The cell growth cycle: resource allocation and subcellular pattern formation in budding yeast.

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Abstract

Metabolic oscillations percolate throughout cellular physiology. The transcriptome oscillates between expression of genes encoding for biosynthesis and growth, and for catabolism and stress response. Long protein half-lives suppress effects on protein abundance level, and the function of periodic transcription remains elusive. We performed RNA-seq analysis during a dynamic state of the system. Short protein half-lives and high transcript abundance amplitude of sulfur uptake genes and carbonic anhydrase, and dynamic changes of pathway intermediates H_2S and CO_2 support a direct role of transcription in cycle dynamics. Substantial changes in the relative duration of expression of the antagonistic co-expression cohorts precede a system bifurcation to a longer period, supporting the idea of a function in cellular resource allocation. The pulse-width modulation model, a mathematical formulation of this idea, can explain a large body of published experimental data on the dependence of the cycle period on the culture growth rate. This pulse-like model of cell growth provides a first theoretical framework, where the phenomenon is understood as a mechanism of cellular resource allocation and protein homeostasis, and is applicable to circadian transcriptome dynamics from all domains of life.

Introduction

When yeast cultures are grown to a high cell density they tend to show collective metabolic dynamics, alternating between phases of high oxygen consumption (HOC) and low oxygen consumption (LOC). Numerous studies on continuously grown yeast have shown that stable oscillatory dynamics propagate throughout the metabolome, transcriptome and impinge on chromatin organization. The cycle period is dependent on growth conditions and the strain employed. However, under equivalent growth conditions experimental data can be categorized into long (τ_{osc} = 4 h–5 h) or short period cycles (τ_{osc} = 0.7 h–1 h; peculiar to the diploid strain IFO 0233). The cell division

cycle (CDC) is tightly or loosely coupled with the long or short period cycles, respectively. The phenomenon is considered a model system for circadian cell biology in the absence of a circadian clock (***Causton et al., 2015***; ***Mellor, 2016***), or, more generally, for multi-scale coherence of cellular processes (***Sonnleitner et al., 1997***; ***Lloyd and Murray, 2007***; ***Aon et al., 2008***). Long period cycles were explained by a partial synchronization of the CDC, revealing the asymmetry of the yeast budding cycle (***Küenzi and Fiechter, 1969***; ***von Meyenburg, 1969a***; ***Münch et al., 1992***; ***Bellgardt, 1994***; ***Hjortso and Nielsen, 1995***; ***Duboc and von Stockar, 2000***; ***Futcher, 2006***). ***Satroutdinov et al.*** (***1992***) then observed much shorter periods in IFO 0233, which questioned these prior models, including a causal role of the glycogen storage cycle.

Despite these differences in periods and metabolic flux patterns, a similar temporal program of gene expression is observed in both short period (IFO 0233) and long period experimental systems (***Machné and Murray, 2012***). Both show maxima and minima of the cellular ATP/ADP ratio during the HOC and early LOC phases, respectively (***von Meyenburg, 1969b***; ***Satroutdinov et al., 1992***; ***Xu et al., 2004***; ***Müller, 2006***; ***Machné and Murray, 2012***; ***Amariei et al., 2014***). Global remodeling of promoter and gene body nucleosome organization occurs during the late LOC phase (***Amariei et al., 2014***; ***Nocetti and Whitehouse, 2016***). During HOC, transcription progresses from a ribosome biogenesis cohort (`Ribi`) and cytoplasmic ribosomal protein genes (`RP`), to amino acid synthesis genes (`AA`) and mitochondrial ribosomal protein genes (`mtRP`) at the transition to LOC. During the LOC phase, transcripts of a large group of stress-response and catabolic proteins (`S/C`) peak. In IFO 0233, the latter overlaps with expression of the `mtRP` cohort, while in long period systems these are separated (***Machné and Murray, 2012***). Several hypotheses on putative functions of the temporal program have been suggested. The functional profiles of co-expressed cohorts match metabolic activity, and the initial hypothesis was a "just-in-time" model of gene expression (**H1**), where enzymes are expressed when required within the metabolic cycle (***Klevecz et al., 2004***; ***Tu et al., 2005***; ***Murray et al., 2007***). However, protein half-lives in yeast are now thought to be much longer than initially reported (***Christiano et al., 2014***). This dampens the effect of periodic transcript on protein abundances (***Lück et al., 2014***). Indeed recent proteomic studies found no (**preprint: *Feltham et al.*** (***2019***)) or only few (***O' Neill et al., 2020***) periodic protein abundances in long period systems. Thus, ***Feltham et al.*** (***2019***) suggest a null hypothesis (**H0**) where periodic mRNA synthesis and degradation produce no significant costs and are a side-effect of the metabolic states the cells traverse. ***Slavov and Botstein*** (***2011***) and ***Burnetti et al.*** (***2016***) suggested an alternative hypothesis (**H2**), based on the observation that the relative duration of the LOC phase varies strongly while HOC phase duration only subtly changes with growth rate. This would result in different absolute abundances of the proteins from HOC- and LOC-specific co-expression cohorts and could underlie growth rate-dependent cellular resource allocation (***Maaløe, 1979***; ***Molenaar et al., 2009***). And finally, the association of protein biosynthesis with HOC phase and of protein aggregation and autophagy with LOC phase led to suggestions of a conserved eukaryotic cell growth cycle with a main role in protein homeostatis (**H3**) (***von Meyenburg, 1969a***; ***Klevecz et al., 2004***; ***Lloyd and Murray, 2007***; ***Murray et al., 2007***; ***Slavov et al., 2012***; ***O' Neill et al., 2020***).

To test these (non-exclusive) hypotheses, we performed strand-specific RNA-sequencing in high temporal resolution during a dynamic state of the short period cycle of the strain IFO 0233; after a multi-generational drift of period and amplitudes and just preceding a distinct bifurcation to longer but unstable periods. Global system variables, such as ATP flux and proton export, oscillated, confirming the cell-wide nature of the cycle. Only few proteins with short half-lives and high transcript abundance amplitudes are compatible with **H1**, and their metabolic substrates dynamically changed over the bifurcation. The bulk of the protein coding transcriptome followed the conserved program and a pronounced shift from HOC phase-specific to LOC phase-specific transcript abundance peaks occurred within just two cycles, compatible with **H2**. A mathemtical formulation of H2, the pulse-width modulation (PWM) model, reproduces the observed relations of cycle periods and of relative proteome fractions to growth rate.

Results

What Oscillates? Multi-generational Dynamics and a Bifurcation to Longer Period

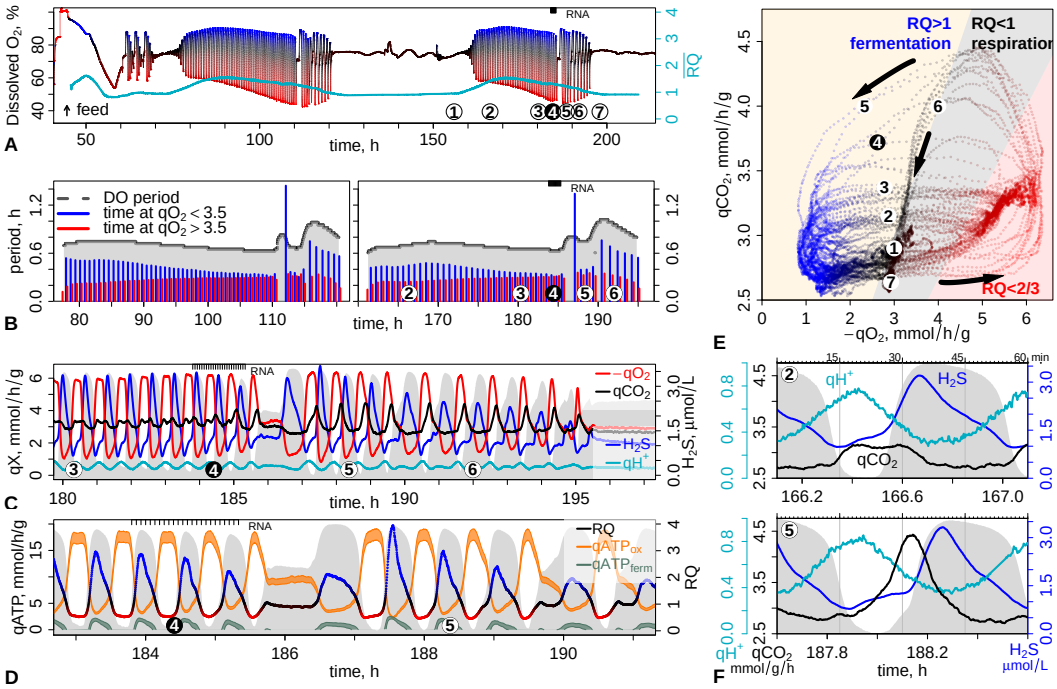

Figure 1. Complex Dynamics: Slow Transients and a Sudden Bifurcation. Metabolic dynamics during continuous culture of the budding yeast strain IFO 0233: panel A shows the full recorded time-series of the culture, and panels B–E zoom in on the time axis; bullet points P1–P7 serve as a guide between panels and are discussed in the text. The gray backgrounds show the dissolved O_2 concentration (see A for axis) and serves as a reference to oscillation phase. **A:** Dissolved O_2 (DO) measurement from the start of continuous feeding (dilution rate $\phi = 0.089\,\mathrm{h}^{-1}$). Line colors are derived from the respiratory quotient RQ (D) and indicate phases of high O_2 consumption (HOC: red) and low O_2 consumption (LOC: blue). The cyan line and right axis show the temporal mean $\overline{\mathrm{RQ}}$, a moving average over ca. 10 h. **B:** The cycle periods for the dynamic transients were derived from a Wavelet transform of the DO signal and the phase lengths are the time spans of each cycle where oxygen uptake ($-q_{O_2}$) stayed below (red) or above (blue) $3.5\,\mathrm{mmol/h/g_{DCW}}$. **C:** Zoom on P3–P6 for measured metabolic rates and concentrations; q_{O_2}, q_{CO_2} and H_2S were measured in the offgas of the reactor, corrected for the measurement delay and H_2S concentration was derived via its solubility. Proton export (q_{H^+}) was calculated from the NaOH addition rate. **D:** Zoom on P4–P5 for calculated rates. The respiratory quotient (RQ) and ATP production rates by respiration ($q_{ATP\,ox}$) or by fermentation ($q_{ATP\,ferm}$) were calculated from q_{O_2} and q_{CO_2}, ranges of q_{ATP} show values for pure glucose oxidation and fermentation, or pure fermentation of glucose and oxidation of only ethanol. The RQ color gradient serves as a reference in (A, E). **E:** Phase portrait of q_{O_2} and q_{CO_2} over the time range indicated by bullet points in (A); colored by RQ (D), arrows indicate time direction; background colors indicate RQ ranges. **F:** One-hour snapshots at different times (bullet points 2 and 5).

We studied short period oscillatory transcriptome dynamics in a continuous culture of the budding yeast strain IFO 0233. The culture traversed complex dynamic transients instead of the more commonly studied stable oscillation (Fig. 1A and S4). The transients occurred fully spontaneously, spanned 30 h–40 h, *i.e.* multiple cell division cycles (generations), and appeared twice with highly similar trajectories (Fig. S6).

During these transients the oscillation followed the typical pattern observed for this strain and these conditions, with period τ_{osc} of 0.6 h–0.7 h (Fig. 1B-C): the culture cycled between a phase of low oxygen consumption (LOC, here: $-q_{O_2} < 3.5\,\mathrm{mmol/h/g_{DCW}}$) with additional fermentation (respiratory quotient $\mathrm{RQ} = \frac{q_{CO_2}}{-q_{O_2}} > 1$), and a phase of high oxygen consumption (HOC). During HOC, RQ decreased below the $\frac{2}{3}$ stoichiometry of ethanol oxidation (Fig. 1D). This is consistent with re-uptake of ethanol (***Satroutdinov et al., 1992***) but could indicate further unknown contributions to CO_2 turnover. Proton export (q_{H^+}, Fig. 1C and E) peaked in early HOC phase, consistent with a higher intracellular

pH during HOC (***Keulers et al., 1996***). The temporal mean $\overline{\mathrm{RQ}} > 1$ (Fig. 1A) indicates net fermentation during the dynamic state, but stoichiometric estimation of ATP production rates q_{ATP} showed that fermentation did not contribute significantly to overall ATP turnover (Fig. 1D). The estimated ATP turnover rates are in phase with previously measured ATP/ADP ratio and energy charge, peaking in early to mid HOC phase (***Machné and Murray, 2012***; ***Amariei et al., 2014***). The concentration of H_2S peaked at $\approx$ 3 µM with a sharp increase upon transition to LOC (Fig. 1C, F), consistent with its release during amino acid biosynthesis in this transition phase (***Murray et al., 2007***) and its suggested role in population synchronization (***Murray et al., 2003***). Both multi-generational transients were characterized by gradual amplitude and period drifts. The overall period and LOC phase duration decreased while maximal oxygen uptake and HOC phase duration increased (bullet points P2-P3, Fig. 1A, B, S4). As periods reached a minimum of $\tau_{osc} \approx 0.6$ h, the plateau of $q_{\mathrm{CO_2}}$ was split into two distinct peaks flanking the HOC phase (P3-P4, Fig. 1C–E). The first $q_{\mathrm{CO_2}}$ peak vanished and a sharper high amplitude peak remained at the transition to LOC, immediately preceding a distinct bifurcation of system dynamics. An additional phase first appeared at this bifurcation: metabolism was respiratory (RQ ≈ 1) and all parameters remained at intermediate values for about one full oscillation cycle (time $t = 186$ h), Fig. 1C, S5). A delayed H_2S peak then preceded a long and pronounced LOC phase. The system then fell back to a shorter period, but $q_{\mathrm{CO_2}}$ remained phase-delayed and with higher amplitude (P5, Fig. 1C–F). Finally, the period suddenly increased to $\tau_{osc} \approx 1$ h, and the pause between HOC and LOC phases re-appeared as a short plateau or shoulder, again at RQ ≈ 1, and flanked by pulses of CO_2 and H_2S release (P6, Fig. 1C–F).

In summary, we confirmed that the metabolic cycle reflects a cell-wide phenomenon where global variables such as ATP flux or proton export oscillate at high amplitude. Here, a gradual uncoupling of CO_2 release from respiratory activity preceded a bifurcation of system dynamics to longer periods and a biphasic LOC. A new phase appeared at the transition from HOC to LOC, characterized by purely respiratory metabolism. Low but purely respiratory activity at RQ ≈ 1 is characteristic of the LOC phase in long period cycles (***Münch et al., 1992***). This bifurcation was accompanied by a novel pulse of CO_2 release before, and a shifted pulse of H_2S release after the intermediate RQ $= 1$ phase. This is compatible with previous observations that gaseoues metabolic intermediates CO_2 (***Keulers et al., 1996***) and H_2S (***Murray et al., 1999***, ***2003***) are involved in oscillation control and population synchronization.

Why does the Transcriptome Oscillate? Testing Hypotheses.

Non-Coding Transcription is Biased to LOC Phase.

Numerous time series of the protein-coding transcriptome have revealed a constant temporal program of transcription cohorts which is unfolded at strongly varying periods from 40 min to 7.5 h (***Machné, 2017***; ***Mellor, 2016***). Neither the effect of transient states of the oscillation nor non-coding transcription have been studied yet. We thus took 24 samples for RNA-seq analysis in high temporal resolution (4 min) over 2.5 cycles, accidentally just preceding the intermediate pause at RQ ≈ 1 (P4 and top axis ticks in Fig. 1). The strand-specific sequencing reads were mapped to the reference genome (strain S288C, R64-1-1), yielding reads for 76 % of the genome (Fig. S8A). We previously developed a similarity-based segmentation algorithm that collapses read-counts into segments which show a similar temporal read-count profiles (***Machné et al., 2017***). The algorithm yields ca. 37k segments (Fig. S8D), each a putative individual transcript. All segments were classified by their oscillation p-values, calculated with the `rain` package (***Thaben and Westermark, 2014***), and by their overlaps with annotated genome features (Tab. S2). 4,489 segments were classified as open reading frame (ORF) transcripts; 3,378 of these showed periodic transcript abundances and reproduced the previously characterized (***Machné and Murray, 2012***) transcriptome program (Fig. 2A). In constrast, periodically expressed non-coding (811 of 9,051) and antisense (232 of 569) segments predominantly peaked in the LOC phase.

Very short and weakly expressed segments were removed from further analyses, and the remaining 11k segments (Fig. S8G–I) were clustered into ten co-expressed cohorts. Clusters were

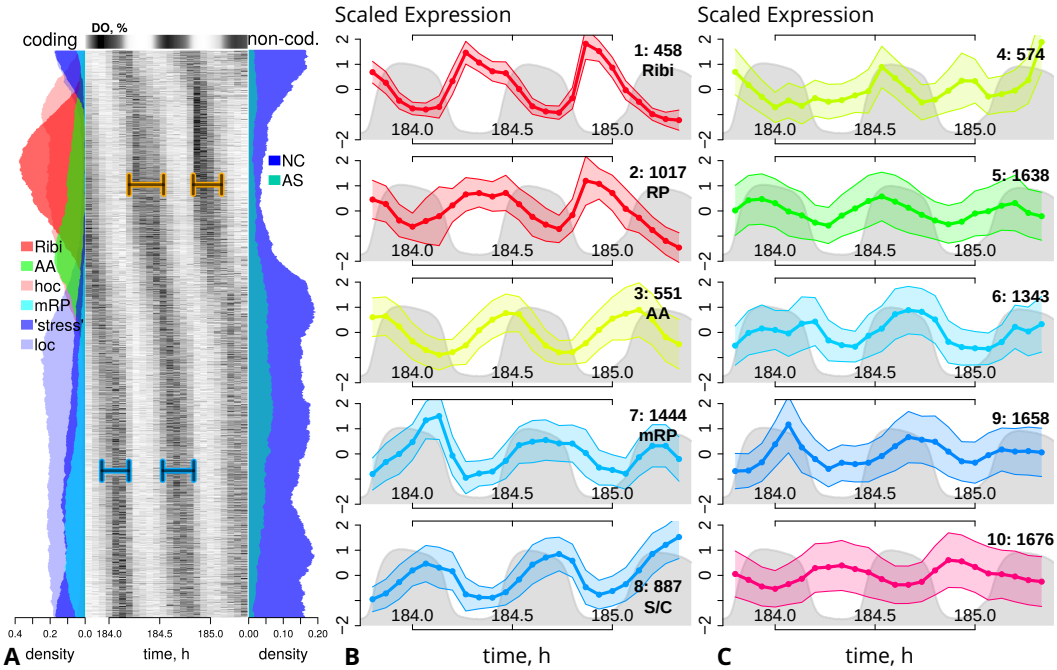

Figure 2. RNA-seq Segmentation and Clustering. A: phase-ordered heatmap of the time-courses of segments with oscillating abundance levels (6344 segments at $p_{\text{rain}} < 0.05$, Fig. S8)). The dissolved O_2 (DO, %) is shown as a color gradient (black: high DO) on the top axis. Left and right panels show local densities (circular moving average of counts over 1/10 of the total number) of segments overlapping with previously defined (***Machné and Murray, 2012***) classes of coding genes (left: Ribi, ribosomal biogenesis and cytoplasmic ribosomal proteins; AA: amino acid synthesis; mRibi: mitochondrial genes, incl. ribosomal proteins; stress: catabolic and protein homeostasis genes), or non-coding segments (right: AS, antisense to ORF; NC, no overlap with any annotated transcribed feature). **B:** time series of the five major periodic co-expression cohorts. Segment time-courses (mean RPM) were normalized to mean of 0 and divided by their standard deviation (R function `scale`). The mean of each cluster is shown as a solid line with points indicating the sampling times, and standard deviations as transparent ranges; the legends indicate the cluster label, the number of segments in the cluster and the posterior functional cohort assignment. The gray background indicates the dissolved O_2 (DO) concentration. **C:** time series for the cohorts 4, 5, 6, 9, 10, which comprise mostly segments without or little overlap with coding regions; plotted as described for (B).

sorted, re-labeled and colored by their mean peak phase (Fig. S9–S11). These ten cohorts can be further classified (Fig. S9C) into two groups of five clusters each. The high amplitude clusters (Fig. 2B and S10) are characterized by long segments, and 61 %–91 % of segments assigned to a protein-coding gene. Low amplitude clusters (Fig. 2C and S11) contain shorter and weakly expressed segments, with 21 %–91 % non-coding, most peaking throughout LOC phase. Thus, the clustering analysis confirms both observations: coding gene co-expression cohorts traverse the temporal program while non-coding transcripts are predominantly expressed in LOC phase.

A Conserved but Adaptable Temporal Program of Transcription.

The temporal sequence of transcript abundance peaks of the five protein-coding gene cohorts (Fig. 2B: clusters 1–3, 7 & 8) and their Gene Ontology (GO) enrichment profiles (Fig. 3A and S12) recapitulate previous results (***Klevecz et al., 2004***; ***Machné and Murray, 2012***): the RiBi regulon of ribosomal biogenesis genes (***Jorgensen et al., 2004***) peaks in early to mid HOC phase (cluster 1: `Ribi`), followed by clusters encoding for cytoplasmic ribosomal proteins (cluster 2: `cRP`), and amino acid biosynthetic pathways (cluster 3: `AA`) at the transition to LOC. During the LOC phase, mitochondrial proteins, including mitochondrial ribosomal proteins (cluster 7: `mRP`) are co-expressed in a regulon associated with stress response (***Gasch et al., 2000***; ***Brauer et al., 2005***) and G1 phase (***O'Duibhir et al., 2014***), and comprising proteins of the general stress response (chaperones) and of carbohydrate, fatty acid and protein catabolism (cluster 8: `S/C`).

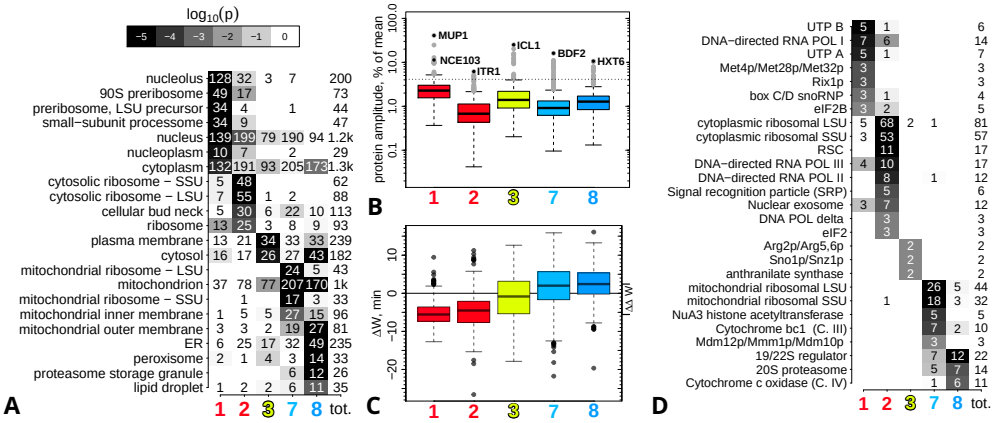

Figure 3. A Conserved and Adaptable Temporal Program. A: Sorted cluster enrichment profiles for the GO category "cellular component". The p-values were calculated by cumulative hypergeometric distribution tests and are shown as gray-scale. Only categories with $p < 0.001$ (white text) and more then 10 genes in one of the clusters are shown. See Figure S12 for all clusters and GO categories. **B:** Box-plots of cluster distributions of predicted relative protein amplitudes A_{protein}, estimated from transcript amplitudes and protein half-lives (Fig. S17). The horizontal line indicates the lowest $A_{\text{protein}} \approx 4\,\%$ of the top 100 genes (Table S3). The top predicted oscillators of each cluster are indicated. **C:** Box-plots of cluster distributions of peak-width changes. $\Delta W = W_2 - W_1$ is the difference of the peak widths (Fig. S19) between the second and first full expression cycle, and $\Delta\Delta W$ is the difference of ΔW medians of clusters 1 and 8. **D:** Cluster enrichment profile ($p_{\text{sort}} = 0.01$) of protein complexes in the CYC2008 annotation (***Pu et al., 2009***), colored as in (A). For a comprehensive picture of complex expression, the segment with the best overlap (highest Jaccard index $J > 0$) was considered for each coding gene. See Figure S20 for all clusters.

Transcriptome Meta-Analysis: Different Time Scales - Same Program.

Similar observations were described in transcriptome analyses from different strains and conditions, with periods ranging from 40 min to 7.5 h (***Li and Klevecz, 2006***; ***Tu et al., 2005***; ***Chin et al., 2012***; ***Kuang et al., 2014***; ***Wang et al., 2015***; ***Slavov et al., 2011***). We used our clustering into five protein-coding co-expression cohorts to analyze seven data sets from five previous publications (Fig. S13–S15). This meta-analysis emphasizes the universality and adaptability of the co-expression cohorts: firstly, a temporally constrained program (0.5 h–2 h) leads from `Ribi`/`cRP` *via* `AA` to `mRP`, ending with the transition from HOC to LOC (left panels in Fig. S14). Secondly, the HOC- and LOC-specific cohorts are always anti-correlated. In long period systems, the duration of `S/C` expression increases with increasing duration of the LOC phase, while `Ribi` forms sharp peaks. Thirdly, the `mRP` and `AA` cohorts always peak at the transition from HOC to LOC but show variable timing between experiments (right panels in Fig. S14–S15). Interestingly, very similar temporal patterns can be observed in six distinct cell cycle arrest & release experiments (Fig. S16) (***Orlando et al., 2008***; ***Bristow et al., 2014***).

Next, we tested three hypotheses on putative functions of this universal yet adaptable "functio-temporal" expression program.

H1: CO_2, Carbonic Anhydrase and the Glyoxylate Cycle: Novel Feedback Candidates.

The temporal order of mRNA abundances makes intuitive sense as a just-in-time functional program (**H1**) coordinated with metabolic dynamics (***von Meyenburg, 1969b***; ***Klevecz et al., 2004***; ***Tu et al., 2005***; ***Murray et al., 2007***; ***Machné and Murray, 2012***). However, the effect of periodic mRNA on protein levels depends on protein half-lives. Thus, we estimated relative protein amplitudes from our RNA abundance profiles and protein half-life data (***Christiano et al., 2014***) using a mathematical model of periodic gene expression (***Lück et al., 2014***). The majority of proteins are predicted to vary by only 0.5 %–1 % of their mean abundance (Fig. 3B and S17). Only 21 proteins have relative protein amplitudes of ≥10 %, most from the `Ribi` and `AA` cohorts. The top 100 list contains several cell wall proteins, nutrient transporters, transcription factors, and a few metabolic enzymes (Fig. S17, Tab. S4 and Dataset S3). Several proteins of the sulfate uptake pathway (MET genes) are

expressed in `Ribi`, peaking prior to the pathway intermediate H_2S at the HOC/LOC transition (Fig. 1). The carbonic anhydrase (NCE103, in `Ribi`) catalyzes the interconversion of carbon dioxide and bicarbonate ($CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$). The early downregulation of the `Ribi` cohort (Fig. 2A, Fig. 3C) in the second sampled cycle correlates with the appearance of the CO_2 pulse at transition to LOC (Fig. 1C–F). In short period cycles, population synchronization likely occurs *via* a gaseous substance, and evidence for both, CO_2 and H_2S, have been previously presented (***Keulers et al., 1996***; ***Murray et al., 1999***, ***2003***). Both are substrates of biosynthetic metabolism (anabolism) and several key enzymes of biosynthesis are among our top predicted protein oscillators, notably three enzymes of the glyoxylate cycle (ICL1, CIT2, MDH2); all expressed with the `AA` cohort. In summary, several short-lived proteins with high RNA amplitudes are involved in the transition from energy generation (catabolism) during HOC phase to a biosynthetic pulse (anabolism) at the transition to LOC phase.

H2: Resource Allocation by Pulse-Width Modulation.

The majority of proteins appear too stable to be affected by periodic transcript abundance. An alternative interpretation of periodic transcription has been suggested (***Slavov and Botstein, 2011***; ***Burnetti et al., 2016***). Growth rate is proportional to the (cytoplasmic) ribosome fraction of total biomass (***Waldron and Lacroute, 1975***; ***Brauer et al., 2005***; ***Metzl-Raz et al., 2017***). Variation of the relative times spent in HOC- and LOC-specific transcription states could serve to tune relative proteome fractions (**H2**). Shorter relative duration of the HOC phase-specific transcription would lead to a lower fraction of ribosome-associated proteins (`Ribi` and `RP` cohorts). Indeed, a pronounced shift to LOC phase-specific expression (`S/C`) with decreasing growth rate is seen in dilution rate shift experiments (Fig. S15D) (***Chin et al., 2012***; ***Wang et al., 2015***). The heatmap (Fig. 2A) and cluster average time-courses (Fig. 2B) indicate a shift of relative times spent with HOC or LOC phase-specific expression within just two cycles of the oscillation. To quantify this observation, we compared changes of the peak widths between the penultimate and the ultimate fully traversed cycles of oscillation before the bifurcation (Fig. 2A, S19). The `S/C` cohort gains on average about 8 min over the `Ribi` cohort (Fig. 3C), 22 % of a full cycle, all within just two cycles of the oscillation. Thus, a pronounced shift to expression of the `S/C` cohort, indicative of a lengthened LOC phase and decreased growth rate, directly preceded the bifurcation in system dynamics to a longer period.

H3: The Temporal Program Reflects the Spatial Structure.

The resource allocation model can account for the observed relations to growth rate, and the model holds for different periods. However, it offers no interpretation of the temporal program, the fixed sequence of co-expression cohorts. Recent insights into the intricate spatio-temporal organization of protein homeostasis (***Langdon et al., 2018***; ***Shiber et al., 2018***; ***Schwarz and Beck, 2019***) offer a novel and complementary perspective. Here, we suggest, as a contribution to the protein homeostasis model (**H3**), a relation of the temporal program to the spatial organization of the cell. The GO analysis by "cellular component" maps the temporal program onto the spatial organization of cell structures (Fig. 3A): from nucleolus (`Ribi`), to nucleus (`Ribi/RP`), to cytosol, to plasma membrane (`AA`); followed by mitochondria (`mRP`), then large cytosolic structures, such as the endoplasmatic reticulum (ER), proteasomes and lipid droplets (`S/C`). Thus, the protein products of temporally co-expressed genes also tend to co-localize at their final subcellular locations. Analysis of the CYC2008 protein complex annotation (***Pu et al., 2009***) confirms that many protein complexes are co-expressed (Fig. 3D). Large complexes (>10 proteins) involved in basal cellular functions are co-expressed in either HOC or LOC phase, while complexes with regulatory function are comprised of differentially expressed subunits (Fig. S20B). In a growing cell, fresh ribosomes are constantly built and loaded with the current set of mRNA. The temporal expression program would lead to spatially structured layers of loaded ribosomes, prior or in parallel to peptide- and mRNA motif-based sorting (***Niessing et al., 2018***), and underpinning co-translational protein complex formation.

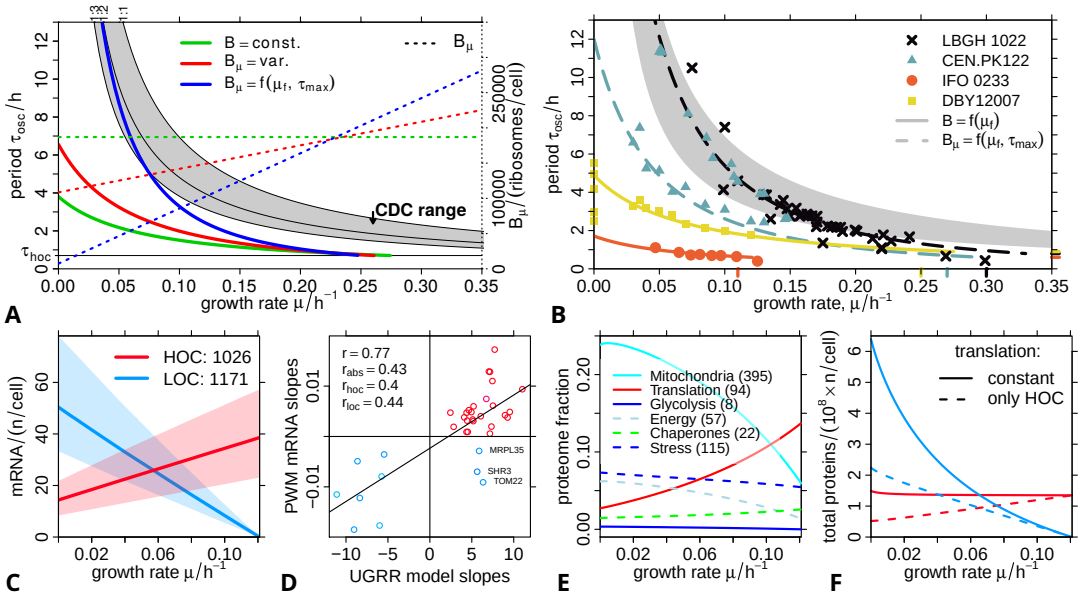

Figure 4. The Period Growth Rate Relation. A: Periods predicted by the base model (Eq. 1, solid green line) and the extended model with variable ribosome concentration $B(\mu)$ (Eq. 27, solid red line), with parameters from Table S5. The colored dashed lines are the ribosome concentrations (right y-axis) used for each model. Alternatively, ribosome parameters can be estimated via the μ_f-constraint (Eq. 28, solid blue line). As a reference the periods expected from CDC-synchronization in modes 1:1, 1:2 and 1:3 are shown (black solid lines, Eq. 33-34, (***Bellgardt, 1994***)) and the gray background (CDC range) serves as a reference in all similar plots. **B:** Oscillation periods observed at different growth rates and for different strains (colored points, cf. Fig. S22A) can be reproduced by the PWM model (colored lines) with the same parameters as in (A) but with ribosome concentrations calculated via strain-specific μ_f (colored ticks on the x-axis) and, for the variable ribosome model (dashed lines), τ_{max}. Additionally τ_{hoc} (colored ticks on the right y-axis) was manually adjusted (Tab. S6). **C:** Median (lines) and 25%/75% quantiles (transparent range) of all mRNA abundances predicted by the PWM model (Eq. 17) from ϕ_{hoc} (IFO 0233 parameters in (B)), and from gene-specific production and degradation rates (Tab. S21), and classification to either HOC (clusters 1, 2 and 10) or LOC (clusters 6, 7, 8 and 8) phase. The legend indicates the number of genes for which all data was available. **D:** Comparison of the mRNA slopes (C and Fig. S25A) and the slopes provided for signature genes of the UGRR model ***Slavov and Botstein*** (***2011***); data required for PWM-based prediction was available for the shown 35 of 58 signature genes. Gene names are provided for the two outliers. The straight line is a linear regression, and r is the Pearson correlation, r_{abs} removes the influence of the classification by taking the absolute slopes in both data sets, and r_{hoc} and r_{loc} are correlations calculated for only the HOC- or LOC-specific signature genes (red and blue point symbols). **E:** Fractions of the total protein abundance predicted by the PWM model (Eq. 18) for the gene lists used to analyze proteome fractions in ***Metzl-Raz et al.*** (***2017***). Gene numbers in brackets. **F:** Total protein abundances predicted by the PWM model for HOC and LOC phase genes ; calculated without (solid lines, Eq. 18) or with (dashed lines, Eq. 29) an additional restriction of translation to HOC phase. All rates required for mRNA and protein prediction are available in Dataset S3.

The PWM Model Explains Period Relation to Growth Rate

Next, we set out to explore the predictive power of the PWM model of gene expression (Appendix B). In short, we assume a step function of transcriptional activity, such that genes are transcribed at maximal rate during their respective expression phase (HOC or LOC) and not transcribed in the other phase (Eq. 17–18). We were intrigued to find that this formulation not only allows to predict mRNA and protein abundances, but yields a strict constraint between oscillation parameters, the life cycle rates and concentration of ribosomes, and growth rate. Ribosomal proteins (RP) are transcribed with the HOC phase clusters (***Klevecz et al., 2004***; ***Machné and Murray, 2012***) (Fig. 3A, D), and assuming that each RP is associated with one ribosome (Appendix B), we get:

$$\frac{\tau_{osc}}{\tau_{hoc}} = \frac{k}{\mu + \delta_R} \frac{\ell}{\mu + \delta_P} \frac{n_c}{B} , \tag{1}$$

where τ_{osc} and τ_{hoc} are the oscillation period and the duration of the HOC phase, μ is the culture growth rate, B is the total concentration of (cytoplasmic) ribosomes, and all other parameters refer to an average RP: k and ℓ are transcription and translation rates, n_c is the ribosome density (ribosomes per RP mRNA); and δ_R and δ_P are the mRNA and protein degradation rates.

Only τ_{loc}, the time spent in LOC phase (here: $\tau_{loc} = \tau_{osc} - \tau_{hoc}$), reflects changes in growth rate (***Slavov and Botstein, 2011***), while τ_{hoc} remains constant, or even slightly increased (***Burnetti et al., 2016***), with increasing growth rate. Similarly τ_{loc} changed consistently with period τ_{osc}, while τ_{hoc} changed less and in opposite direction during our experiment (Fig. 1B). Thus, we considered a constant τ_{hoc}, and gathered published measurements for all other parameters (Tab. S5, Fig. S21A) to calculate τ_{osc} at different growth rates. Remarkably, these parameters already yield realistic periods (green line, Fig. 4A) and the non-linear dependence of periods on growth rates (***Bellgardt, 1994***; ***Burnetti et al., 2016***; ***Machné, 2017***). Linearly varying the ribosome concentration with growth rate (Fig. S21C) (***Waldron and Lacroute, 1975***) makes the period function steeper (red line, Fig. 4A).

Periods reach a minimum towards the growth rates μ_f, where yeast metabolism switches from purely respiratory to respiro-fermentative metabolism of glucose (***Burnetti et al., 2016***; ***Machné, 2017***). In IFO 0233, fermentation set in early, at growth rates $\mu_f = 0.11\,\mathrm{h}^{-1}$–$0.15\,\mathrm{h}^{-1}$ (***Hansson and Häggström, 1983***; ***Satroutdinov et al., 1992***) consistent with its short period cycles. This constraint allows to estimate strain-specific specific values for the RP and ribosome-related parameters via published values for μ_f (Fig. 4B, S22D, Tab. S6). The model with variable ribosomes is required to fit data from the two strains with longer periods (CDC range), or alternatively very low degradation rates (Fig. S22C). This pattern is confirmed when fitting Eq. 1 separately to 20 consistent period data sets (Fig. S23, S24). Long period data sets require to set at least one of the degradation parameters in the denominator to 0. This may be due to a phase-locking with the CDC (***Bellgardt, 1994***) (gray areas in Fig. 4A, B), where the HOC phase aligns with the budding phase of the CDC and LOC phase is purely respiratory (RQ ≈ 1) and corresponds to the G1 phase of the CDC (***Münch et al., 1992***). When such phase-locking with the CDC occurs, PWM and oscillation parameters may not be directly coupled in this range.

Qualitative Consistency with Transcriptome and Proteome Data

Next, we used the fractional HOC phase length ($\phi_{hoc} = \tau_{hoc}/\tau_{osc}$) calculated for IFO 0233 (Fig. 4B), and gene-specific parameters from literature (Fig. S21) to predict mRNA and protein abundances (Eq. 17, 18) for 1,197 genes classified to either HOC or LOC phase clusters (Fig. 4C–F, S25). The Universal Growth Rate Response (UGRR) model allows to predict growth rate based on the relative mRNA abundances of 58 signature genes selected by a strong negative or positive correlation of mRNA abundances with growth rate (***Airoldi et al., 2009***; ***Slavov and Botstein, 2011***). The mRNA abundance slopes calculated from the PWM prediction (Fig. 4C) correlated well with the growth rate-dependent slopes of the UGRR model's signature genes (Fig. 4D). Similarly, we found good overall agreement of the relative proteome fractions at different growth rates of gene groups selected by ***Metzl-Raz et al.*** (***2017***) (Fig. 4E). For example, the proteome fraction of mitochondrial genes decreases, while the fraction of genes involved with translation increase with growth rate, reflecting measurements (***Paulo et al., 2016***; ***Metzl-Raz et al., 2017***).

Quantitative Discrepancies: Chromatin Structure and Energetic Constraints

However, quantitively our predictions are off for some gene groups. For example the "translation" gene group occupies only 3% to 14% of the proteome in the PWM prediction, while this ranged from 19% to 38% in the proteome data evaluated by ***Metzl-Raz et al.*** (***2017***). In contrast, proteins from the "stress" group are overestimated ≈2x by the PWM prediction. Total mRNA abundances are within the range of published estimates (***Hereford and Rosbash, 1977***; ***Zenklusen et al., 2008***) at high growth rates, but 1.5x too high at low growth rates (Fig. S25). Total protein abundances are within the estimated range (***Milo, 2013***) only at the highest growth rate, and 6x fold too high at slow growth (Fig. 4F, S25). When comparing all gene-wise predictions with data, the main contribution for

the good overall correlation stems from correct classification into HOC phase or LOC phase-specific genes, while correlations are low, absent or even negative within the two groups (Fig. S25). This only slightly improves when focusing on the subsets of genes in a previously defined consensus clustering (***Machné and Murray, 2012***) of co-expression cohorts (Fig. S25).

These errors help to pinpoint weaknesses of the model, and suggest directions for future extensions. The `Ribi` and `RP` cohorts (HOC) are characterized by large nucleosome-free regions (NFR) in their promoter, while the `S/C` (LOC) cohorts have occupied promoters and a less defined gene body nucleosome structure (***Lee et al., 2007***; ***Machné and Murray, 2012***). Total nucleosome occupancy (histone H3) at transcription start sites was lower in HOC and reached a maximum in LOC phase, anti-phase to histone acetylation (H3K9ac) and RNA polymerase II binding (***Amariei et al., 2014***). Nucleosomes act as a barrier to RNA polymerase (***Jimeno-González et al., 2015***), and different chromatin structures are accompanied by different modes of transcription (***Kristjuhan and Svejstrup, 2004***; ***Zenklusen et al., 2008***). These observations all suggest that transcription of LOC phase-associated genes occurs at lower overall rates, which would rectify some of the observed discrepancies between model and data.

Similarly, translation rates could differ between the co-expression cohorts; e.g. RP genes are often assumed to have optimized codon usage, contributing to high RP abundances (***Jansen et al., 2003***). However, we believe a more significant over-simplification of the model leads to the overestimation of protein abundances at low growth rates. Protein biosynthesis is globally regulated by nutrient availability via the TOR1 kinase. Its inhibitor rapamycin immediately stops HOC phase and induces a long phase of low respiratory activity in both IFO 0233 and CDC-coupled systems (***Murray et al., 2007***; ***O' Neill et al., 2020***). Cumulative evidence suggests (see Appendix B) that a TOR1-mediated translational pulse occurs during HOC phase or at the transition from HOC to LOC phase, when the ATP/ADP ratio is high and then rapidly drops. As a first approximation of this phenomenon, we additionally assumed that translation of all transcripts occurs exclusively in HOC. Indeed, this assumption (Eq. 29) leads to more realistic total protein abundances at low growth rates (dashed lines in Fig. 4F, S25F,J). However, when using this assumption in period estimation, the obtained results are confined to short periods (Fig. S25K). Future work based on this novel theoretical framework should explicitly account for energetic constraints (Fig. 1) on the protein synthesis capacity during the cycle.

Discussion

The phenomenon of metabolic auto-synchronization in budding yeast continuous culture was instrumental for the clarification of the asymmetric CDC of budding yeast (***Küenzi and Fiechter, 1969***; ***von Meyenburg, 1969a***). The discovery of stable short period cycles in the distillery strain IFO 0233 (***Satroutdinov et al., 1992***) fortified early indications (***von Meyenburg, 1969b***; ***Mochan and Pye, 1973***) that the system is more than just synchronization of the CDC. Here, we first explored the complexity of dynamics observable in budding yeast continuous culture, here, a long-term transient and a sudden bifurcation, then tested the two main hypotheses on putative functions of the periodic transcriptome (**H1** and **H2**), and developed a novel model (**H3**).

Filtering for short-lived proteins with high mRNA amplitude confirms previously suggested candidate pathways (***Keulers et al., 1996***; ***Murray et al., 1999***, ***2003***, ***2007***) that may play a role in the control of metabolic dynamics and population synchronization (**H1**). These pathways also show distinct flux changes during the observed bifurcation of system dynamics: specifically H_2S, the gaseous intermediate of sulfate uptake and an inhibitor of the O_2-reaction in respiration; and perhaps more intriguingly, the prime waste product of heterotrophic organisms, CO_2. ***Aguilera et al.*** (***2005***) found that HCO_3^- can be a limiting anabolic substrate in aerated liquid cultures and colonies in the absence of carbonic anhydrase (CA). Potential roles of the periodic abundances of CA and enzymes of the auto-catalytic glyoxylate cycle, e.g. in a feedforward switch between catabolic and anabolic phases of the cycle, are promising candidates for further research into the mechanistics of metabolic dynamics.

Our calculations agree with two recent proteome measurements (***O' Neill et al., 2020***; ***Feltham et al., 2019***) that the majority of protein abundances oscillate only at negligible amplitudes. ***Slavov and Botstein*** (***2011***) suggested that periodic transcript abundances could serve to balance steady-state protein abundances with growth rate by differential variation of the length of the expression phases (**H2**). When translating this idea to a mathematical model, parameterized with published data, we find that it can indeed account for the relation of growth rate to the ribosomal biomass fraction (***Schaechter et al., 1958***; ***Molenaar et al., 2009***; ***Scott et al., 2010***), and to the relative abundances of large, functionally coherent classes of transcripts and proteins (***Brauer et al., 2005***; ***Paulo et al., 2016***; ***Metzl-Raz et al., 2017***). We were suprised to find that the model can also explain the relation of cycle periods to growth rate. This is the first mechanistic model for this relation since the CDC-based population synchrony models (***Bellgardt, 1994***; ***Hjortso and Nielsen, 1995***; ***Duboc and von Stockar, 2000***), and provides a novel theoretical framework to understand this phenomenon, essentially as a mechanism of cellular resource allocation. The same problem exists in diurnal context, where only few protein abundances oscillate, which questions the function of circadian clock-driven transcript dynamics (***Lück et al., 2014***; ***Wang et al., 2018***; ***Krahmer et al., 2021***; ***Karlsen et al., 2021***). The 24 h period is constant, but seasonal changes in light/dark phase lengths could mediate PWM-based control of steady state protein abundances.

Ideas and Speculation

Slavov et al. (***2012***) suggested the existence of a conserved eukaryotic cell growth cycle which, at slow growth, can be traversed multiple times during the biomass accumulation phases (G1 or G2) of the CDC. Nitrogen uptake (***von Meyenburg, 1969b***), and amino acid (***Hans et al., 2003***) and protein biosynthesis (***O' Neill et al., 2020***) were associated with HOC phase or with transition to LOC phase (***Murray et al., 2007***). This coincides with the G1/S transition in dividing cells (***Münch et al., 1992***; ***Klevecz et al., 2004***). Pulses of biomass growth or protein production were directly observable in single cell studies at the G1/S transition (***Cookson et al., 2010***; ***Bryan et al., 2010***; ***Litsios et al., 2019***), and multiple pulses per CDC were observed in mammalian cells (***Liu et al., 2020***). Here, we further suggest that these translational bursts act on spatially pre-organized layers of mRNA:ribosome complexes (**H3**), facilitating co-translational protein folding and complex formation (***Shiber et al., 2018***; ***Schwarz and Beck, 2019***) and spatial sorting of the proteome. Such a spatial pre-sorting could be generated by the temporal sequence of transcript synthesis and loading of fresh ribosomes in the growing cell – a reaction-diffusion mechanism (***Turing, 1952***) for subcellular pattern formation. In LOC phase, increased protein aggregation (***O' Neill et al., 2020***) at low ATP/ADP ratios (***Amariei et al., 2014***; ***Xu et al., 2004***) and low pH (***Keulers et al., 1996***; ***O' Neill et al., 2020***) could then serve to remove misfolded or otherwise damaged protein complexes. Knock-out or mutation of the prion-like (Q/N-rich) protein Gts1p (***Sanada et al., 2011***; ***Ripaud et al., 2014***) affected oscillations in all period ranges (***Wang et al., 1998***; ***Adams et al., 2003***; ***Liu et al., 2002***; ***Saito et al., 2002***). This perspective, a function of the cycle in protein homeostasis (***O' Neill et al., 2020***) and spatial organization, may also provide a framework to understand our observation that non-coding RNA transcript abundances peaked in LOC phase, where they could help to organize membrane-less compartmentation (***Maharana et al., 2018***; ***Quinodoz et al., 2021***). We speculate that, at low cellular energy, LOC phase may be a phase of assembly, clean-up and spatial self-organization of the growing cell.

Supporting Information and Data

Supporting Information File:

Appendices A and B, and all Supporting Figures.

Dataset S1:

Reactor data, including all calculated rates and RNA-seq samples IDs.

Dataset S2:

All 36,928 segments reported by `segmenTier`, incl. genome coordinates, cluster labels, read-counts, oscillation values (amplitude A_2, phase ϕ_2, p-value p_{rain}), coding gene and SUT overlaps, and all time points, using the sampling IDs (2–25) indicated in the reactor data.

Dataset S3:

Data for 3,849 coding genes that overlap with a segment with $J > 0$: relative mRNA and protein amplitudes and protein half-lives for prediction of protein amplitudes (Fig. S17), and production and degradation rates as used for period, mRNA and protein abundance predictions, incl. cluster associations and classification as RP gene (Fig. S21A/B, Tab. S5).

Materials and Methods

Strain History

Kuriyama's lab first reported oscillations in continuous culture of the *Saccharomyces cerevisiae* strain IFO 0233 (***Satroutdinov et al., 1992***). The strain number is from the Japanese culture collection NBRC and is identified there as "`Distillery yeast Rasse II`", "accepted" in 1941, and as ATCC 560 in the US American culture collection. These strains can be traced back to the "Brennereihefe, Rasse II" isolated as "Hefe 128" axenic culture by Paul Lindner at the Berlin Institut für Gärungsgewerbe in 1889 from samples of a distillery in Gronowo (West Prussia, now Poland) which obtained their yeast from a dry yeast supplier in the city Thorn (now Toruń, Poland) (***Lindner, 1895***). The strain and its descendant "Rasse XII" became commercially successful distillery strains within hybrid formulations ("Rasse M"), and was at the time an intensively studied strain in basic research, *e.g.*, in the search for the nature of "bios" (***Lindner, 1919***).

Continuous Culture

Pre-Culture

Saccharomyces cerevisiae (strain IFO 0233) were maintained on yeast nitrogen base agar plates (2 % glucose, 1.5 % agar; Difco, Japan) at 4 °C, sub-cultured from frozen stock cultures (-80 °C; 1 mL; 15 % glycerol; 5×10^8 cells). Pre-cultures were inoculated into Yeast Extract Peptone Dextrose media (10 mL; 1 % yeast extract, 2 % peptone, 2 % glucose) and grown at 30 °C in an orbital incubator (200 rpm) for 24 h.

Continuous Culture Medium & Inoculation

The culture medium consisted of D-glucose (20 g L^{-1}), (NH4)2SO4 (5 g L^{-1}), KH2PO4 (2 g L^{-1}), MgSO4.7H2O (0.5 g L^{-1}), CaCl2.2H2O (0.1 g L^{-1}), FeSO4.7H2O (20 mg L^{-1}), ZnSO4.7H2O (10 mg L^{-1}), CuSO4.5H2O (5 mg L^{-1}), MnCl2.4H2O (1 mg L^{-1}), 70 % H2SO4, (1 mL L^{-1}), Difco yeast extract (1 g L^{-1}) and Sigma Antifoam A (0.2 mL L^{-1}). All chemicals were supplied by Wako Pure Chemical Industries Ltd., Japan. The medium prepared with this recipe has a pH of ca. 2.5 which allows for autoclaving of media with both sugar and ammonium without browning (caramelization) and further avoids precipitation of salts in feed medium bottles during continuous culture. A custom-built bioreactor as outlined below was filled with 0.635 L of medium and autoclaved (121 °C; 15 min). Aeration (0.15 L min^{-1}), agitation (750 rpm), and temperature (30 °C) and pH (3.4) control were switched on, until the system was equilibrated. Then, the dissolved oxygen probe was 2-point calibrated by flushing with pure nitrogen (0 %) and switching back to air (100 %). The equilibrated and fully calibrated reactor was inoculated with $\approx 1 \times 10^9$ pre-culture yeast cells. A batch phase continued for ≈40 h until the cells had reached stationary phase, indicated by a sharp decrease in respiratory activity. Then continuous culture, *i.e.*, feeding with fresh medium, was initiated (at 44.5 h in Figure 1).

Culture Control & Monitoring

Continuous culture was performed in a custom-built bioreactor. The culture vessel was a jar fermentor (Eyela, Japan) with a total volume of 2.667 L. Culture volume was measured using a balance

(SB16001, Mettler Toledo, Japan), and continuous dilution with fresh medium was performed using a peristaltic pump (AC2110, ATTA, Japan) with a six roller planetary design which minimizes pulsing during rotation (about 10 rpm), and medium was pumped through 1 mm tubing (inner diameter; Masterflex, Cole Palmer, USA) and a 23 gauge steel needle. This ensured that the media was introduced in a stream of <20 µL droplets and just under a droplet per second at the operating dilution rate. Feed medium bottle weight was monitored by a balance (PMK-16, Mettler Toledo, Japan), set up to read from unstable environments and shielded from direct breezes. The culture was agitated at 750 rpm and aerated at 0.150 L min^{-1} by a mass flow controller (B.E. Marubishi, Japan). Dissolved oxygen was measured using an InPro 6800 sensor and pH with an InPro 3030 (both: Mettler Toledo, Japan). Culture pH was maintained at 3.4 by the automatic addition of 2.5 mol L^{-1} NaOH, and the weight of the NaOH bottle was monitored on a balance (PM400). Local control of agitation and pH was carried out by Labo controllers (B.E. Marubishi, Japan). The reactor pressure was monitored by a manometer (DM-760, Comfix, Japan) installed on a split outlet flow stream. The culture temperature was controlled at 30 °C by an external sensor connected to a circulating water bath (F25-ME, Julabo, Japan). Partial pressure of oxygen and carbon dioxide in the off-gas were measured by an Enoki-III gas analyzer (Figaro engineering, Japan). The partial pressure of hydrogen sulfide in the off-gas was measured using an electrode based gas monitor (HSC-1050HL, GASTEC, Japan). Instruments were calibrated as per manufacturer's instruction.

Reactor Data Acquisition and Calculation of Metabolic Rates

Data were acquired *via* the in-house `FERMtastic` software at 0.1 Hz. Metabolic rates were calculated as described previously (***von Meyenburg, 1969a***; ***Heinzle, 1987***; ***Verduyn et al., 1991***; ***Marison et al., 1998***; ***Murray et al., 2007***) from the online recorded data. Details and all equations are provided in Appendix A of the supporting information. All data were processed in the script `samplingSeq_2019.R` of the `yeastSeq2016` git repository. All calculated rates are provided in Dataset S1.

RNA Sequencing & Read Mapping

Sampling, RNA Extraction & Sequencing Library Generation

Total RNA was extracted as previously described (***Sasidharan et al., 2012***) from 24 samples taken every 4 min, covering ca. 2.5 cycles of the respiratory oscillation.

Culture samples were immediately quenched in ethanol and disrupted using acid-washed zirconia/silica beads (0.5 mm; Tomy Seiko Co., Ltd., Japan) with sodium acetate buffer (250 µL; sodium acetate 300 mM, Na2-EDTA 10 mM , pH 4.5–5.0) and one volume of TE-saturated phenol (Nacalai Tesque) equilibrated with sodium acetate buffer (250 µL).

The samples were then centrifuged (12 000 g, 15 min, 4 °C) and the aqueous phase transferred to fresh 1.5 mL microcentrifuge tubes. Back-extraction was performed by adding sodium acetate buffer (125 µL) to the bead-beat tubes, vortex (10 s), centrifuging (12 000 g, 15 min, 4 °C) and adding the aqueous phase to the first aqueous phase. 2.5 volumes ice-cold 99.5 % ethanol were added to the aqueous phase and RNA/DNA precipitated at −20 °C overnight. The samples were then centrifuged (12 000 g, 30 min, 4 °C), the supernatant removed by aspiration, and pellets washed 3× in 500 µL 70 % ethanol and air-dried (10 min, room temperature). DNA was removed (RNase-Free DNase Set; Qiagen, Japan) and RNA recovered by column purification (QIAquick PCR Purification Kit; Qiagen, Japan) in 50 µL UltraPure water, and stored at −80 °C prior to analysis. Total RNA had an RNA integrity number >7 and 260nm:230nm and 260nm:230nm ratios >2.14. All cDNA libraries were then generated and sequenced by the Beijing Genome Institute (BGI), China. Strand specific cDNA libraries were created using the "dUTP method" (***Parkhomchuk et al., 2009***; ***Levin et al., 2010***) and sequencing was carried out on an Illumina 1G sequencer.

RNA-seq Read Mapping

RNA-seq reads were mapped against the yeast reference genome (strain S288C, release R64-1-1) using `segemehl` (version 0.1.4) (***Hoffmann et al., 2014***) with default parameters and spliced read

mapping enabled. Initially unmatched reads were mapped again using the remapping tool from the `segemehl` package and the resulting files were merged. Coverage (read-counts per nucleotide) was normalized for total library size to reads-per-million (RPM) and RPM values were stored in a bedgraph file for further analysis.

RNA-seq Time Series Analysis

Analysis Strategy and R Packages

All analyses were performed with bash and R. The full analysis pipeline is available in a git repository at https://gitlab.com/raim/yeastSeq2016. Analysis and plotting tools developed for this work are available in an git repository with scripts and an R package available at https://github.com/raim/segmenTools. RNA-seq segmentation was performed with the `segmenTier` R package (***Machné et al., 2017***), available at https://cran.r-project.org/package=segmenTier. Scripts for genome-wide data collections and mapping to the yeast S288C reference genome (release R64-1-1) as well as the `genomeBrowser` plots are available at the git repository https://gitlab.com/raim/genomeBrowser.

Additional Data Sources

Genome annotations including Gene Ontology (GO) terms were taken directly from the gff genome file from the *Saccharomyces* genome database (SGD, release R64-1-1, 2011-02-08, same as for RNA-seq mapping). Published transcript data sets (XUT, SUT, etc.) were also obtained from SGD for the same genome release. Protein complex annotation CYC2008 (***Pu et al., 2009***) was downloaded from http://wodaklab.org/cyc2008/resources/CYC2008_complex.tab on 2019-06-04. Published period and growth rate-related data were obtained from https://gitlab.com/raim/ChemostatData, a data collection for ***Machné*** (***2017***), which also contains R scripts for analysis, incl. period models described in Appendix B. All other data were obtained from the supporting material of publications: half-live data for mRNAs and proteins from ***Geisberg et al.*** (***2014***) and ***Christiano et al.*** (***2014***); ribosome density data from ***Arava et al.*** (***2003***); the consensus clustering of periodically expressed transcripts from ***Machné and Murray*** (***2012***); UGRR expression data and slopes from ***Slavov and Botstein*** (***2011***); protein abundance data from ***Paulo et al.*** (***2016***), where growth rate data was sent in personal communication; and functional gene groups from (***Metzl-Raz et al., 2017***).

Discrete Fourier Transform.

A time series of N measurements $x = \{x_0, \dots, x_{N-1}\}$, taken at equally spaced time points $\{t_0, \dots, t_{N-1}\}$, can be transformed to frequency-space by the Discrete Fourier Transform (DFT):

$$X_k = \sum_{n=0}^{N-1} x_n e^{-2\pi i \frac{kn}{N}} \quad , \quad k = \{0, \dots, N-1\} \tag{2}$$

where X_k is a vector of complex numbers representing the decomposition of the original time series into a constant (mean) component (at $k = 0$) and a series of harmonic oscillations around this mean with periods P_k, amplitudes A_k and phase angles ϕ_k:

$$\begin{aligned} P_k &= (t_{N-1} - t_0)/k \,, \\ A_k &= |X_k|/N \,, \\ \phi_k &= -\text{atan2}(\text{Im}(X_k), \text{Re}(X_k)) \,. \end{aligned} \tag{3}$$

All DFT were performed with R's `fft` function.

For DFT-based clustering and segmentation analysis, it proved useful to scale DFT components by the mean amplitude of all other components $k > 0$:

$$X'_{k>0} = \frac{X_{k>0}}{\overline{|X|}_{k \neq \{0,k\}}} \,, \tag{4}$$

and the constant component ($k = 0$) by the `arcsinh` transformation:

$$X'_0 = \ln\left(|X_0| + \sqrt{X_0^2 + 1}\right). \tag{5}$$

For analysis of read-count data x_n were the raw read-counts, for analysis of segments x_n were the mean of all read-counts of the segment.

The index k corresponds to the number of full cycles with period P_k in the time series. Only the first 19 time points, covering two full cycles of the oscillation were used for the calculation of phases and p-values, such that $k = 2$ reflects the main oscillation. For all plots, phases were shifted such that $\phi_2 = 0$ corresponds to the transition from LOC to HOC.

Oscillation p-Values

For calculation of oscillation p-values p_{DFT} on read-count level the time series were permutated $N_p = 10,000$ times, and random amplitude $\tilde{A}_2$ calculated. The p-value was estimated as the fraction of permutations for which the random amplitude was larger than the observed amplitude A_2 (eqn. 3). This analysis was performed with the script `genomeOscillation.R` from the `segmenTools` git repository. Oscillation p-values p_{rain} on segment level were calculated with the R package `rain` (***Thaben and Westermark, 2014***) using period $P = 0.65$ h and time step $\delta t = 4$ min. This analysis was done with the script `segmentDynamics.R` from the `segmenTools` git repository.

Segmentation of RNA-seq Read-Counts & Segment Classification

The data were pre-segmented into expressed and weakly expressed chromosomal domains by a previously described heuristic (***Machné et al., 2017***) with a minor correction that splits pre-segments at chromosome ends. Pre-segmentation was done with the script `presegment.R` from the `segmenTools` script collection; Figure S7 provides pre-segment length distributions and run parameters. Pre-segments were then individually split into non-overlapping segments with coherent temporal expression profiles by the `segmenTier` algorithm, using optimized parameters from our previous study (***Machné et al., 2017***). Shortly, the `arcsinh`-transformed read-count data was Fourier-transformed (Eq. 2); the first component ($k = 0$), reflecting the mean expression level, was `arcsinh`-transformed (Eq. 5); and all other ($k > 0$) components were amplitude-scaled (Eq. 4). The real and imaginary parts of the scaled DFT components $X'_{k=0,\ldots,6}$ were then clustered into 12 groups with R's implementation of `k-means` (using the `Hartigan-Wong` method or if that failed, the `MacQueen` method). This clustering then provided the anchors for the similarity-based segmentation by the `segmenTier`, where we used the `icor` scoring function with exponent $\epsilon = 2$, length penalty $M = 150$, nuissance cluster penalty $M_0 = 100$, and nuissance cluster exponent $\nu = 3$. This combination of parameters is achieved by arguments `-trafo "ash" -dc.trafo "ash" -dft.range 1,2,3,4,5,6,7 -K 12 -Mn 100 -scores "icor" -scales 2 -M 150 -nui.cr 3` to the `runSegmentier.R` script in the `segmenTools/scripts` collection. All segments are provided in Dataset S2.

The resulting segments were then filtered and classified by their oscillation p-values (p_{rain}, see above) and their overlaps with transcribed features annotated in the reference genome (release R64-1-1), using `segmentOverlaps.R` and `segmentAnnotation.R` in the `segmenTools/scripts` collection. Overlaps were quantified as the Jaccard index, $J = \frac{I}{U}$, where I is the intersect, the number of overlapping nucleotides, and U the union, the number of nucleotides covered by both, the segment and the annotated feature. Table S2 provides details on filtering and the resulting sizes (numbers) of analyzed segment sets. Figure S8 provides the full data structure which guided these threshold choices.

Segment Clustering

The means of read-counts covered by a segment were taken as segment time series. Periodic expression was analyzed by permutation analysis and DFT and by the R package `rain`. 11,248 segments with $p_{\text{rain}} < 0.85$ were chosen for further analysis (Fig. S8E-F). The DFT of the segment time series was amplitude-scaled (Eq. 4, Fig. S9A) and the first (constant) component ($k = 0$) was

`arcsinh`-transformed (Eq. 5). Real and imaginary parts of the scaled DFT components $X'_{k=0,..,6}$ were then clustered with the `flowClust` algorithm (***Lo et al., 2009***) for cluster numbers $K = 2, \ldots, 16$. The clustering with the maximal Bayesian Information Criterion, as reported by `flowClust` (Fig. S9B), was selected for further analysis. Clustering was performed by `clusterTimeseries2` function of `segmenTools` *via* the `segmentDynamics.R` script). The resulting clustering was sorted, re-labeled and colored automatically based on the means of their segments' expression phases (Eq. 3). The clustering was further sub-divided into high-amplitude clusters enriched for coding genes and low-amplitude clusters (compare Fig. S10 and S11).

Relative Protein Amplitudes

3,189 segments overlapping with a coding region with Jaccard index $J > 0.5$ and with protein half-live ($\tau_{1/2}$) annotation in (***Christiano et al., 2014***) were considered. Proteins with half-life annotation ">=100" were treated as $\tau_{1/2} = \infty$. The relative mRNA amplitudes were calculated from the DFT X_k (Eq. 2-3) of the first 19 time points (2 full cycles) of the RNA-seq read count time series as $A_{\text{mRNA}} = X_2/X_0$, *i.e.*, the ratio of the amplitudes of the 2nd component X_2 (2 cycles) over the 0th component X_0 (corresponds to the mean over all time points) of the DFT. Relative protein amplitudes A_{protein} were then calculated with the analytical solution to an ordinary differential equation of rhythmic production, after equation S8 of (***Lück et al., 2014***), as

$$A_{\text{protein}} = A_{\text{mRNA}} \frac{\gamma}{\sqrt{\gamma^2 + \omega^2}}, \tag{6}$$

with oscillation frequency $\omega = \frac{1}{0.67}\text{h}^{-1}$ and total protein degradation rate $\gamma = \delta + \phi$, where the actual protein degradation rates δ were taken from (***Christiano et al., 2014***) and dilution by growth is the chemostat dilution rate $\phi = 0.089\,\text{h}^{-1}$. In this model, the relative amplitude A_{mRNA} is assumed to directly reflect periodic production,*i.e.*, translational activity. Total amounts or translation rates are not required, and only a relative amplitude of protein amount can be calculated. Predicted protein amplitudes are provided in Dataset S3.

Peak Width Analysis

For each high-amplitude segment (2,505 segments with $p_{\text{rain}} < 0.0001$) the time series was interpolated to 1° resolution (0.105 min), and the oscillation phase ϕ_2 (Eq. 3) was used as anchors to scan for times spent above the temporal median $\tilde{x}$ during the first and the second full cycle in the data set (horizontal arrows in Fig. 2A). These times were recorded as the peak widths W_1 and W_2. The peak width change is the difference $\Delta W = W_2 - W_1$. Only segments with peak phases with ≥60° distance to the start or end of the timeseries and where the median expression was traversed twice within one cycle were considered, resulting in 2,357 segments with ΔW values. $\Delta\Delta W$ was calculated as the difference of the medians of ΔW of the two extreme segment clusters 1 and 8.

Cluster Enrichment Analyses

Cluster-Cluster Enrichment Tests

Categorical enrichments, *e.g.* coding gene co-expression cohorts *vs.* gene annotations, were analyzed by cumulative hypergeometric distribution tests (R's `phyper`) using `segmenTools`'s `clusterCluster` function and the `clusterAnnotation` wrapper for GO and and protein complex analysis, which compares overlaps of each pair of two distinct classifications into multiple classes, and stores overlap counts and p-values ("enrichment tables") for informative plots (see "Enrichment Profiles").

In these tests, the complete set of ORF annotated in the reference genome was analyzed (urn size: 5,795). Of these, 4,489 ORF that overlapped with an segment (Tab. S2) with a Jaccard index $J > 0.5$ were assigned to this segment's cluster, where non-clustered segments ($p_{\text{rain}} \geq 0.85$) were assigned to cluster "0", and all non-overlapping ORF ($J_{\text{ORF,max}} < 0.5$) assigned to the "n.a" cluster. For the analysis of protein complex analysis, all 5,524 ORF that overlapped with a segment with $J_{\text{ORF}} > 0$,

and the one with the maximal J_{ORF} was used for cluster assignment. This relaxed assignment was used to comprehensively capture complex co-expression and differential expression.

Enrichment Profiles

The results of multi-class enrichment tests (segment overlaps or cluster-cluster categorical overlaps) were visualized as colored table plots, *e.g.* Figure 3A), using `segmenTools`' function `plotOverlaps`. The total counts of overlapping pairs are plotted as text, where the text color is selected based on a p-value cutoff p_{txt} (as indicated). The background color gray level of each field scales with $\log_2(p)$, such that fields with a minimal p-value p_{min} (as indicated) are black.

For intuitively informative plots the enrichment tables were sorted. Table rows were sorted along the other dimension (table columns) such that all categories enriched above a certain threshold p_{sort} in the first column cluster are moved to the top, and, within, sorted by increasing p-values. Next, the same sorting is applied to all remaining row clusters for the second column cluster, and so on until the last column cluster. Remaining row clusters are either plotted unsorted below a red line or removed. This is especially useful to visualize enrichment of functional categories along the temporal program of co-expression cohorts, *e.g.*, Figure 3A and D. This sorting is implemented in `segmenTools`' function `sortOverlaps`.

Author Contributions

DBM designed and performed the experiment, SHB performed the RNA-seq read mapping, RM and PFS developed the segmentation algorithm. RM and IMA analyzed the time series data. All authors contributed to data interpretation and to writing of the manuscript.

Acknowledgments

We thank Sarah Lück, Oliver Ebenhöh, Wolfram Liebermeister, Ovidiu Popa, Chilperic Armel Foko Kuate and St. Elmo Wilken for inspiring discussions of the data and critical review of the manuscript. We are grateful to Martin Senz from the Berliner Institut f. Gärungsgewerbe und Biotechnologie, a successor of Paul Lindner, for help with clarifying the origin of the IFO 0233 strain.

Funding

DBM was funded by a partnering award from Japan Science and Technology Agency, Yamagata prefectural government and the City of Tsuruoka. RM was funded by the *Deutsche Forschungsgemeinschaft*, grants AX 84/4-1 and STA 850/30-1. IMA and RM were funded by EXC-2048/1–project ID 390686111 (CEPLAS).

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