GROWTH-RATE DEPENDENT AND NUTRIENT-SPECIFIC GENE EXPRESSION RESOURCE ALLOCATION IN FISSION YEAST

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ABSTRACT

Cellular resources are limited and their relative allocation to gene expression programmes determines physiological states and global properties such as the growth rate. Quantitative studies using various growth conditions have singled out growth rate as a major physiological variable explaining relative protein abundances. Here, we used the simple eukaryote *Schizosaccharomyces pombe* to determine the importance of growth rate in explaining relative changes in protein and mRNA levels during growth on a series of non-limiting nitrogen sources. Although half of fission yeast genes were significantly correlated with the growth rate, this came alongside wide-spread nutrient-specific regulation. Proteome and transcriptome often showed coordinated regulation but with notable exceptions, such as metabolic enzymes. Genes positively correlated with growth rate participated in every level of protein production with the notable exception of RNA polymerase II, whereas those negatively correlated mainly belonged to the environmental stress response programme. Critically, metabolic enzymes, which represent ~55-70% of the proteome by mass, showed mainly condition-specific regulation. Specifically, many enzymes involved in glycolysis and NAD-dependent metabolism as well as the fermentative and respiratory pathways were condition-dependent and not consistently correlated with growth. In summary, we provide a rich account of resource allocation to gene expression in a simple eukaryote, advancing our basic understanding of the interplay between growth-rate dependent and nutrient-specific gene expression.

INTRODUCTION

Cellular growth is the process by which cells increase in mass. It is a fundamental systemic process that impacts most aspects of cell physiology. Growth can be very fast: for example, yeast cells can double in mass every few hours, and certain bacteria only require minutes. Conversely, slower growth is observed in multicellular organisms, in which several cell types take days to grow and divide. Crucially, the cellular growth rate changes in response to external cues such as nutrient quality, stressing agents, or growth factors.

Measurements of biomass composition in unicellular organisms have long-established cellular growth rates as a covariate of cell physiology (Schaechter, Maaløe, and Kjeldgaard 1958; Mitchison and Lark 1962; Waldron and Lacroute 1975; Fantes and Nurse 1977; Neidhardt, Ingraham, and Schaechter 1990; Bremer and Dennis 2008). In the last decade, quantitative experimental work, together with mathematical modelling, have described this relationship (reviewed in (Klumpp and Hwa 2014; Shahrezaei and Marguerat 2015; Jun et al. 2018; Bruggeman et al. 2020)). This body of work has emphasised how the macromolecular composition of the cell is tightly connected to growth rate. Specifically, for cultures undergoing balanced exponential growth modulated by external nutrients, the total RNA abundance per unit of biomass and the growth rate are correlated linearly. This phenomenological relationship is called the first or ribosomal growth law and reflects an increased requirement for ribosomes during faster growth to support protein synthesis. The demand for ribosomes is also felt at the protein level, where it induces a trade-off between proteins involved in translation and those involved in catabolism. It was shown that about half of the total protein mass in *Escherichia coli* responded to growth modulations by nutrient limitation and translational inhibition (Scott et al. 2010; You et al. 2013). These observations were formalised in a phenomenological model separating the proteome into three broad sectors based on their growth rate correlations. Proteins that are positively correlated with the cellular growth rate during nutrient limitation and negatively during translational inhibition comprise the R-sector, whereas proteins showing the opposite behaviour comprise the P-sector. Proteins that do not respond to the growth rate belong to the Q-sector (Scott et al. 2010). The concept of proteome sectors has been the basis of several phenomenological and coarse-grained mechanistic models relating optimal resource allocation to protein abundance and cellular growth rates (Molenaar et al. 2009; Scott et al. 2014; Maitra and Dill 2015; Weiße et al. 2015; Pandey and Jain 2016; Liao, Blanchard, and Lu 2017; Bertaux et al. 2020; Hu et al. 2020).

The molecular mechanisms behind the phenomenological assignment to the three proteome sectors remain less well understood. R-sector proteins are universally involved in translation and ribosome biogenesis and many of them are controlled by global signalling pathways such as guanosine tetraphosphate (ppGpp) in prokaryotes or the target of rapamycin complex 1

(TORC1) in eukaryotes (Irving, Choudhury, and Corrigan 2020; Petibon et al. 2020). P-sector proteins, on the other hand, are more diverse and often involved in metabolic adaptation and stress response (Brauer et al. 2008; You et al. 2013; Hui et al. 2015; A. Schmidt et al. 2016). In *E. coli*, the master regulator CRP-cAMP has been proposed to control the P-sector assignments of carbon catabolism enzymes when growth rate was modulated by the quality of abundant carbon sources (You et al. 2013). Under other growth modulations and in other organisms, whether the regulation of P-sector proteins is as directly mechanistically linked to the growth rate as for R-proteins is less clear.

Transcriptomics and proteomics have been instrumental in characterising the coordination between gene expression and cellular growth. The ribosomal growth law was first confirmed in the *E. coli* proteome in continuous cultures limited by carbon availability (Peebo et al. 2015), under titrations of carbon, nitrogen, and translational inhibition (Hui et al. 2015), and in an extensive study of 22 growth conditions (A. Schmidt et al. 2016). In addition, the Hui study proposed that the P sector could be divided into subsectors related to different metabolic functions depending on the type of nutrient limitation. In the budding yeast *Saccharomyces cerevisiae*, a seminal microarray study showed strong correlations between hundreds of transcripts with the chemostat dilution rate across six nutrient titrations (Brauer et al. 2008). The observed correlations agreed with the ribosomal growth law and highlighted stress response as a component of the P-sector alongside metabolic functions. More recently, Metzl-Raz and colleagues observed the ribosomal growth law in the proteome of budding yeast after combining existing data sets of cultures grown in a variety of carbon sources (Paulo et al. 2015; 2016) with data obtained under nitrogen and phosphorus limitation (Metzl-Raz et al. 2017). They also proposed that a pool of non-translating ribosomes is available as a buffer during changing growth conditions, a strategy also observed in prokaryotes (Dai et al. 2016; Mori et al. 2017; Kohanim et al. 2018). This suggest that resource allocation may not be fully optimised for maximal cell growth. Signs of excess capacity have also been reported for metabolic pathways, including glucose catabolism (Yu et al. 2020). Further omics studies in *S. cerevisiae* have defined additional characteristics of resource allocation such as reallocation of proteome mass from amino acid biosynthesis to protein translation upon amino acid supplementation (Björkeroth et al. 2020), or the respective contribution of transcription and translation to different allocation strategies (Yu et al. 2021). Thus, genome-wide omics experiments have been key to improve our understanding of resource allocation in *E. coli* and *S. cerevisiae* by connecting proteome sectors to specific physiological functions.

The cellular growth rate reflects the metabolic state of the cell and in limiting nutrient conditions metabolic enzymes are often part of the P-sector (Hui et al. 2015; A. Schmidt et al. 2016). This suggests that expression levels of specific metabolic enzymes when responding to external

conditions can be directly regulated alongside the growth rate. The cell metabolism however is an exquisitely complex network of interconnected processes and perturbation of single pathways can have wide-spread systemic effects. Central carbon metabolism (CCM) relies on three pathways: glycolysis, the pentose phosphate pathway, and the tricarboxylic acid (TCA) cycle. Together, these generate energy in the form of ATP, in a process mediated by reducing agents such as NADH, and produce building blocks for biosynthesis. ATP can be generated anaerobically via fermentation; a process which consists of glycolysis and the subsequent degradation of pyruvate, or aerobically via respiration, which requires the TCA cycle and subsequent oxidative phosphorylation (OXPHOS). The extent of fermentative versus respiratory metabolism affects the NAD+/NADH redox balance and vice versa, as NAD+ reduction during glycolysis and the TCA cycle must be balanced by NADH oxidation occurring during pyruvate degradation and OXPHOS (Vemuri et al. 2007; van Hoek and Merks 2012; Campbell et al. 2018; Luengo et al. 2020). In eukaryotes, these reactions are compartmentalised between the cytoplasm and the mitochondria, with the latter housing the respiratory enzymes and functioning as hubs that connect diverse metabolic pathways including CCM and amino acid metabolism (Spinelli and Haigis 2018). For instance, amino acid degradation enables the assimilation of nitrogen as ammonium or glutamate via de- or transamination reactions. The remaining carbon backbone is recycled into the cell's biomass or excreted, and the associated metabolites affect carbon metabolism (Godard et al. 2007). Importantly, mitochondrial intermediates are required for amino acid biosynthesis even during fermentative energy generation (Malecki et al. 2020). In fission yeast, a single point mutation in the pyruvate kinase Pyk1, affecting its activity, has been shown to rebalance the fluxes through the fermentation and respiration pathways alongside shifts in the transcriptome and proteome composition (Kamrad et al. 2020), giving a prime example of how the cell co-adjusts perturbations in metabolic fluxes and expression burdens. Taken together, shifts in the metabolic demand propagate throughout the cell, as most metabolic pathways are tightly interlinked (Chubukov et al. 2014).

The expression levels of CCM enzymes, and therefore the fluxes through the pathways depend on external conditions and stress levels. As a result, cellular states and metabolic strategies are linked to resource allocation to different gene expression programmes. For example, during rapid growth on glucose, yeast utilises the fermentative pathway alongside the TCA cycle even in the presence of oxygen, a phenomenon known as aerobic glycolysis or the Crabtree effect (Shimizu and Matsuoka 2018). Aerobic glycolysis is also a characteristic of tumour cells, for which it is known as the Warburg effect (Heiden, Cantley, and Thompson 2009). This strategy appears counterintuitive as fermentation generates fewer molecules of ATP per glucose molecule than respiration. Several hypotheses have been proposed to

resolve this paradox. All require a second growth-limiting constraint besides glucose uptake which would be specific to respiro-fermentative growth (de Groot et al. 2019). Examples include the cytoplasmic density of macromolecules (Vazquez et al. 2008; Goelzer et al. 2015), total proteome allocation (Basan et al. 2015), and membrane area availability (Szenk, Dill, and de Graff 2017). Thus, a whole-cell understanding of cellular trade-offs between multiple constraints must take into account gene expression alongside metabolic maps (Goelzer and Fromion 2017; Yang et al. 2018; Dahal, Zhao, and Yang 2020). Resource allocation constraints have been successfully introduced into genome-wide metabolic models of several organisms as more high-quality expression data has become available (O'Brien et al. 2013; Sánchez et al. 2017; Y. Chen et al. 2020). In summary, quantitative surveys of the gene expression cost of metabolic pathways are key to understanding cell physiology.

Here, we define the growth-rate dependent and nutrient-specific resource allocation to the fission yeast *Schizosaccharomyces pombe* proteome and transcriptome. We find that both types of regulation are interconnected and define protein synthesis and stress response as the processes positively and negatively regulated with the growth rate. We then study the plasticity of the gene expression burden of metabolic pathways in response to changes in nutrients and their reliance on transcriptional and post-transcriptional regulation. Altogether we provide a rich account of resource allocation in a simple eukaryote as a function of external conditions.

RESULTS

Fission yeast gene expression shows growth-rate-related and condition-specific components

To generate cell populations that grow at different rates while not limited for nutrients, we used eight defined culture media each containing a unique source of nitrogen. These media have been extensively characterised elsewhere (Fantes and Nurse 1977; Carlson et al. 1999; Petersen and Russell 2016). Seven media contained 20 mM of a single amino acid (Trp, Gly, Phe, Ser, Ile, Pro, Glu), and one 93.5 mM of ammonium chloride (NH_4Cl, referred to as Amm) as a reference (**Fig. 1**). In our hands, this design achieved growth rates ranging 0.05 - 0.28 h^{-1}. *S. pombe 972h*$^-$ prototroph wild-type cells were grown in turbidostats at constant concentrations of OD_{600} ~0.4 (3 - 5×10^6 cells/ml) in triplicates for 43 - 143 h (6 - 28 generations depending on the nitrogen source) (**Fig. 1C-E, Supp. Table S1**) (Takahashi et al. 2015). Like in chemostats, turbidostat cultures are diluted by the addition of fresh media. In the case of the turbidostat system, however, it is the cell concentration that is directly measured and maintained constant and not the proliferation rate. This ensures that cellular growth is not limited by a lack of nutrients, but rather determined by the quality of the provided nitrogen

source and the resulting internal allocation patterns. Growth rates were measured halfway through the procedure during a two-fold dilution cycle (**Fig. 1B, Supp. Table S1**). To measure the proteome and transcriptome allocation as a function of the growth rate, we performed label-free proteomics and RNA sequencing (RNA-Seq) analysis of cells from each culture condition (**Methods, Supp. Table S2-S5)**.

We first asked whether the fission yeast proteome composition differed significantly between the eight growth conditions. Strikingly, ~44% of the 2045 proteins robustly detected in all samples were significantly more variable across conditions than among biological replicates (Holm-adjusted $p_{ANOVA} < 0.05$). This pervasive level of gene regulation was also apparent at the transcriptome level where ~52% of mRNAs showed significant variability. These results indicate that the composition of the proteome and transcriptome are both strongly affected by conditions that change the growth rate.

To investigate this variability further, we used the *z*-score transformed protein fraction of each gene for hierarchical clustering (**Fig. 2A, Methods**). This treatment enabled normalisation for protein expression levels across the proteome while preserving the variation of each protein between conditions. We defined 10 clusters that revealed two major features of the datasets (**Fig. 2A-C**). First, most clusters showed a clear change in protein expression in one or more conditions (clusters 3-10). Second, the expression of several proteins was not strictly condition-specific but instead showed a coordinated linear increase with growth rate (clusters 1-2). Interestingly, the total baseline expression of the condition-specific clusters was positively (clusters 3, 4, 6, 10), or negatively (clusters 5, 7, 8, 9) correlated with the growth rate. Apart from cluster 6, clusters were enriched for defined functional categories, indicating that the shifting balance between condition-specific regulation and growth rate regulation may have physiological consequences related to the enriched functions **(Fig. 2A, Supp. Fig. S2.1)**.

Both modes of regulation were also apparent in the transcriptome data for coding and non-coding RNA (ncRNA) (**Fig. 2C, Supp. Figs. S2.2-S2.3)**. Interestingly, most ncRNAs showed clear and reproducible condition-specific expression between replicates, suggesting the presence of active regulation, consistent with analyses using different genetic and physiological conditions (**Fig. S2.3**) (Atkinson et al. 2018). To test this hypothesis, we compared the expression patterns of ncRNA from each cluster with the expression of their flanking coding genes (**Supp. Fig. S2.3C-D**). We found that for 4 out of 9 clusters, ncRNA expression patterns were not mirrored by the neighbouring mRNA. This indicates that many ncRNA are subjected to some level of independent regulation. In summary, we find that regulation of gene expression programmes across conditions that affect the growth rate has

two components; one which is condition-specific and another which is coordinated with growth rate.

Growth dependent gene expression is an important determinant of the cell protein and mRNA composition

We first focused our analysis on the growth-dependent component of fission yeast gene expression. Linear correlations between the expression of individual genes and the growth rate have been observed in several organisms under different types of growth limitation (Brauer et al. 2008; Hui et al. 2015; Metzl-Raz et al. 2017; Peebo et al. 2015; A. Schmidt et al. 2016; Zavřel et al. 2019). Following the terminology used in prokaryotes, we divided proteins and mRNA into three sectors depending on whether they show a growth-dependent component that was positively (R), negatively (P), or not significantly (Q) correlated with the growth rate (Scott et al. 2014; 2010). We used repeated-median linear models to quantify the linear coordination of each protein and mRNA quantity with growth. This model fits a linear dependence in the presence of large numbers of outliers and is therefore robust to the condition-specific component of gene expression (**Methods, Supp. Fig. S2.4, Supp. Table S6**).

The linear fits generated two useful parameters. First, the slope of the linear regression is a measure of the strength of the dependence of a protein's concentration on the growth rate. Second, its *y*-intercept represents the fraction of the protein numbers that is not directly dependent on growth. Both parameters are directly correlated with expression levels making it difficult to disentangle the strength of the growth-rate-related regulation from an mRNA or protein from its abundance. To take this into account, we developed a normalised measure of growth dependence called FC (**Methods**, **Supp. Fig. S2.4G-H**). FC values are a combination of the regression slope and *y*-intercept which do not scale with abundance, thereby enabling a direct comparison of the growth-dependence of single genes or groups thereof.

Repeated-median linear models captured the growth-dependent component of the 10 clusters from **Fig. 2**, and proteins from the R and P sectors dominated the clusters that were positively and negatively correlated with growth, respectively (**Supp. Fig. S2.5**). Of all the genes detected in the proteome across the eight conditions examined, we found that 24% of proteins and 37% of mRNA belonged to the R sector; similarly, 27% and 21% of the proteins and mRNA belonged to the P sector, respectively. The protein and mRNA of a given gene belonged to the same sector in 51% of the cases (**Fig. 2D**). When they did not, the mRNA of P or R proteins were mostly assigned to the Q sector and vice versa, with only 23 R proteins having P sector mRNA, and 74 P proteins having R sector mRNA, out of the 2077 proteins detected.

In quantitative terms, the total proteome mass fraction of the fission yeast R sector ranged between ~20% at zero growth and 55% for the fastest measured growth rate, whereas the mass fraction of the P sector similarly ranged from ~30% to 10% (**Fig. 2E)**. The sum of all Q sector proteins was negatively correlated with the growth rate because proteome fractions add up to one by definition. However, none of the individual proteins showed significant correlation with the growth rate. At the mRNA level, the R fraction ranged from 38% to 59% of the total normalised counts, and the P fraction from 19% to 10% (**Fig. 2F)**. Thus, during fast growth, over half of the gene expression burden is dedicated to factors that increase in concentration with growth rate and may therefore be limiting. Moreover, the amplitude of the variability in the concentration of fission yeast proteins and mRNA that depend on the growth rate alone is in the order of magnitude of the cut-offs that are commonly used for differential expression analysis. Therefore, differences in growth rate are important factors that affect interpretation of transcriptomics and proteomics data (Yu et al. 2021).

R sector proteins participate in all steps of the protein synthesis process

We next queried the cellular processes that had a strong R component and could therefore be either limiting for growth or regulated by it. We used a curated list of macromolecular complexes spanning most cellular processes and calculated the proportion of each complex subunit that was growth rate-dependent in each category (**Fig. 3A, Supp. Table S7)** (Gene Ontology Consortium 2019; Lock et al. 2019). As observed in prokaryotes and budding yeast, the top 4 categories relying the most on R proteins belonged to a single process: the synthesis of proteins (**Fig. 3AB**). Strikingly, R complexes were found at every single step of protein synthesis: the transcription of rRNAs and tRNAs and their processing, assembly and post-translational modification of the ribosome, and initiation and termination of translation (**Fig. 3B)**. Interestingly, expression of the chromatin-modifying complexes NuA4 and Ino80 were part of the R sector (**Fig. 3C**), suggesting they may be involved in ribosome biogenesis in fission yeast as has been proposed for NuA4 in budding yeast (Uprety, Sen, and Bhaumik 2015). Alternatively, these results could indicate that the chromatin structure and levels of histone modification may be limiting for growth.

The overall correlation between growth and the factors involved in protein synthesis had a notable exception. Although RNA polymerase (RNAP) I and specific subunits of RNAPIII were part of the R sector, RNA polymerase II specific subunits were not significantly correlated with growth rate (**Fig. 3B-D**). Therefore, the number of RNAP II complexes is unlikely to be a limiting step in protein production during growth. Interestingly, RNAP II numbers were found to be limiting for the scaling of gene expression to cell size, indicating that coordination of gene

expression to cell size and growth rate follow different mechanisms (Padovan-Merhar et al. 2015; Sun et al. 2020).

The stoichiometry of translation complexes changes with the growth rate

Differences in FC values between protein complexes indicate that their relative levels or stoichiometry changes with the growth rate. We hypothesised that these variations could provide mechanistic insights into the functioning of these complexes. To investigate this in the context of protein translation, we analysed three non-overlapping subclasses of translation proteins: the ribosomal proteins (RP), the ribosome biogenesis regulon (RiBi), and the translation initiation, elongation and termination factors (IET) (**Methods, Supp. Table S8**). The RiBi and IET classes had similar FC values, whereas the trendline for RPs was significantly steeper (**Fig. 4A, Supp. Fig. S4.1A**). As a result, the ratios between IET and RPs, and between RiBi proteins and RPs were higher at slow growth (**Fig. 4B**). These results suggest that ribosome biogenesis may become more efficient with increasing growth rates as the ratio RiBi/RP diminishes. Alternatively, a fraction of IET and RiBi proteins larger than of RPs could be held in reserve at slower growth (Metzl-Raz et al. 2017). The relative abundances in EMM of IET:RiBi:RP were approximately 4:1:8 for the proteome mass fractions and 5:4:64 for the transcriptome RPKMs (**Fig. 4A-B**). This confirms earlier observations that the burden on transcription for RP synthesis is higher than for the rest of the proteome (Marguerat et al. 2012; M. W. Schmidt et al. 2007). The growth laws for the initiation and elongation factors were almost identical to each other, suggesting constant stoichiometry with the growth rate (**Supp. Fig. S4.1B-C**). Within the IET category, elongation factors were about three times as abundant as initiation factors, and about fifty times compared to termination factors (**Supp. Fig. S4.1B-C**). This is in line with biochemical evidence showing that translation initiation is a limiting step for protein synthesis (Aylett and Ban 2017). Taken together, we have shown how the growth law can inform on the regulation of gene expression through changes in the stoichiometry of factors with the growth rate.

Furthermore, the large burden of RPs during fast growth resulted from the coordinated growth-related expression of most individual RPs and from a growth dependence component steeper than that of IET and RiBi (**Fig. 4C**). This indicates that the aggregate burden of RPs results from coordinated regulation at the level of single genes (Petibon et al. 2020). The IET and RiBi categories also contained more proteins that were assigned to the P- and Q-sectors, and/or whose expression data was not well explained by the robust model due to significant condition-dependent expression (**Supp. Fig. S4.2**). For instance, the initiation factor eIF3E was present in sub-stoichiometric amounts relative to eIF3A. Interestingly eIF3E has been shown to selectively regulate the translation of transcripts coding for metabolic enzymes (Shah et al. 2016).

Principles of proteome allocation are often conserved in prokaryotes and eukaryotes despite significant mechanistic differences in the way genes are transcribed and translated (Dai and Zhu 2020). Therefore, we thought to compare our findings in fission yeast with published datasets from the budding yeast *S. cerevisiae* and the bacterium *E. coli* (A. Schmidt et al. 2016; Metzl-Raz et al. 2017). We reanalysed the proteomics data for *E. coli* cells growing at different rates in a series of environmental conditions and extracted the growth law parameters for translational proteins (**Methods, Supp. Table S9**) (A. Schmidt et al. 2016). For *S. cerevisiae,* we used growth law parameters of ribosomal proteins published elsewhere (Metzl-Raz et al. 2017). We found that *E. coli* could sustain a given growth rate with a smaller fraction of RPs than both yeasts (note the smaller slope, **Fig. 4D**). This suggests that the effective translation rate in the yeasts is lower than that of *E. coli.* Among the two yeasts, fission yeast used its RPs significantly more efficiently than the budding yeast trendline, but the effect could not be assigned to a significant difference in either the slope or the intercept parameter specifically. Next, we asked whether the changes in stoichiometry of translational proteins during slow growth were conserved in *E. coli*. Again, both the IET/RP and RiBi/RP ratios were higher during slower growth (**Supp. Fig. S4.3A-B**), because the individual RPs had steeper growth laws (**Supp. Fig. S4.3C**). A steeper growth law of RPs than that of elongation factors was recently predicted by a model of *E. coli* that minimised the total expression cost (Hu et al. 2020). Our results indicate that allocation strategies are conserved even though protein production differs mechanistically between the two kingdoms.

P sector proteins are part of the core environmental stress response programme

To complement our analysis of the R sector, we next examined fission yeast proteins from the P sector, i.e., proteins with a negative growth-dependent component. In contrast to the R sector clusters 1 and 2, we could not identify P sector clusters whose expression could be explained exclusively by a negative growth rate correlation (**Fig. 1A-C**). This indicates that proteins with a strong P component are also often regulated in response to specific nitrogen sources. Moreover, the growth component for P proteins was less significant overall than for R proteins (**Supp. Fig. S4.4A-B**). These results suggest that regulation of the R and P sectors may differ mechanistically.

Unlike R proteins, which are mostly involved in protein production, P proteins belonged to a diverse set of complexes participating in a large array of functions (**Fig. 3A**). As individual proteins, they showed weaker correlations than R sector complexes (**Supp. Fig. S4.4C-D**). To analyse whether this diverse set of P proteins was participating in a common higher-level functional programme we analysed the fission yeast GO-slims alongside 21 lists covering fission yeast physiology and environmental responses (**Fig. 4E, Supp. Fig. S4.5A)** (Mata et al. 2002; D. Chen et al. 2003; Rustici et al. 2004; Marguerat et al. 2012; Rallis, Codlin, and

Bähler 2013; Saint et al. 2019; Kamrad et al. 2020). Functional classes with strong P-sector components included vacuole biology, endosome and phagosome, transport and genes induced in the adaptation to nitrogen removal, and/or after treatment with caffeine and rapamycin. The latter two classes, which had the strongest response, are thought to be controlled by TORC1 (Mata et al. 2002; Rallis, Codlin, and Bähler 2013). This suggests that nitrogen sources supporting slower growth rates trigger a form of metabolic stress response. Accordingly, the total expression of the fission yeast core environmental stress response programme upregulated genes (CESR up) was negatively correlated with the growth rate (**Fig. 4F**). This stress module comprises genes induced in response to a wide range of environmental and genetic perturbations (D. Chen et al. 2003; Pancaldi, Schubert, and Bähler 2010). Conversely, genes downregulated as part of the CESR response (CESR down, also called growth module) belonged to the R sector (**Fig. 4F-G, Supp. Fig. S4.5B)**. This finding validates the longstanding hypothesis that the balanced expression of the fission yeast stress response is quantitively connected with the growth rate (López-Maury, Marguerat, and Bähler 2008). Additionally, P proteins were enriched for factors regulated during the S phases of the cell cycle, which is consistent with evidence that the cell-cycle phase length differs between nitrogen sources, in particular growth on Trp (**Fig. 4E and Supp. Fig. S4.5C-D**) (Carlson et al. 1999; Rustici et al. 2004).

Notably, the functional classes involved in metabolism were not strongly negatively correlated with the growth rate (**Fig. 4E**), and the fission yeast P sector was only marginally enriched in proteins involved in central and energy metabolism (**Supp. Fig. 4.6**). This contrasts with previous data from *E. coli* and *S. cerevisiae* where metabolic genes have been reported to be important components of the P sector (Hui et al. 2015; A. Schmidt et al. 2016; Metzl-Raz et al. 2017). However, when considered globally, the sum of protein mass fractions dedicated to metabolic enzymes was clearly anti-correlated with growth in fission yeast, ranging from ~70% of the proteome in poor nitrogen sources to ~55% in the fastest media (**Fig. 5A**). This indicates that in our system which does not rely on titration of a limiting nutrient to modulate the growth rate, the total protein burden on metabolism is linked to the growth rate, whereas allocation to specific enzymes is not. Therefore, the global anti-correlation of metabolic enzymes with growth rate observed in our data may be a manifestation of the trade-off between metabolism and translation, and not the result of the direct quantitative regulation of metabolic enzymes expression with the growth rate.

The burden of specific metabolic pathways is principally condition-dependent

On top of the growth-dependent components, many fission yeast proteins show clear condition-specific gene regulation (**Fig. 2A-C**, clusters 3-10). Functional analysis indicated an

enrichment of these genes for functions related to metabolism. This is consistent with the adoption of distinct metabolic allocation strategies in response to growth with different nitrogen sources (Alam et al. 2016; Mülleder et al. 2016). We classified metabolic genes into six non-overlapping classes based on the following GO terms: canonical glycolysis (GO:0061621), generation of precursors and energy (GO:0006091), cellular amino acid metabolic process (GO:0006520, which includes the interconversion of ammonium, glutamate, and glutamine), lipid metabolic process (GO:0006629), vitamin metabolic process (GO:0006766), and all other metabolic pathways (including transport of metabolites) (**Fig. 5B, Supp. Fig. S5.1, Supp. Table S10**). To avoid overestimating the burden of gene expression by double-counting genes assigned to multiple terms, each protein was assigned only to the first of these GO-terms it was annotated with. The relative allocation to each class was condition-specific, indicating that metabolic states rely differentially on specific pathways (**Fig. 5B**). We note that similar growth rates can be supported by different allocation strategies, as in the case of the Trp and Gly containing media in which cells channelled resources preferentially towards glycolysis (Trp) or amino acid metabolism (Gly) (**Fig. 5B, Supp. Fig. S5.1**). The growth-related components of those categories were weak, except for the vitamin metabolism proteins which belonged to the R sector and the precursor/energy proteins that showed a significant P component (see below, **Supp. Fig. S5.1**). Most coenzymes are stable molecules synthetised only as much as necessary to support growth (Hartl et al. 2017). The strong positive correlation of vitamin metabolism expression with growth rate suggests that cells also minimise the translation burden of vitamin metabolic enzymes. In summary expression of metabolic enzymes in our system, although connected to the growth rate, is mainly condition- and pathway-specific.

We next took a closer look at the energy metabolism pathways and their negative correlation with the growth rate. Nutrient quality, cell growth, and energy metabolism are intimately connected. The generation of ATP through fermentation is often favoured in conditions that support faster growth whereas slow-growing cells in limiting conditions tend to switch to respiratory metabolism (Heiden, Cantley, and Thompson 2009; Shimizu and Matsuoka 2018). Therefore, we asked whether protein allocation to either energy metabolism pathway was correlated with the nitrogen sources used and/or growth rate. To this end, we split the non-glycolytic generation of precursors and energy category into the fermentative enzymes pyruvate decarboxylase (Pdc101) and alcohol dehydrogenase (Adh1), and the respiration process into tricarboxylic acid cycle (TCA, GO: 0006099) and oxidative phosphorylation (OXPHOS, GO:0006119) enzymes (**Fig. 5C, Supp. Fig. S5.2)**. Surprisingly, none of the categories were consistently correlated with the growth rate. Instead, condition-specific expression was dominant, and a clear repression of all OXPHOS complexes upon growth on serine was observed (**Supp. Fig. S5.3**). A recent report showed that serine catabolism

generates high levels of reactive oxygen species (ROS) in *S. pombe*, suggesting that respiration may be repressed upon growth on serine to avoid a further increase in ROS (Kanou et al. 2020). Notably, expression of the fermentative enzymes Adh1 and Pdc101, although variable between conditions, was consistently higher than the total expression of the respiratory enzymes. Moreover, respiratory enzymes were not induced in nitrogen sources supporting slow growth. Taken together, the expression balance between fermentation and respiratory enzymes was not quantitatively connected to the growth rate, but depended on the nutrient properties.

To complement this analysis, we searched for condition-specific patterns of protein expression that were not related to the growth rate in our proteomics dataset using principal component analysis (PCA) (**Supp. Fig. S5.4**). The first principal component (PC1) explained 29% of the total variance and split the culture conditions in two irrespective of the growth rate with Trp (W), Phe (F), Ser (S), and Pro (P) in one group (from here on termed the WFSP media) and Gly (G), Ile (I), Glu (E) and Amm in the other (**Fig. 5D**). Strikingly 24% (495/2045) of proteins had more than 50% of their variance explained by PC1. We defined two large classes of protein based on their response to this component: i) WFSP+ consisting of 275 proteins that were positively correlated with PC1 and therefore induced in the WFSP media; ii) WFSP- characterised by 220 proteins with expression negatively correlated with PC1 and therefore repressed in the WFSP media (**Supp. Table S11**). Interestingly, no single principal component was dominated by growth rate correlation (**Supp. Fig. S5.4E**), reinforcing the point that nutrient-specific and growth-dependent components of gene expression coexist for many proteins.

Glycolytic and NAD-dependent enzymes were the two major classes of proteins overrepresented in the WFSP lists. First, most glycolytic enzymes belonged to one of the two WFSP classes (**Fig. 5E-F, Supp. Fig. S5.5**). These enzymes were highly expressed across conditions, amounting to ~15%–30% of the total proteome mass (**Fig. 5B, Supp. Fig. S5.1**). Therefore, the total gene expression burden of cellular metabolism across the WFSP conditions was heavily affected by the abundance of a small number of enzymes. Second, the two enzymes glyceraldehyde-3-phosphate (G3P) dehydrogenase Tdh1 and alcohol dehydrogenase Adh1 were assigned to opposing WFSP lists, and the ratio of Adh1/Tdh1 expression was highly elevated in the WFSP conditions (**Fig. 5F-G**). Fermentation of a single molecule of glucose generates two molecules of ethanol and carbon dioxide. During the process, Tdh1 reduces two NAD+ molecules and Adh1 oxidises two NADH molecules. Therefore, the elevated Adh1/Tdh1 balance exerts a pressure on the NAD+/NADH equilibrium towards the NAD+ side. The induction of Adh1 and repression of Tdh1 proteins may be a controlled response to maintain homeostasis under disruptions to the NAD+/NADH redox

balance. This way, differential resource allocation towards the NAD-cycling glycolytic–fermentation pathway may indicate that the metabolic rewiring invoked by the WFSP nitrogen sources could result from changes in the cell redox balance.

To follow up on this observation, we further investigated the burden of NAD-dependent pathways. NADH is oxidised by NADH dehydrogenases that are situated in the inner mitochondrial membrane; the enzyme transfers two electrons per NADH molecule to the electron transport chain to power ATP synthesis. On the other hand, NAD+ is reduced several times during each iteration of the TCA cycle by the α-ketoglutarate (αKG) dehydrogenase complex (KGDHC), the isocitrate dehydrogenase (IDH) complex, and the malic enzymes. Fission yeast is thought to have two separate NADH dehydrogenase enzymes, Ndi1 and Nde1, with the NAD-binding domain of Ndi1 facing the mitochondrion and Nde1 facing the cytosol. We examined the expression burden of these enzymes in our data and found that, although neither belonged to one of the WFSP lists, the ratio of Nde1/Ndi1 expression was strongly elevated in the WFSP conditions (**Fig. 5H, Supp. Fig. S5.6**). The IDH complex comprises the two subunits Idh1 and Idh2, and KGDHC consists of four subunits: Kgd1, Kgd2, Ymr31, and Dld1, the latter being part of multiple complexes. Dld1 and Idh2 were part of the WFSP+ class, unlike any of the other subunits. As above, the ratio of protein abundances for Dld1/Kgd1 and Idh2/Idh1 were elevated in the WFSP conditions (**Fig. 5I-J, Supp. Fig. S5.6**). Therefore, the response to the WFSP nitrogen sources altered the stoichiometry of NAD-dependent enzymatic complexes.

Importantly, these signatures were not detected in our transcriptomics data, suggesting a role for post-transcriptional regulation. In line with this, ubiquitin and its related pathways, as well as the translation factors eIF3e and eIF5A, showed strong WFSP patterns suggesting a role for protein stability (**Supp. Fig. S2.4E, Supp. Fig. S4.2B-D, Supp. Table S11**). In summary, we identified two distinct cellular states that differed in the expression of enzymes involved in fermentation and the cell's redox balance that were not correlated with the growth rate.

Correcting for growth-rate dependence revealed additional transcriptional signatures of growth on single amino acid sources

Defining the heterogeneity of metabolic states is key to a mechanistic understanding of cell population evolution, but this requires disentangling the gene signatures that depend on the growth rate from those that are purely nutrient specific. Our dataset has the unique capacity to achieve this. We performed differential expression analysis on our RNA-Seq dataset, by comparing each growth condition to a reference transcriptome obtained via averaging all the conditions, and corrected for the growth-dependent component of gene expression (**Methods**). We defined 10 signatures (termed R1–R10) by clustering the $\log_2$-transformed

fold change ratios with respect to the synthetic reference of all genes that were significantly enriched in at least one condition (**Fig. 6A, Supp. Fig. S6.1, Supp. Table S12**).

The 10 signatures covered the differential expression of 2269 genes in total, representing 44% of the fission yeast transcriptome. Five signatures (R3, R4, R7, R8, and R9) were also visible at the proteome level (**Fig. 6B**). About 69% of the mRNA present in the transcriptomic signatures were quantified in at least one condition in the proteome and ~40% were detected in all conditions, indicating that this relatively limited agreement was not due to the lower coverage of the proteomics data.

We next performed functional enrichment analyses of the transcriptomics clusters (**Methods**), using Gene Ontology annotations (Gene Ontology Consortium 2019; Lock et al. 2019). Broader functional categories were captured using GO-slim analysis (**Fig. 6C)**, and specific pathways using terms from the biological_process ontology with at most 50 annotations (**Supp. Fig. S6.2**). In agreement with our observation that respiratory genes were repressed in Ser medium, the Ser repressed cluster R4 was strongly enriched for genes related to mitochondrial metabolism. Additionally, genes from the Ser induced cluster R8 were enriched for iron ion homeostasis. Both parts of the Ser response contained oxidoreductases, which is compatible with the recently reported high levels of ROS generated by serine catabolism (Kanou et al. 2020). The Trp repressed cluster R3 was enriched for genes related to amino acid metabolism, again suggesting that the slow growth sustained by the Trp medium was not due to any additional burden of disrupted amino acid synthesis. The smaller cluster R9 was enriched for genes related to pheromone activity (M-factor precursors), signalling, and the induction of meiosis (**Supp. Fig. S6.2**). Interestingly, the signature expression across conditions for these genes (induced in Trp, Phe, Pro, and Glu containing media) mirrored that of Mae2 (**Supp. Fig. S2.4F**), which removes excess carbon from the TCA cycle. As meiosis is usually induced by nitrogen starvation (Petersen and Russell 2016), this result suggests that the state of central carbon metabolism may also play a role in the meiotic transition, as (elemental) nitrogen was abundant in all growth media used. Altogether, we identified a rich set of metabolic signatures that were not dependent on the growth rate, but exclusively reflect changes in external nutrients.

CONCLUSIONS

In this study we quantified the proteome and transcriptome of the fission yeast *S. pombe* grown in eight defined media that affect the growth rate. Each medium contained a single nonlimiting source of nitrogen, such that variations in gene expression were determined by system-level resource allocation and not by the response of a single pathway to the titration of a limiting nutrient. This set up is in contrast to other studies which relied on a specific limiting

nutrient to perturb resource allocation while affecting the growth rate (Brauer et al. 2008; Hui et al. 2015), or leaving it constant (Yu et al. 2020) .

Using this orthogonal approach, we propose a model in which shifts in resource allocation trigger two layers of gene expression regulation. The first layer consists of gene expression that is significantly correlated with growth rate and the second is condition-specific depending solely on nutrients. Many proteins and mRNAs showed a combination of both layers of regulation. This suggests that condition-specific responses occur on top of a global level gene regulation that is coordinated with the growth rate (Shahrezaei and Marguerat 2015). Importantly, the global layer of regulation discussed here affects relative abundances of proteins and of mRNAs, and is distinct from the scaling of gene expression to the growth rate which ensures constant biomolecule concentrations (Chávez et al. 2016). The mechanisms behind the observation that a large number of mRNA and proteins show some level of global scaling with the growth rate are not entirely clear. It could be related to the fact that expression of the protein production machinery itself increases with the growth rate and to changes in levels of TOR signalling for instance (see below). This could result in different cellular states that feedback globally on gene expression (Keren et al. 2013). It is of note that the growth-rate-dependent component defined in this study might in some cases complicate the interpretation of condition-specific responses and should then be taken into account (Pancaldi, Schubert, and Bähler 2010; Yu et al. 2021).

Eukaryotic growth-rate-related gene expression depends to some extent on the TORC1 axis of gene regulation, which is widely conserved across eukaryotes (Weisman 2016; González and Hall 2017; Morozumi and Shiozaki 2021). TORC1 activity is affected by a variety of stressors including nutrient starvation. Upstream of TORC1, the adenosine monophosphate kinase AMPK has been proposed to mediate the response to nitrogen starvation, and intriguingly, the two complexes can inhibit each other (Davie, Forte, and Petersen 2015; Ling et al. 2020). Downstream, the TORC1 pathway is a key regulator of the balance between the stress and growth modules (López-Maury, Marguerat, and Bähler 2008; Rallis, Codlin, and Bähler 2013; Rallis et al. 2014), with targets including eukaryotic initiation factor 2 subunit alpha (eIF2α) (Valbuena, Rozalén, and Moreno 2012), the SAGA complex (Laboucarié et al. 2017), and the rate of fermentation through Greatwall and $PP2A^{B55\delta}$ (Watanabe et al. 2019). These questions are often studied during adaptation to changing conditions and our system using continuous culture in turbidostats provides an attractive set up for future studies of the mechanisms that maintain the stress vs growth gene expression balance in steady-state conditions.

We found that known chromatin modifiers belonged to the R-sector. This is intriguing as expression of histones themselves were not dependent on the growth rate (**Supp. Table S6**). This may suggest that number of histones modifying enzymes and levels of modifications are rate limiting for transcription, or alternatively mediate an orthogonal function such as signalling the cell metabolic state through covalent protein modifications (Mellor 2016; Figlia, Willnow, and Teleman 2020; Morgan and Shilatifard 2020). This illustrates the intricate relationship of chromatin structure with the cell metabolism. Moreover, we found that RNAPII expression was not increasing with the growth rate suggesting that, unlike for gene expression scaling to cell size, its numbers are not limiting for the rate of growth (Padovan-Merhar et al. 2015; Sun et al. 2020). Yet, maintaining constant mRNA concentrations requires synthesis or degradation rates to adjust to cell growth. Therefore, other mechanisms such as transcription elongation or mRNA decay rates are likely to be modulated with the growth rate as suggested in budding yeast (Chávez et al. 2016).

Discussing protein allocation in term of factors limiting for growth relies on the assumption that expression of all proteins is optimised for growth in any given condition. Recent evidence has challenged this view and has suggested that significant parts of *E. coli* (Valgepea et al. 2013; Peebo et al. 2015; Mori et al. 2017) and budding yeast (Metzl-Raz et al. 2017; Yu et al. 2020) gene expression are not immediately required for sustaining the growth rate and are instead held in reserve. This reserve pool of protein could support cell adaption to sudden environmental changes. It has furthermore been suggested that central carbon metabolism has a large reserve capacity, suggesting that many enzymes may also not be utilised solely to maximise metabolic fluxes (O'Brien, Utrilla, and Palsson 2016; Christodoulou et al. 2018; Yu et al. 2020). In this study, whereas several nutrient-specific regulatory programmes were detected in both the transcriptome and the proteome, such as specific responses to Ser and Trp, this was not true for the WFSP pattern and other transcriptomics signatures (**Figs. 5 and 6**). This disconnect could means that metabolic pathways are differentially buffered through protein levels and stability which could in turn be interpreted in term of reserve capacity. A better understanding of post-transcriptional regulation in fission yeast will be important to fully understand what causes the high translational burden of metabolism.

We found that expression of metabolic enzymes was strongly condition-specific and only marginally anti-correlated with the growth-rate. This condition-specific regulation represented a large change in the gene expression burden, driven by glycolytic proteins and enzymes and complexes relying on NAD turnover. Interestingly, this large variation in expression burden of the carbon metabolism resulted from changes in nitrogen source and occurred in the presence of abundant external glucose. This highlights the fact that metabolic adaptation to external condition is pervasive not only in term of fluxes but also in term of gene expression burden.

The catabolism of the backbones of the amino acids used as nitrogen sources could provide a link between nitrogen and carbon metabolism in our system. Our data provide a rich resource to constrain future genome-scale models of fission yeast that integrate metabolism and gene expression, which will allow testing this hypothesis (O'Brien et al. 2013; Sánchez et al. 2017; Y. Chen et al. 2020).

An improved understanding of the fundamental principles behind cellular growth and the physiological and translational burden of metabolism across evolutionarily diverse biological systems would influence a wide range of research areas such as microbiology, synthetic biology, and cancer research. Cellular models of growth should integrate strategies used by a variety of organisms under a wide range of conditions, in order to identify common principles. Beyond its contribution to our understanding of gene regulation, this work will support future experimental and modelling efforts aimed at defining the nature of the trade-offs involved in growth, stress resistance, and metabolism across the tree of life.

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FIGURE LEGENDS

Figure 1: Characterisation of culture growth in turbidostats across eight minimal media. **A.** Illustration of the turbidostat culture chamber with the control flow and analysis pipeline. **B.** Example growth curve (Ile replicate 2) showing different growth phases in the turbidostat. **C.** Estimated growth rates μ based on a two-fold dilution and regrowth cycle for the 8 growth media using 3 biological replicates each. Amm: ammonium chloride, equal to standard EMM2 medium. **D.** Total number of generations each culture spent in a turbidostat. **E.** Total time in hours each culture spent in a turbidostat, with the duration of individual growth phases coloured as in B. Note that, with N_G the number of generations, T the time spent in the turbidostat, μ the growth rate, and T_d the doubling time, $T_d = \ln(2)/\mu$ and $N_G = T/T_d$.

Figure 2: Fission yeast gene expression shows growth-rate dependent and nutrient-specific components. A. Hierarchical clustering of *z*-score transformed protein expression fractions for the 2045 protein groups detected across all conditions for cells grown in 7 single amino acids or NH_4Cl (Amm) using 3 biological replicates. Growth conditions are ordered by increasing growth rate. Ten clusters are labelled on the left together with manual summary of enriched functional categories (see Supp. Fig. S2.1). **B.** Summed protein mass fractions for the 10 clusters defined in A as a function of the growth rate. Repeated-median linear model (RMLM) fit is shown as a black line and the predicted 2.5^{th}–97.5^{th} percentile confidence interval (CI) of the fit as the grey shaded area. **C.** As shown in B, for DESeq2-normalised RNA-Seq counts. **D.** Assignment of 2077 proteins detected across all conditions and their respective transcripts to the R (orange), P (blue), and Q (grey) sectors based on protein fractions (left) and DESeq2-normalised counts (right). Each protein is connected to its corresponding transcript by a line and colours are according to the protein sectors. **E.** Sum of protein fractions for the R (orange), P (blue), and Q (grey) sectors as a function of growth conditions. The figure includes all 3510 protein groups detected in at least one condition. Best fit and predicted CI are plotted for the ordinary least squares (OLS) linear model. **F.** As shown in E, for DESeq2-normalised RNA-Seq counts for 5135 detected genes. Abbreviations: P_P, Q_P, R_P: protein groups assigned to P-, Q-, and R-sector. P_T', Q_T', R_T': transcripts corresponding to protein groups detected across all conditions assigned to P-, Q-, and R-sector. P_T, Q_T, R_T: all transcripts assigned to P-, Q-, and R-sector.

Figure 3: Proteins from the R sector are involved in every level of the protein production programme. A. Fraction of R (orange), P (blue), and Q (grey) genes in manually curated broad categories of protein complexes. The number of complexes (C) and genes (G) in each category are shown in parentheses. The 4 leftmost categories encompass the protein production programme. **B.** Volcano plot of protein complexes belonging to the broad categories "snoRNA regulation", "Protein translation", "Ribosomal proteins", and "Ribosome biogenesis" in the protein production programme. The plot shows the $-\log_{10}$ of the *q*-value of the repeated-median linear model (RMLM) fit on the sum of normalised counts in each protein complex as a function of the growth rate against a normalised estimate of the slope of the fit (see **Methods**). **C.** As shown in B for complexes belonging to the “mRNA regulation” and “Chromatin regulation” categories. **D.** Sums of DESeq2-normalised counts for subunits of RNAPI (left), II (middle) and III (right) are plotted as a function of the growth rate. The sums of subunits unique to a given complex are plotted in orange and of all subunits are plotted in grey. RMLM fits are shown as lines and the predicted 2.5^{th}–97.5^{th} percentile confidence interval (CI) of the fit as shaded areas.

Figure 4: Stoichiometries of translation complexes, comparison of ribosomal growth law with other species, and functional analysis of P sector. A. Sum of the protein fractions

plotted as a function of the growth rate for factors involved in translation initiation, elongation, and termination (IET; left), ribosome biogenesis (RiBi; middle), or ribosomal proteins (RP; right). The best fit and bootstrapped 95% confidence interval (CI) are shown in black and grey, respectively. The fold change (FC) values ± standard deviations of the bootstrapped values are shown. **B.** Proteome mass ratio plotted as a function of the growth rate for the following comparisons: IET vs RP (left), RiBi vs IET (middle), and RiBi vs RP (right). Shown in black/grey are the predictions and 95% CIs as given by the linear models fitted to the data in A. **C.** FC values for proteins of the IET, RiBi, and RP categories plotted as a function of their median expression. Proteins assigned to the R, P, and Q sectors are coloured in orange, blue, and grey, respectively. **D.** Total proteome mass fraction allocated to ribosomal proteins as a function of growth rate for *S. cerevisiae* (red) (Metzl-Raz et al. 2017), *S. pombe* (green), and *E. coli* (grey) (data from (A. Schmidt et al. 2016). RMLM fits and 95% CIs are shown as lines and shaded areas, respectively. **E.** The $-\log_{10}$ Q-value of repeated-median linear model (RMLM) fits plotted against their respective FC values for proteins belonging to GO-slim and literature lists (Mata et al. 2002; D. Chen et al. 2003; Rustici et al. 2004; Rallis, Codlin, and Bähler 2013; Kamrad et al. 2020). List with a significant negative slope (q-value < 0.001) are highlighted in blue. BP GO-slim terms related to metabolism are highlighted in green, stress/growth modules from (D. Chen et al. 2003) in vermillion, and cell cycle induced modules from (Rustici et al. 2004) in orange. **F.** Sum of protein fractions plotted as a function of the growth rate for the Core Environmental Stress Response (CESR) repressed (growth module) or induced (stress module) genes. RMLM fit and predicted 95% CI as in A. **G.** Assignment of growth and stress module proteins (Prot) detected in all samples and their respective transcripts (Trans) to the R (orange), P (blue), and Q (grey) sectors based on protein fraction expression and DESeq2 normalised counts. Each protein is connected to its corresponding transcript by a line and the colours correspond to the protein sectors.

Figure 5: The coordination of energy metabolism enzymes with the growth rate is marginal A. Sum of protein fractions of proteins involved in translation and ribosome biogenesis (red, see Figure 4A), energy metabolism and transport (green, see Figure 4E) or all other genes (grey) plotted as a function of the growth rate. **B.** Relative proteome fractions of 5 categories of proteins involved in metabolism. The median of the three replicates from each condition was used for calculating the protein fractions and plotting growth rates. **C.** As shown in B, for proteins of the OXPHOS and TCA pathways, the Adh1 and Pdc101 fermentation proteins, and proteins annotated as "generation of precursor metabolites and energy" and not included in the other four categories or glycolysis. **D.** Protein expression as a function of growth rate as exhibited by the first principal component (PC1). **E.** Comparison of the first two principal components (PCA biplot) for each protein group detected in the proteome across all conditions. Areas with >50% variance explained by PC1 correlation are highlighted in yellow (negative correlation, WFSP-) and pink (positive correlation, WFSP+). Genes related to glycolysis and ethanol fermentation are indicated in blue. **F.** Topology of the glycolysis and ethanol fermentation pathway showing genes, cofactors, and selected metabolites, with colours as in E. **G.** Left: ratio of protein fractions for Adh1/Tdh1 plotted as a function of the growth rate. Right: diagram showing Adh1 and Tdh1 functioning together with median proteome fractions of both proteins in each condition. Colours are as annotated in D. **H.** As shown in G for Nde1 and Ndi1. **I.** Ratio of protein fractions of Idh2 and Idh1 plotted as a function of the growth rate. Colours are as annotated in D. **J.** As shown in I for the ratio of the protein fractions ofDld1 and Kgd1 plotted as a function of growth rate.

Figure 6: Transcriptomic signatures for growth on amino acid sources. A. DESeq2 $\log_2$ fold change ratios (scale capped at abs($\log_2(fc)$) = 5) for the 10 signatures R1-R10. Fold changes are relative to the RMLM-predicted synthetic reference (**Methods**). Columns are ordered according to the growth rate and rows are ordered by hierarchical clustering (**Supp.**

Fig. 6.1). **B.** The log_2-transformed ratios of observed versus RMLM-predicted protein fractions for genes in the R1-R10 signatures. Row and column orders are as described in A. Genes missing from the proteomics data are in grey. **C.** Functional analysis of the transcriptomics clusters R1-R10 as shown in A. Enrichment for GO slim terms belonging to the "biological process" (top), "cellular component" (middle), and "molecular function" (bottom) categories are shown. The colour scheme denotes the local false discovery rate (*lfdr,* capped at $1e^{-6}$ and printed on the figure if capped) from a Fisher exact one-sided test for the overlap of each cluster with functional lists. Only significant lists are shown (*lfdr* < 0.05) and the number of genes in each category and cluster are shown in parentheses.

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S2.1: Functional analysis of the proteome clusters from Figure 2A. Enrichment for GO slim terms belonging to the "biological process" (top), "cellular component" (middle), and "molecular function" (bottom) categories are shown. The colour scheme denotes the local false discovery rate (*lfdr*, capped at $1e^{-6}$ and printed on the figure if capped) of a Fisher exact one-sided test for the overlap of each cluster with the functional lists. Only significant lists are shown (*lfdr* < 0.05) and the number of genes in each category and cluster are shown in parentheses.

Supplementary Figure S2.2: mRNA dependence on the growth rate. Hierarchical clustering of the *z*-score transformed DESeq-normalised RNA-Seq counts for 4979 mRNAs expressed across all conditions in the RNA-Seq dataset for cells grown on 7 single amino acids or NH_4Cl (Amm). The growth conditions are ordered by increasing growth rate. The twelve clusters are indicated on the left. **B.** Sum of DESeq-normalised RNA-Seq counts for the 12 clusters defined in A as a function of growth conditions. The growth conditions are ordered by increasing growth rate. The RMLM best fit is shown in black and the predicted 95% confidence interval in grey.

Supplementary Figure S2.3: ncRNA dependence on the growth rate I. Hierarchical clustering of *z*-score transformed DESeq-normalised RNA-Seq counts for 1211 ncRNAs for cells grown on 7 single aamino acids or NH_4Cl (Amm). The growth conditions are ordered by increasing growth rate. The nine clusters are labelled on the left. **B.** Sum of DESeq2-normalised RNA-Seq counts for the 9 clusters defined in A as a function of growth conditions. The growth conditions are ordered by increasing growth rate. The RMLM best fit is shown in black and the predicted 95% confidence interval in grey. **C.** The *z*-score transformed DESeq2 normalised RNA-Seq counts of all genes that neighbour the ncRNAs from the clusters defined in A are shown. Annotations of flanking genes were taken from (Atkinson et al. 2018). **D.** Sum of DESeq2-normalised RNA-Seq counts (NC) for the neighbouring genes for each cluster defined in A.

Supplementary Figure S2.4: Illustration of repeated-median linear model (RMLM) fits on single genes. A. Example R-protein Rpl402. **B.** Example P protein Suc22. **C.** Example Q protein group comprising Hht1, Hht2, and Hht3. **D.** Example of P protein with additional medium-specific expression Snz1. **E.** Example WFSP+ pattern (see **Fig. 5D**) with a poor RMLM fit for the protein group comprising Ubi3, Ubi4, and Ubi5. **F.** Additional example of a poor RMLM fit for the protein Mae2. The best fit for the RMLM is shown as solid black lines and the predicted 95% CI in grey. The best fit for the ordinary least squares (OLS) linear model is shown as dashed grey lines. **G.** Illustration of the fold change (*FC)* calculation for the example protein Rpl402 indicating the relationship between the *FC* values, slope, and the y-intercept of the fit. **H.** Growth law shapes corresponding to a series of example *FC* values.

Supplementary Figure S2.5: Growth category assignment of proteins and the corresponding transcripts from clusters defined in Figure 2A. Assignment of proteins from the clusters defined in Figure 2A (Prot) and their respective transcripts (Trans) in the R (orange), P (blue), and Q (grey) sectors based on protein fraction expression and DESeq2-normalised counts. Each protein is connected to its corresponding transcript by a line coloured as per the protein classification.

Supplementary Figure S3.1 Volcano plot of protein complexes that do not belong to those illustrated in Figure 3B-C. The $-\log_{10}$ of the *q*-value of the repeated-median linear

model (RMLM) fit on the sum of normalised counts in each protein complex as a function of the growth rate are plotted against a normalised estimate of the slope of the fit (see **Methods**).

Supplementary Figure S4.1: Stoichiometries of translation complexes as a function of the growth rate. A. Bootstrapped parameter densities of fold change (FC), slope and intercept values for the IET (blue), RiBi (pink), and RP (red) categories. The analysis is based on the 1000 bootstrap samples used in Figures 4A and B. **B.** The sum of protein fractions in the translation "Initiation", "Elongation", and "Termination" categories plotted as a function of the growth rate. The best fit and bootstrapped 95% CI are shown in black and grey, respectively. The FC values ± standard deviations of bootstrapped values are shown. **C.** The proteome mass ratio plotted as a function of the growth rate for the following comparisons: Elongation vs Initiation (left), Termination vs Initiation (middle), and Termination vs Elongation (right). Shown in black and grey are the predictions and 95% CIs, respectively, as given by the linear models fitted to the data in B.

Supplementary Figure S4.2: Analysis of translational proteins with non-positive or weak growth-rate correlations. A. Normalised sum of squared residuals (SSR) versus coefficient of determination (R^2) for repeated-median linear model (RMLM) fits to protein groups involved in translation initiation, elongation, and termination (IET, left), ribosome biogenesis (RiBi, middle), and to ribosomal proteins (RP, right). All P-sector proteins were labelled, R-sector proteins were labelled if their normalised SSR was greater than 0.1; for Q-sector proteins the threshold was 0.2. **B.** Proteome burden associated with translation elongation and termination factor eIF5A (Tif512) as a function of growth rate. The best fitted RMLM is shown as a solid black line, with its predicted 95% CI in grey. The best fitted ordinary least squares model is shown as a dashed grey line. **C.** As shown in B for translation initiation factor eIF3e (Int6). **D.** Ratio of protein mass fractions for Int6 and the major eIF3 subunit Tif301 plotted as a function of the growth rate.

Supplementary Figure S4.3: Stoichiometries of translation complexes as a function of the growth rate in *E. coli* (A. Schmidt et al. 2016)**. A.** Sum of the protein fractions plotted as a function of the growth rate for factors involved in translation initiation, elongation, and termination (IET, left), ribosome biogenesis (RiBi, middle), or for ribosomal proteins (RP, right). The best fit and bootstrapped 95% confidence interval (CI) are shown in black and grey, respectively. The fold change (FC) values ± standard deviations of the bootstrapped values are shown. The type of nutrient or perturbation used to modulate the growth rate are colour coded as per the legend on the right. Data from cultures in stationary phase was not included in the fits. **B.** The proteome mass ratio plotted as a function of the growth rate for the following comparisons: IET vs RP (left), RiBi vs IET (middle), and RiBi vs RP (right). Shown in black and grey are the predictions and 95% CIs, respectively, as given by the linear models fitted to the data in A. **C.** FC values for proteins of the IET, RiBi, and RP categories plotted as a function of their median expression. Proteins assigned to the R, P, and Q sectors are coloured in orange, blue, and grey, respectively.

Supplementary Figure S4.4: Analysis of the residuals of the R and P sectors. A. Violin plots and box plots of the normalised sum of squared residuals (SSR) of the RMLM best fit for mRNA counts or protein fractions belonging to the R (orange) or P (blue) sectors. The *p*-values for the two-sided Wilcoxon rank-sum test are indicated. **B.** As shown in A for the R^2 values. **C.** Normalised SSR of the RMLM fit plotted against their respective R^2 values for all proteins belonging to the R (orange), P (blue), or Q (grey) sectors. **D.** As shown in C for clusters 1 (left), 4 (middle) and 8 (right) of Figure 2A.

Supplementary Figure S4.5: Functional analysis of P sector mRNAs. A. The -$\log_{10}$ *q*-value of RMLM fits plotted against their respective FC values for mRNAs belonging to the GO-

slim and literature lists (Mata et al. 2002; D. Chen et al. 2003; Rustici et al. 2004; Rallis, Codlin, and Bähler 2013; Kamrad et al. 2020). The lists with a significant negative slope (*q*-value < 0.001) are highlighted in blue. The BP GO-slim terms related to metabolism are highlighted in green, stress/growth modules from (D. Chen et al. 2003) in vermillion, and cell cycle induced modules from (Rustici et al. 2004) in orange. **B.** Sum of the DESeq2-normalised counts plotted as a function of the growth rate for the Core Environmental Stress Response (CESR) repressed (growth module), or the induced (stress module) genes. The RMLM fit and predicted 95% CI are indicated as in Figure 4F. **C.** The sum of protein fractions plotted as a function of the growth rate for S phase induced periodic genes (Rustici et al. 2004). **D.** As shown in C for the DESeq2 normalised counts. **E.** Total expression in RNA-Seq (DESeq2-normalised counts, left panel) and proteomics data sets (proteome fraction, right panel) for gene lists induced in the M phase versus G1 phase (Rustici et al. 2004).

Supplementary Figure S4.6: Functional enrichment of P sector proteins. The -$\log_{10}$ of *q*-value (tail-based false discovery rate) of the one-sided Fisher exact enrichment test plotted as a function of the number of genes detected across all conditions in the proteome for all *S. pombe* GO-slim categories. Significantly enriched lists (*q*-value < 0.05) are highlighted in cyan. The BP GO-slim terms related to metabolism are highlighted in green.

Supplementary Figure S5.1: Growth rate specificity of metabolism proteins (related to Figure 5B). Sum of protein fractions plotted as a function of the growth rate for 6 categories covering metabolism (see Figure 5B and Methods).

Supplementary Figure S5.2: Growth rate specificity of energy metabolism proteins (related to Figure 5C). Top left two panels: protein fractions as a function of growth rate for the ethanol fermentation enzymes Adh1 and Pdc101. Remaining three panels: sum of protein fractions as a function of growth rate for the proteins involved in oxidative phosphorylation, TCA cycle, and for those annotated as "generation of precursor metabolites and energy" and that were neither included in the first four panels nor glycolysis.

Supplementary Figure S5.3: Condition specific expression of complexes forming the respiratory electron transport chain and proton pumps. The sum of protein mass fractions as a function of the growth rate (top panels) and median proteome fraction of components of the complex in each condition (bottom panels), for internal NADH dehydrogenase, the succinate dehydrogenase complex, the cytochrome C reductase complex, cytochrome C, the cytochrome C oxidase complex, and the ATP synthases.

Supplementary Figure S5.4: PCA analysis of the proteomics data. A. Cumulative variance explained by the first 9 principal components of the proteomics dataset (**Methods**). **B.** PC1 plotted against PC2 for all proteins belonging to the R (orange), P (blue), or Q (grey) sectors. **C.** As shown in B for PC3 and PC1. **D.** As shown in B for PC3 and PC2. **E.** The relative contribution of each experimental condition plotted as a function of the growth rate for the first 9 principal components of the proteomics dataset. Note that PC1 shows a clear WFSP+ pattern, as repeated in Figure 5D.

Supplementary Figure S5.5: Proteome burdens of enzymes in the glycolysis and ethanol fermentation pathways. Repeated-median linear model fits are shown as black lines and the predicted 2.5^{th}–97.5^{th} percentile confidence intervals of the fits as the grey shaded areas.

Supplementary Figure S5.6: Proteome burdens of selected enzymes with NAD cofactor (related to Figure 5I–J). Repeated-median linear model fits are shown as black lines and the predicted 2.5^{th}–97.5^{th} percentile confidence intervals of the fits as the grey shaded areas.

Supplementary Figure S6.1: Hierarchical clustering of RNA-Seq data after correction for growth-rate dependent gene expression. Counts were normalised to the growth-dependent linear model in DESeq2 and the fold change ratios *fc* were calculated with respect to a synthetic average sample as a reference (**Methods**). Only genes with at least one condition meeting abs($\log_2$(*fc*)) > 0.5 and adjusted *p*-values < 0.01 were selected. Gene-condition pairs not meeting this significance threshold are shown in grey. The colour scale is capped at abs($\log_2$(*fc*)) = 5. The R1-R10 signatures from Figure 6 are shown on the left.

Supplementary Figure S6.2: Functional analysis focusing on informative terms of R1-R10 signatures from Figure 6. Enrichment for GO terms belonging to the "biological process" category with no more than 50 annotations are shown. The colour scheme denotes the -$\log_{10}$ local fdr (*lfdr*, capped at $1e^{-9}$) for Fisher exact one-sided tests for the overlap of each cluster with functional lists. Only significant lists are shown (*lfdr* < 0.01) and the number of genes in each category and clusters are shown in parentheses.

SUPPLEMENTARY TABLE LEGENDS

Supplementary Table S1: Summary of growth conditions for the 24 cultures studied. medium: three-letter abbreviation of growth medium; replicate: biological replicate index; growth_rate: calculated growth rate (in h^{-1}); doubling_time: doubling time as calculated from the growth rate (in h); OD: mean OD_{600} during second phase of stable OD; total_doublings: total number of doubling times elapsed during turbidostat growth; growth_1_h: elapsed time (in h) between inoculation and first meeting of the OD setpoint; constant_2_h: elapsed time (in h) during the initial phase of stable growth; dilution_h: elapsed time (in h) during the approximate twofold dilution; growth_2_h: elapsed time (in h) during regrowth phase following the dilution; constant_2_h: elapsed time (in h) during the second phase of stable OD; total_h: total elapsed time (in h) during turbidostat growth; nitrogen_source: full name of nitrogen source in growth medium; turbidostat: index of physical device that the culture was grown in.

Supplementary Table S2: Summary of protein groups detected in proteomics analysis. PomBaseIDs: database identifiers of the proteins comprising the protein group, i.e that were indistinguishable based on the detected peptides; n_proteins: number of proteins comprising the group; Protein IDs: FASTA headers of the proteins; total_peptides_all: number of different peptides detected in at least one sample that could have been assigned to the group; total_peptides_razor: number of different peptides detected in at least one sample that were assigned to the group; total_peptides_unique: number of different peptides detected in at least one sample that were assigned to the group and could not have been assigned to another group; sequence_coverage: percentage of the protein sequence that overlapped with at least one peptide; sequence_lengths: number of amino acids comprising the group's proteins' peptide chain; mol_weight: molecular weight of protein (in kDa).

Supplementary Table S3: Relative protein expression levels as determined by the proteomics analysis. PomBaseIDs: database identifiers of the protein group; medium: three-letter abbreviation of growth medium; replicate: biological replicate index; iBAQ: intensity based absolute quantification; raw: raw detected intensity; lfq: label-free quantification as reported by MaxQuant.

Supplementary Table S4: Proteome mass fractions as determined from intensity-based absolute quantifications (iBAQ). PomBaseIDs: database identifiers of the protein group; medium: three-letter abbreviation of growth medium; replicate: biological replicate index; proteome_fraction: proteome mass fraction.

Supplementary Table S5: Transcript abundance as determined by RNA-Seq analysis and subsequent normalisation. PomBaseIDs: database identifiers of the protein group; medium: three-letter abbreviation of growth medium; replicate: biological replicate index; normalised_counts: abundance after DESeq2-based normalisation; rpkm: reads per kilobase per million; raw_counts: raw counts detected in sequencing analysis.

Supplementary Table S6: Summary statistics of repeated-median linear model (RMLM) fits and of gene expression. PomBaseID: systematic database identifier of the gene; primary_name: gene standard name; description: gene product description; PomBaseIDs: proteomics protein group identifier; sector.proteins: proteome sector the protein group was assigned to (q-value < 0.1); confident.proteins: whether proteome assignment was confident (local false discovery rate *lfdr < 0.1*); cluster.proteins: cluster the protein group was assigned to (see Fig. 2A); sector.transcripts: transcriptome sector the protein group was assigned to (q-value < 0.1); confident.transcripts: whether transcriptome assignment was confident (*lfdr* <

0.1); cluster.transcripts: cluster the transcript was assigned to (see Supp. Fig. 2.2A); q.value.proteins: *q*-value assigned to the protein RMLM fit; local_fdr.proteins: *lfdr* assigned to the protein RMLM fit; slope.proteins: slope of the protein RMLM fit; intercept.proteins: *y*-intercept of the protein RMLM fit; p.value.proteins: *p*-value associated with the protein RMLM fit; r.squared.proteins: coefficient of determination (R^2) of the protein RMLM fit; fc.proteins: fold change (FC) calculated from the protein RMLM fit; median.proteins: median proteome mass fraction; max.proteins: largest detected proteome mass fraction; min.proteins: smallest detected proteome mass fraction; spread.proteins: difference between largest and smallest detected proteome mass fraction; n_conditions.proteins: number of conditions that the protein was detected in; mean.proteins: mean proteome mass fraction; var.proteins: variance of proteome mass fraction; cv.proteins: coefficient of variation (CV) of proteome mass fraction; ssr.proteins: sum of squared residuals (SSR) to the RMLM for proteome mass fractions; norm_ssr.proteins: normalised SSR for proteome mass fractions (see **Methods**, equation (7)); q.value.transcripts: *q*-value assigned to the transcript RMLM fit; local_fdr.transcripts: *lfdr* assigned to the transcript RMLM fit; slope.transcripts: slope of the transcript RMLM fit; intercept.transcripts: *y*-intercept of the transcript RMLM fit; p.value.transcripts: *p*-value associated with the transcript RMLM fit; r.squared.transcripts: R^2 of the transcript RMLM fit; fc.transcripts: FC calculated from the transcript RMLM fit; median.transcripts: median RNA-Seq normalised counts; max.transcripts: largest detected RNA-Seq normalised counts; min.transcripts: smallest detected RNA-Seq normalised counts; spread.transcripts: difference between largest and smallest detected RNA-Seq normalised counts; n_conditions.transcripts: number of conditions that the transcript was detected in; mean.transcripts: mean RNA-Seq normalised counts; var.transcripts: variance of RNA-Seq normalised counts; cv.transcripts: CV of RNA-Seq normalised counts; ssr.transcripts: SSR to the RMLM for RNA-Seq normalised counts; norm_ssr.transcripts: normalised SSR for RNA-Seq normalised counts (see **Methods**, equation (7)).

Supplementary Table S7: Manual assignment of complexes to broader functional categories. GOID: Gene Ontology (GO) term accession identifier; description: GO term name; category: manually assigned category.

Supplementary Table S8: Assignment of *S. pombe* translation proteins to non-overlapping functional classes. PomBaseIDs: proteomics protein group identifier; annotation: annotation to ribosomal protein (RP), initiation/elongation/termination factors (IET), or ribosome biogenesis (RiBi).

Supplementary Table S9: Assignment of *E. coli* translation genes to non-overlapping functional classes. gene: gene identifier; annotation: annotation to ribosomal protein (RP), initiation/elongation/termination factors (IET), or ribosome biogenesis (RiBi).

Supplementary Table S10: Assignment of metabolic proteins to non-overlapping functional classes. PomBaseIDs: proteomics protein group identifier; annotations: all relevant GO-slim annotations present for the protein group (semi-colon-separated); class: assignment to broad non-overlapping groups (**Fig. 5B**, **Supp. Fig. S5.1**); subclass: for Precursors/Energy class, subdivision into non-overlapping subgroups (**Fig. 5C, Supp. Fig. S5.2**).

Supplementary Table S11: Proteins induced and repressed in Trp (W), Phe (F), Ser (S), and Pro (P) media. PomBaseIDs: proteomics protein group identifier; correlation: Pearson correlation coefficient *R* between *z*-score transformed proteome mass fractions and protein principal component 1 (PC1); r.squared: R^2, WFSP: whether *R>0 (+)* or *R<0 (-)*; PomBaseID:

systematic database identifier of the gene; primary_name: gene standard name; description: gene product description.

Supplementary Table S12: Differential expression analysis after removal of growth rate correlations and subsequent assignment to transcriptional signatures. PomBaseID: gene identifier; cluster: assignment to transcriptional signature; medium: ; baseMean: baseline transformed expression in synthetic reference; log2fc: log_2-transformed fold change; stderror: standard error of the log_2-transformed fold change; statistic: test statistic of the DE analysis; p.value: raw *p*-value of Wald test; p.adjusted: Benjamini–Hochberg adjusted *p*-value.

MATERIALS AND METHODS

Culture conditions

Cells were grown in continuous culture in turbidostats using Edinburgh minimal media (EMM2) with saturating amounts of carbon and nitrogen (Petersen and Russell 2016). This ensured that the cells could reach balanced exponential growth, limited only by internal gene expression patterns. In addition to the standard EMM2 media where nitrogen is provided by 93.5 mM of ammonium chloride (NH_4Cl, referred to as Amm), we used seven alternative nitrogen sources where 20 mM of a single amino acid replaced the NH_4Cl: glutamate (Glu), proline (Pro), isoleucine (Ile), serine (Ser), phenylalanine (Phe), glycine (Gly), and tryptophan (Trp) (Sigma).

Cells were grown and harvested as follows: $972h^-$ cells from frozen glycerol stocks were precultured on YES agar plates. Single colonies were inoculated in 5-10 ml of EMM2 in glass flasks and grown overnight at 32 °C. Approximately 1 ml of culture was transferred to a fresh flask containing EMM2 and the final nitrogen source and grown to large ~5 x 10^6 cells/ml. These cells were used to inoculate the continuous culture setup at 0.5-1 x 10^6 cells/ml. The process was repeated for biological triplicates grown from three different colonies.

To generate the final cultures, cells were grown in turbidostats (Takahashi et al. 2015), with media flow controlled using customised Python scripts (Saint et al. 2019). Cell cultures were monitored every 30 s and fresh growth medium was added whenever the optical density OD_{600} exceeded 0.4. This resulted in 1%–2% dilution cycles, keeping the total culture volume constant throughout. Cells were kept in the turbidostats for ~10 generations at 32 °C. To measure the growth rate, cells were diluted twofold approximately halfway through the experiment and regrown to the reference level of OD_{600} = 0.4. The growth rate for each sample was determined by fitting an exponential curve to the OD measures acquired every 30 s during the regrowth phase. The final culture volumes were ~30 ml, from which 10 ml was used for transcriptomics, 10 ml for proteomics analysis, and 10 ml was saved as a backup. The cells were harvested by centrifugation, washed twice with PBS and stored at –80 °C until RNA-Seq and proteomics sample preparation was performed.

RNA-Seq

A 10 ml aliquot of the culture was centrifuged at 3000 rpm for 3 min. After removing the supernatant, cell pellets were frozen in dry ice and kept at -80 °C until the library preparation was performed. Total RNA from the pellets was extracted using the hot-phenol method (Lyne et al. 2003) and the RNA obtained was quantified using a BioDrop (biochrom, Cambridge,

UK). Poly(A) enrichment was performed using 500 ng of total RNA with the NEBNext Poly(A) mRNA Magnetic Isolation Module (NEB, Ipswich, USA) kit according to the manufacturer's instructions. The remaining mRNA was used for stranded RNA-seq library preparation using the NEBNext® Ultra™ II Directional RNA Library Prep Kit for Illumina® (NEB, Ipswich, USA) according to the manufacturer's instructions. The resulting libraries were quality checked and quantified using the Bioanalyser (Agilent, Santa Clara, USA) and a Qubit™ dsDNA BR Assay Kit (Invitrogen) respectively.

Libraries were sequenced on an Illumina HiSeq 2500 instrument (Illumina, San Diego, USA). Data were processed using RTA version 1.18.54 and 1.18.64, with default filter and quality settings. The reads were demultiplexed with CASAVA 1.8.4 and 2.17 (allowing 0 mismatches). Transcripts were mapped to the genome sequences (available from PomBase) using TopHat2 (Kim et al. 2013; Lock et al. 2019). HTSeq was used to count the number of reads per exon (gff3, PomBase) (Anders, Pyl, and Huber 2015; Lock et al. 2019). The reads across exons were summed to obtain the total number of reads per gene. This procedure yielded raw counts c_{ijk} for each gene *i,* growth medium *j,* and biological replicate *k.* Per sample normalisation was performed using the DESeq2 *estimateSizeFactors* function, yielding size factors S_{jk} for each sample (Love, Huber, and Anders 2014). The normalised counts were calculated as follows:

$$n_{ijk} = \frac{c_{ijk}}{S_{jk}}. \quad (1)$$

Unless otherwise noted, RNA-Seq analyses were performed using these normalised counts, which enabled between-sample comparison of the expression of genes or sets of genes.

Proteomics

Cell pellets from 10 ml of each turbidostat culture was frozen in dry ice and stored at -80 °C until sample preparation. Once thawed, cells were resuspended in lysis buffer (1% sodium deoxycholate, 1% ammonium bicarbonate). Lysis was performed in a FastPrep instrument (MP Biomedical) for 5 pulses at a speed of 6 m/s. Total cell extracts were treated with 5 mM tris(2-carboxyethyl)phosphine (TCEP) for 15 min at room temperature to reduce the disulphide bonds. An alkylation reaction was performed with the addition of 10 mM iodoacetamide for 30 min at 25 °C in the dark. The reaction was quenched using 12 mM N-acetyl-cysteine for 10 min. The proteins were quantified using a BCA Protein Assay Reducing Agent Compatible kit (ThermoFisher Scientific) and 100 µg of total protein was used for digestion. To improve the cleavage efficiency, protein extracts underwent a double digestion, first with Lys-C (Wako chemicals, USA) for 4 h at 37 °C using a 1:200 (w/w) ratio, and then overnight with porcine trypsin at 37 °C using a 1:100 (w/w) ratio. Digestion was stopped by lowering the pH with

trifluoroacetic acid (TFA) at a final volume of 1%. The sodium deoxycholate precipitate formed due to the lowered pH was removed by centrifuging the samples at 4 °C for 15 min at 14,000 rpm. The precipitated detergent was then discarded. The digested peptides were vacuum dried and stored at -80 °C until required for analysis.

The protein digests were analysed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) via an untargeted analysis approach using Data-Dependent Acquisition (DDA) (Ducret et al. 1998). The raw MS data was analysed using MaxQuant (Cox and Mann 2008) and applying the Label-free Quantification algorithm (Cox et al. 2014) for DDA data analysis.

Protein digests were reconstituted in 0.1% trifluoroacetic acid (TFA) and transferred to autosampler vials for LC-MS/MS analysis. The tryptic peptides were separated using an Ultimate 3000 RSLC nano liquid chromatography system (Thermo Scientific) coupled to a Q-Exactive tandem mass spectrometer (Thermo Scientific) via an EASY-Spray source. Sample volumes were loaded onto a trap column (Acclaim PepMap 100 C18, 100 um x 2 cm) at 8 ul/min of 2% acetonitrile, 0.1% TFA. Peptides were eluted on-line to an analytical column (EASY-Spray PepMap C18, 75 um x 75 cm). Peptides were separated at 200 nl/min with a ramped 180 min gradient using 4%-30% buffer B (buffer A: 2% acetonitrile, 0.1% formic acid; buffer B: 80% acetonitrile, 0.1% formic acid) over 150 min, and 30%-45% buffer B over 30 min. Eluted peptides were analysed by operating in positive polarity using a data-dependent acquisition mode. Ions for fragmentation were determined from an initial MS1 survey scan at 70,000 resolution (at m/z 200) in the Orbitrap followed by Higher-energy Collisional Dissociation (HCD) of the top 12 most abundant. MS1 and MS2 scan AGC targets set to 3e6 and 5e4 for maximum injection times of 50 ms and 110 ms, respectively. A survey scan covering the range of 400–1800 m/z was used, with HCD parameters of isolation width 2.0 m/z and a normalised collision energy of 27%.

DDA data was processed using the MaxQuant software platform (v1.6.2.3) (Cox and Mann 2008) with database searches performed by the in-built Andromeda search engine against the PomBase database (5,138 entries, v.20190507) (Lock et al. 2019). A reverse decoy database was created, and the results displayed at a 1% false discovery rate (fdr) for peptide spectrum matches and identified proteins. The search parameters included trypsin, two missed cleavages, fixed modification of cysteine carbamidomethylation, and variable modifications of methionine oxidation, asparagine deamidation, N-terminal glutamine to pyroglutamate modification, and protein N-terminal acetylation. Label-free quantification was enabled with an LFQ minimum ratio count of 2. The ‘match between runs’ function was used with match and alignment time limits of 0.7 and 20 min, respectively.

Intensities were based on identified unique and razor peptides, and intensity-based absolute quantification (iBAQ) was calculated as the raw intensity/number of obtainable tryptic peptides. For the post-processing of the MaxQuant output, the data was filtered for detection in all three biological replicates. Subsequently, proteome mass fractions ϕ_{ij} were calculated for each protein group *i*, sample from growth medium *j*, replicate *k* from the reported protein masses m_i, and the iBAQ quantities B_{ijk} as follows:

$$\phi_{ijk} = \frac{m_i B_{ijk}}{\sum_l m_l B_{ljk}}. \quad (2)$$

Repeated median linear models

As shown in the main text, several genes were enriched in one or more growth conditions in addition to growth-rate correlations. The presence of such outliers affected the fit quality of the standard ordinary least squares (OLS) linear model fits. To account for this, we used repeated median linear models (RMLM) for fitting regression lines (Siegel 1982), as implemented in the R package `mblm` (Komsta 2019). This method is robust when up to 50% of outliers are present in the data, and the working is described below.

In general, the data can be described as *N* pairs of the growth rate μ and some expression value *y* (*N* = 24 if expression was detected across all samples, or a smaller multiple of 3 when data was missing). From each observation $(\mu, y)_i$, a line is drawn to each of the other *N – 1* points $(\mu, y)_j$, and the median slope and *y*-intercept of these *N – 1* lines is associated with the data point *i*. The regression coefficients for the slope and *y*-intercept of the repeated median linear model are defined as the medians of all *N* slopes and *y*-intercepts. To compare the growth law shape of protein groups with varying absolute abundances, the fold-change *FC* was defined from the RMLM as the ratio

$$FC = \frac{y(\mu = \mu_{\max}) - y(\mu = 0)}{y(\mu = 0.5\mu_{\max})}, \quad (3)$$

with μ_{max} = 0.3 h^{-1}. This can be expressed in terms of the fitted slope *a* and the *y*-intercept *b* as follows:

$$FC = \frac{\mu_{\max}}{0.5\mu_{\max} + {}^{b}/_{a}}. \quad (4)$$

Hierarchical clustering

We used *z*-scores to normalise for variations in absolute expression levels. For each gene or protein group *i* in the sample with medium *j* and replicate *k*, the *z*-score was calculated as

$$z_{ijk} = \frac{y_{ijk} - \mu_i}{\sigma_i} \quad (5)$$

from the expression values y_{ijk}, where μ_i and σ_i are the mean and standard deviations across all samples. The analysis was performed only on genes or protein groups that were detected across all 24 samples. The resulting matrices of the *z*-scores were analysed using hierarchical clustering and principal component analysis.

Hierarchical clustering on genes/protein groups was performed using the Euclidean distance and Ward linkage ("*ward.D2*"), using the `*hclust*` implementation of the R statistical language (v.3.5.3). In the transcriptome analysis, separate dendrograms were constructed for coding and non-coding RNAs, using the protein-coding list from PomBase and selecting ncRNAs from the presence of "NCRNA." in the systematic IDs.

Sector assignment

For each gene or protein group *i,* we calculated R-squared (R^2), defined as

$$R_i^2 = 1 - \frac{\sum_{j,k} r_{ijk}^2}{\sum_{j,k}\left(y_{ijk} - \mu_i\right)^2}, \quad (6)$$

and the associated *p*-values using the `summary.lm` method. Here r_{ijk} denotes the residuals from the RMLM fit, y_{ijk} the expression (normalised counts or proteome fractions), μ_i the mean expression across samples, and the summation was performed across all *N* samples where the gene was detected. We calculated the tail-based false discovery rates (*fdr*, or *q*-values) and local false discovery rates (*fdr*) using the `fdrtool` R package and the false non-discovery rate cut-off method (Strimmer, 2008). Genes or protein groups were assigned to the P or R sector when their tail-based *fdr* < 0.1. R and P sector genes had positive and negative slopes, respectively, as determined by the fitted RMLM. In **Supp. Table S6**, hits with local *fdr* < 0.1 were flagged as confident.

To assess fit quality, in addition to R^2, we used a normalised sum of squared residuals, defined as

$$SSR_{\mathrm{norm},i} = \frac{1}{N-1}\frac{\sqrt{\sum_{j,k} r_{ijk}^2}}{\mu_i}, \quad (7)$$

with the notations as described in the previous paragraph.

Bootstrapping

For the analysis illustrated in **Fig. 4AB** and **Supp. Fig. S4.1 and S4.3**, 1000 bootstrap samples were generated using the *bootstraps* function from the `rsample` package (v0.0.8) (Silge et al.

2021). The RMLM analysis was repeated on the bootstrapped samples, resulting in sample distributions for the RMLM slopes, intercepts, and *FCs*. Plots of the 2.5%-97.5% confidence interval were drawn using the RMLM predictions on a 101-point grid spanning 0 to 0.3 h^{-1}.

Other confidence intervals were drawn using the *geom_smooth* function in ggplot2 (v3.3.2) (Wickham 2016) with the default 95% confidence interval and the RMLM method, unless otherwise noted.

Barcode plots

For the barcode plots in **Supp Fig. S4.3 and S4.6**, the directed length l_{ij} of the bar for protein *i* and medium *j* was calculated from the median proteome mass fractions across the three biological replicates,

$$x_{ij} = \underset{k=1,2,3}{\text{median}}\, \phi_{ijk}\,, \tag{8}$$

and the median across all samples,

$$M_i = \underset{j,k}{\text{median}}\, \phi_{ijk}\,, \tag{9}$$

in the following way:

$$l_{ij} = \frac{x_{ij} - M_i}{M_i}, \tag{10}$$

with missing data imputed to zero. The scale was capped at $-1 < l_{ij} < 2$.

Differential expression analysis

To identify differential expression in the transcriptome on top of growth rate mediated effects, we performed an analysis using `DESeq2` (v1.22.2) from the Bioconductor suite (v3.8) (Love, Huber, and Anders 2014; Huber et al. 2015), comparing the residual expression in each condition to a synthetic reference condition. The fold change obtained by this procedure can be interpreted as the ratio of observed normalised counts and the counts predicted by the RMLM, and the associated *p*-value provides an interpretable estimate of significance.

The DESeq2 analysis pipeline enables the introduction of per-gene, per-sample normalisation factors that are commonly used to correct for batch-dependent GC-content or length biases. We adapted this functionality to normalise the growth rate bias of each gene, by introducing factors N_{ijk} that converted between the measured raw counts c_{ijk} and RMLM-predicted raw counts q_{ijk}:

$$q_{ijk} = \frac{c_{ijk}}{N_{ijk}}, \tag{11}$$

in analogy to the definition of size factors in Equation (1). However, the fitting of RMLMs yielded per-gene, per-sample predictions p_{ijk} of the normalised counts. Using the sample-dependent size factors, we converted these to predictions of raw counts as follows:

$$q_{ijk} = p_{ijk} S_{jk}. \tag{12}$$

Therefore, the normalisation factors were calculated as

$$N_{ijk} = \frac{c_{ijk}}{S_{jk} p_{ijk}} = \frac{n_{ijk}}{p_{ijk}}. \tag{13}$$

We excluded genes with negative predicted raw counts and rescaled the normalisation factors across samples for each gene to have a geometric mean of 1 for numerical accuracy.

Using the RMLM-predicted raw counts, we further defined a synthetic reference condition with three biological replicates by using the median predicted count across all growth media for each replicate as follows:

$$s_{ik} = \mathrm{int}\left(\underset{j}{\mathrm{median}}\, q_{ijk}\right). \tag{14}$$

These reference counts were rounded to the nearest integer, as they represent raw counts in the DESeq2 pipeline. By design, the q_{ijk} have no residual growth-rate trend.

Subsequently, the analysis proceeded on the constructed data set with 9 conditions: the original 8 and the synthetic one, with each set having 3 biological replicates. Pairwise fold-changes *F* and the associated *p*-values (both uncorrected and adjusted p_{adj}) are reported between the 8 growth media and the synthetic reference. Genes were reported as differentially expressed (DE) if $p_{\mathrm{adj}} < 0.01$ and $|\log_2 F| > 0.5$ for at least one condition.

Functional enrichment

We performed one-sided Fisher exact tests to assess the enrichment of DE genes across the *S. pombe* GO-slims and terms from the biological_process GO with at most 50 annotations in *S. pombe* (Gene Ontology Consortium 2019; Lock et al. 2019) . From the resulting *p*-values, local false discovery rates *lfdr* were calculated using the `fdrtool`s false non-discovery rate method (Strimmer 2008).

In the enrichment plots for the GO-slim terms (**Fig. 6B, Supp. Fig. S2.1**), terms with *lfdr*<0.05 were deemed significant, and the terms were ordered from top to bottom by increasing smallest *lfdr* to aid interpretation. For the biological_process enrichment plot (**Supp Fig. S5.3**), the significance threshold was *local fdr*<0.001. The significant terms were clustered hierarchically using the Euclidean distance and Ward linkage ("*ward.D2*"), using the `*hclust*`

implementation of the R statistical language (v.3.5.3).The terms were ordered by the smallest *lfdr* as much as possible while remaining consistent with the clustering constraint.