dilutionStudy.m*. Finally, as several proteins showed to be a big limitation due to an extremely low detected measurement, the mentioned models were flexibilized so they could at least grow at the desired biomass growth rate with the available glucose. This implementation is available in *flexibilizeModels.m*.

Total Protein Content Measurement

Total protein contents of each condition were measured using the modified Lowry method ⁴⁹. The total protein content measured at all dilution rates (n=3, $\pm$ standard deviation) are as follows:

Specific growth rate (h ⁻¹ , AVG $\pm$ SD)	0.027 $\pm$ 0.002	0.044 $\pm$ 0.001	0.102 $\pm$ 0.001	0.152 $\pm$ 0.003	0.214 $\pm$ 0.011	0.254 $\pm$ 0.003	0.284 $\pm$ 0.013	0.334 $\pm$ 0.009	0.379 $\pm$ 0.011
Total protein content (% , AVG $\pm$ SD)	29.9 $\pm$ 0.3	31.2 $\pm$ 0.2	37.1 $\pm$ 0.7	41.9 $\pm$ 2.4	32.0 $\pm$ 4.8	37.8 $\pm$ 0.8	39.1 $\pm$ 0.9	41.9 $\pm$ 0.7	39.1 $\pm$ 6.5

References

1. Nielsen J. Yeast cell factories on the horizon. *Sci* **349**, 1050-1051 (2015).
2. Meadows AL, *et al.* Rewriting yeast central carbon metabolism for industrial isoprenoid production. *Nature* **537**, 694-697 (2016).
3. Yu T, *et al.* Reprogramming Yeast Metabolism from Alcoholic Fermentation to Lipogenesis. *Cell* **174**, 1549-1558.e1514 (2018).
4. Nielsen J. Production of biopharmaceutical proteins by yeast: advances through metabolic engineering. *Bioengineered* **4**, 207-211 (2013).
5. Dai Z, Huang M, Chen Y, Siewers V, Nielsen J. Global rewiring of cellular metabolism renders *Saccharomyces cerevisiae* Crabtree negative. *Nature Communications* **9**, 3059 (2018).

6. Fendt SM, Sauer U. Transcriptional regulation of respiration in yeast metabolizing differently repressive carbon substrates. *BMC Syst Biol* **4**, 12 (2010).
7. Gonçalves E, *et al.* Systematic analysis of transcriptional and post-transcriptional regulation of metabolism in yeast. *PLoS Comp Biol* **13**, e1005297-e1005297 (2017).
8. Metzl-Raz E, Kafri M, Yaakov G, Soifer I, Gurvich Y, Barkai N. Principles of cellular resource allocation revealed by condition-dependent proteome profiling. *eLife* **6**, e28034 (2017).
9. Alberts B, *et al.* *Molecular biology of the cell*, 5th edn. Garland Science (2008).
10. Schmidt A. Merged map of the yeast proteome. *Cell Systems* **6**, 151 (2018).
11. Scott M, Gunderson CW, Mateescu EM, Zhang Z, Hwa T. Interdependence of cell growth and gene expression: origins and consequences. *Sci* **330**, 1099-1102 (2010).
12. Scott M, Hwa T. Bacterial growth laws and their applications. *Curr Opin Biotechnol* **22**, 559-565 (2011).
13. Hackett SR, *et al.* Systems-level analysis of mechanisms regulating yeast metabolic flux. *Sci* **354**, aaf2786 (2016).
14. De Deken RH. The Crabtree effect: a regulatory system in yeast. *Journal of General Microbiology* **44**, 149-156 (1966).
15. Crabtree HG. Observations on the carbohydrate metabolism of tumours. *The Biochemical Journal* **23**, 536-545 (1929).
16. Nilsson A, Nielsen J. Metabolic trade-offs in yeast are caused by F1F0-ATP synthase. *Scientific Reports* **6**, 22264 (2016).
17. Deken RHD. The Crabtree Effect: A Regulatory System in Yeast. *Microbiology* **44**, 149-156 (1966).
18. Swanson WH, Clifton C. Growth and assimilation in cultures of *Saccharomyces cerevisiae*. *Journal of bacteriology* **56**, 115 (1948).
19. Canelas AB, Ras C, ten Pierick A, van Gulik WM, Heijnen JJ. An in vivo data-driven framework for classification and quantification of enzyme kinetics and determination of apparent thermodynamic data. *Metab Eng* **13**, 294-306 (2011).
20. Liebermeister W, Noor E, Flamholz A, Davidi D, Bernhardt J, Milo R. Visual account of protein investment in cellular functions. *Proc Natl Acad Sci USA* **111**, 8488-8493 (2014).
21. Scott M, Gunderson CW, Mateescu EM, Zhang Z, Hwa T. Interdependence of cell growth and gene expression: origins and consequences. *Science* **330**, 1099-1102 (2010).
22. Milo R, Jorgensen P, Moran U, Weber G, Springer M. BioNumbers—the database of key numbers in molecular and cell biology. *Nucleic Acids Res* **38**, D750-D753, BNID 100986 (2010).
23. Waldron C, Lacroute F. Effect of growth rate on the amounts of ribosomal and transfer ribonucleic acids in yeast. *J Bacteriol* **122**, 855-865 (1975).
24. Lahtvee P-J, *et al.* Absolute Quantification of Protein and mRNA Abundances Demonstrate Variability in Gene-Specific Translation Efficiency in Yeast. *Cell Systems* **4**, 495-504.e495 (2017).
25. Liu Y, Beyer A, Aebersold R. On the Dependency of Cellular Protein Levels on mRNA Abundance. *Cell* **165**, 535-550 (2016).
26. Csárdi G, Franks A, Choi DS, Airoidi EM, Drummond DA. Accounting for experimental noise reveals that mRNA levels, amplified by post-transcriptional processes, largely determine steady-state protein levels in

- yeast. *PLoS Genet* **11**, e1005206 (2015).
27. Sharma AK, Ahmed N, O'Brien EP. Determinants of translation speed are randomly distributed across transcripts resulting in a universal scaling of protein synthesis times. *Physical Review E* **97**, 022409 (2018).
 28. Yan X, Hoek Tim A, Vale Ronald D, Tanenbaum Marvin E. Dynamics of translation of single mRNA molecules *in vivo*. *Cell* **165**, 976-989 (2016).
 29. Darzi Y, Letunic I, Bork P, Yamada T. iPath3.0: interactive pathways explorer v3. *Nucleic Acids Res* **46**, 510-513 (2018).
 30. Vlastaridis P, Papakyriakou A, Chaliotis A, Stratikos E, Oliver SG, Amoutzias GD. The pivotal role of protein phosphorylation in the control of yeast central metabolism. *G3 (Bethesda)* **7**, 1239-1249 (2017).
 31. Chen Y, Wang Y-H, Nielsen J. Systematic inference of functional phosphorylation events in yeast metabolism. *Bioinformatics*, (2017).
 32. Chen Y, Nielsen J. Flux control through protein phosphorylation in yeast. *FEMS Yeast Res* **16**, (2016).
 33. Oliveira AP, Ludwig C, Picotti P, Kogadeeva M, Aebersold R, Sauer U. Regulation of yeast central metabolism by enzyme phosphorylation. *Mol Syst Biol* **8**, 623 (2012).
 34. van Eunen K, Rossell S, Bouwman J, Westerhoff HV, Bakker BM. Quantitative analysis of flux regulation through hierarchical regulation analysis. In: *Methods in Enzymology* (eds Jameson D, Verma M, Westerhoff HV). Academic Press (2011).
 35. Sanchez BJ, Zhang C, Nilsson A, Lahtvee PJ, Kerkhoven EJ, Nielsen J. Improving the phenotype predictions of a yeast genome-scale metabolic model by incorporating enzymatic constraints. *Mol Syst Biol* **13**, 935 (2017).
 36. Moxley JF, *et al.* Linking high-resolution metabolic flux phenotypes and transcriptional regulation in yeast modulated by the global regulator Gcn4p. *Proceedings of the National Academy of Sciences* **106**, 6477-6482 (2009).
 37. Emilsson V, Näslund AK, Kurland CG. Growth-rate-dependent Accumulation of Twelve tRNA Species in *Escherichia coli*. *J Mol Biol* **230**, 483-491 (1993).
 38. Wilusz JE. Controlling translation via modulation of tRNA levels. *Wiley Interdisciplinary Reviews: RNA* **6**, 453-470 (2015).
 39. Wolfson AD, Pleiss JA, Uhlenbeck OC. A new assay for tRNA aminoacylation kinetics. *RNA* **4**, 1019-1023 (1998).
 40. Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg SL. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biology* **14**, R36 (2013).
 41. Trapnell C, *et al.* Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nature Protocols* **7**, 562 (2012).
 42. Quinlan RR, Hall IM. BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics* **26**, 841-842 (2010).
 43. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* **15**, 550 (2014).

44. Humphrey SJ, Azimifar SB, Mann M. High-throughput phosphoproteomics reveals in vivo insulin signaling dynamics. *Nat Biotechnol* **33**, 990 (2015).
45. Cox J, Mann M. MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. *Nat Biotechnol* **26**, 1367 (2008).
46. Schwanhäusser B, *et al.* Global quantification of mammalian gene expression control. *Nature* **473**, 337 (2011).
47. Chen Y, Wang Y, Nielsen J. Systematic inference of functional phosphorylation events in yeast metabolism. *Bioinformatics* **33**, 1995-2001 (2017).
48. Evans AM, *et al.* High resolution mass spectrometry improves data quantity and quality as compared to unit mass resolution mass spectrometry in high-throughput profiling metabolomics. *Metabolomics: Open Access* **4**, 132 (2014).
49. Verduyn C, Postma E, Scheffers WA, van Dijken JP. Physiology of *Saccharomyces Cerevisiae* in Anaerobic Glucose-Limited Chemostat Cultures. *Microbiology* **136**, 395-403 (1990).

Figures

Figure 1

Experimental design, series chemostat experimental results and linear relationship between different protein categories and specific growth rates. (A) Experimental design was based on chemostat cultures operated as glucose-limited with a range of dilution rates (equal to specific growth rates, 9 different values) from 0.025 h⁻¹ to ~ 0.4 h⁻¹. Line width of arrow represents flow of feed and effluent. (B) Key exchange fluxes measured at different dilution rates. All data are based on biological triplicates. (C) Changes in the fraction of the proteome allocated to translations (ribosome protein), mitochondrial, and amino acid biosynthesis across different specific growth rates. It is clear that the protein allocation in these regions is proportional to the specific growth rates. (D) Changes in the fraction of the proteome allocated to chaperons, glycolysis, and lipid biosynthesis across different specific growth rates. It is clear that the protein allocation in these regions is inversely proportional to the specific growth rate.

Figure 2

mRNA level determine its protein level by individual or in functional groups. (A) Illustration of strong ($R^2 > 0.99$) linear correlation between absolute protein and mRNA abundance across different growth rates. Illustrated with the four genes PRB1, GOR1, DCS1 and MDH2. (B) Distribution of Pearson correlation

coefficients for linear regression of individual protein levels versus their corresponding mRNA levels across different dilution rates. (C) Changes in the fraction of the mRNA allocated to translations, mitochondrial, and amino acid biosynthesis across different specific growth rates. It is clear that the mRNA fractions allocated in these groups are proportional to the specific growth rate. (D) Changes in the fraction of the protein allocated to chaperons, glycolysis, and lipid metabolism across different specific growth rates. It is clear that the mRNA fractions allocated in these groups are reversely proportional to specific growth rates.

Figure 3

Correlation analysis between reaction fluxes and abundance of corresponding mRNA and protein. (A) Pearson correlation coefficients for both mRNA/flux and protein/flux pairs, with distribution of Pearson correlation coefficients for protein/flux pair showing on the right margin and for mRNA/flux pair showing on the top margin. (B) Reaction map showing correlations between both mRNA/flux and protein/flux pair information is

plotted using iPath3 tool²⁹. In the map, reactions with positive correlations are shown as pink lines, and that with negative correlations as blue lines, reactions with both positive and negative correlations as red lines, other non-significantly correlated reactions shown as green lines. (C) Detail overview of the EMP pathway reactions with dynamics of absolute reaction fluxes, protein phosphorylation levels, absolute protein abundance and absolute mRNA abundance. The abscissas for all bar plots are specific growth rates of the yeast cell.

Figure 4

Enzyme saturation and correlation analysis for various metabolic pathways of *S. cerevisiae* with a range of specific growth rates covering both respiratory and respire-fermentative metabolism. (A) Enzyme usage of all metabolic proteins as a function of specific growth rate (from ~0.025 h⁻¹ to ~ 0.4h⁻¹). (B) Metabolic pathway enrichment of enzymes that correlate with the increasing specific growth rate. Values in parenthesis are number of enzymes in each KEGG pathway, the P values were gotten from Fisher test (asterisk * indicates p-value less than 0.01). (C) Enzyme usage changing trends with different biomass yield for individual enzymes. (D) Correlation of the abundance of intermediates in amino acid biosynthesis pathway and specific growth rate under respiratory (blue) and both respiratory and respire-fermentative conditions (orange).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Suppdata1proteome.xlsx](#)

- [Suppdata211groups.xlsx](#)
- [Suppdata3transcriptome.xlsx](#)
- [Suppdata4averageAminoAcidMW.xlsx](#)
- [Supplementarytext20210320submission.docx](#)
- [nrsoftwarepolicynielsen.pdf](#)
- [nielsen.pdf](#)
- [scriptsandcodesJianye.zip](#)