Pulse-Width Modulation of Gene Expression and Protein Homeostasis

Rainer Machné, Douglas B. Murray

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A Tale of Cell Biology, Told by Budding Yeast (and a Cyanobacterium)

A lecture series beyond the **known knowns** of (cell) biology, exploring the **known unknows**, the **unknown unknowns**, . . . and some **unknown knowns** 👋.

0. Quantitative Microbiology: Exponential growth is rarely balanced.

I. Pervasive transcription during the low energy phase of respiratory oscillations.
II. Transcription at LTR retrotransposons. **TRANSCRIPTION**

III. DNA as a metabolic sensor, and
IV. Chromosomal domains and mobile elements. **GENOME HOMEOSTASIS**

V. Protein homeostasis by a transcriptional oscillator, and
VI. Pulse-width modulation of gene expression. **PROTEOME HOMEOSTASIS**

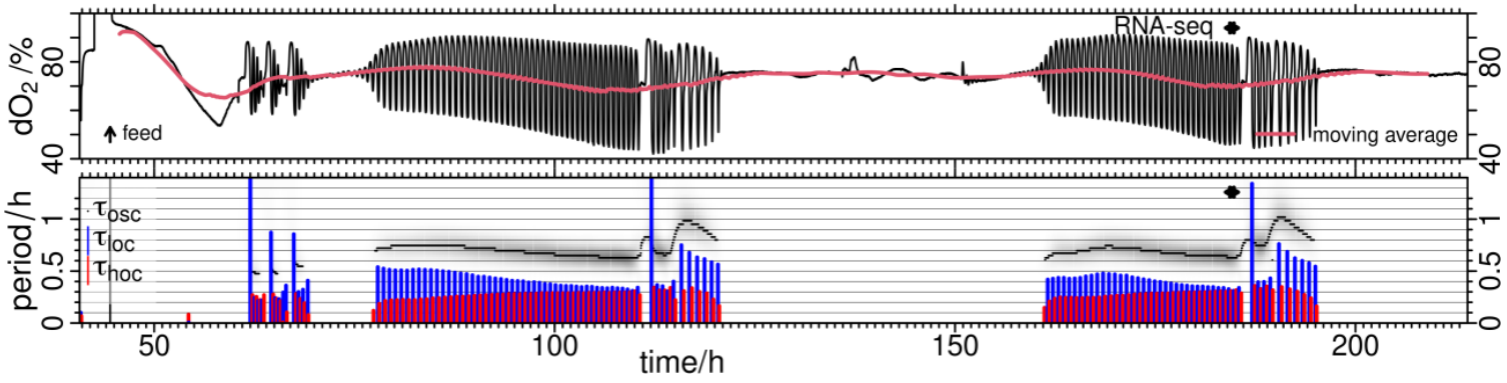
VII. Metabolism: feedbacks and the auto-catalytic cycles of life, and
VIII. The cell growth cycle as a cell-structural proofreading loop. **METABOLISM**

IX. Same, same in a cyanobacterium (circadian DNA supercoiling homeostasis).
X. Other eukaryotes: circadian and developmental clocks. **OTHER SPECIES**

Discussion: *Do yeast cells dream of metabolic sheep?*

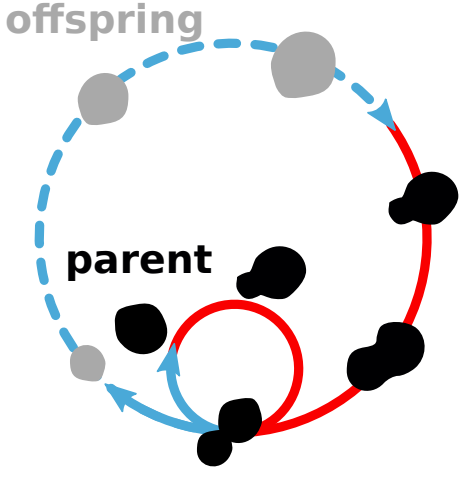
All slides, incl. references, at https://www.bioinf.uni-leipzig.de/~raim/lecture_series/

Respiratory (Metabolic) Oscillation in Budding Yeast



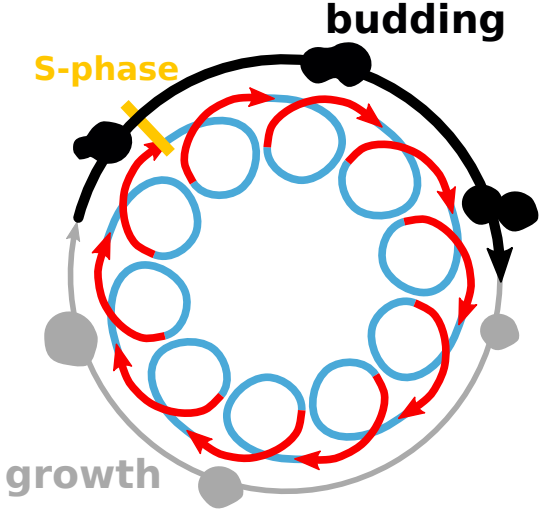
At high cell density: phases of High (HOC) and Low Oxygen Consumption (LOC).
here: distillery strain IFO 0233: exceptionally short periods, and **regular cycles or complex dynamics**.

Long period (v.Meyenburg, 1969):



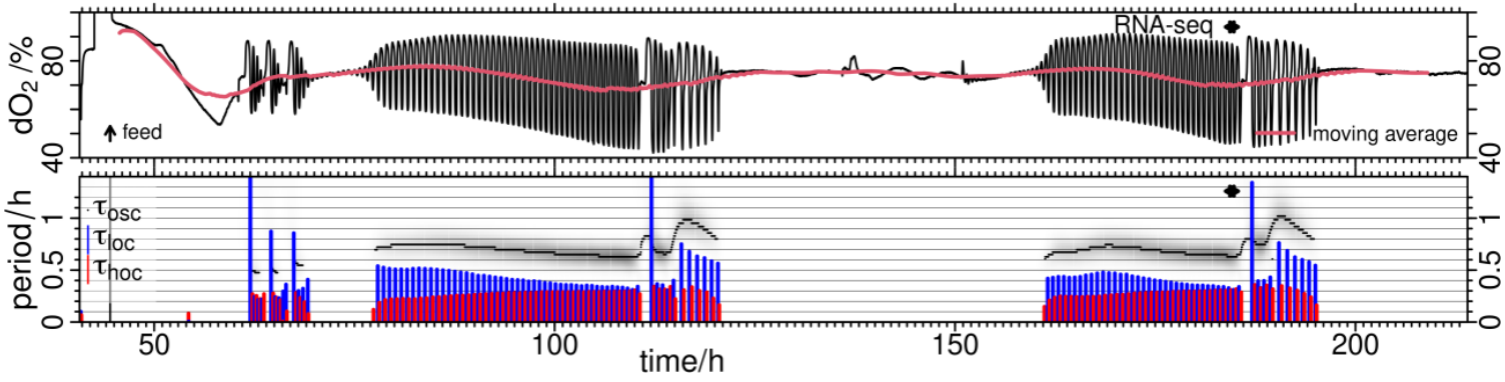
$\tau_{osc} = \tau_{parent}$, $\tau_{hoc} \approx \tau_{bud}$

Short Period, IFO 0233 (Kuriyama, 1992):



$\tau_{osc} \ll \tau_{bud} \approx \frac{\tau_{doubling}}{2}$

Respiratory (Metabolic) Oscillation in Budding Yeast



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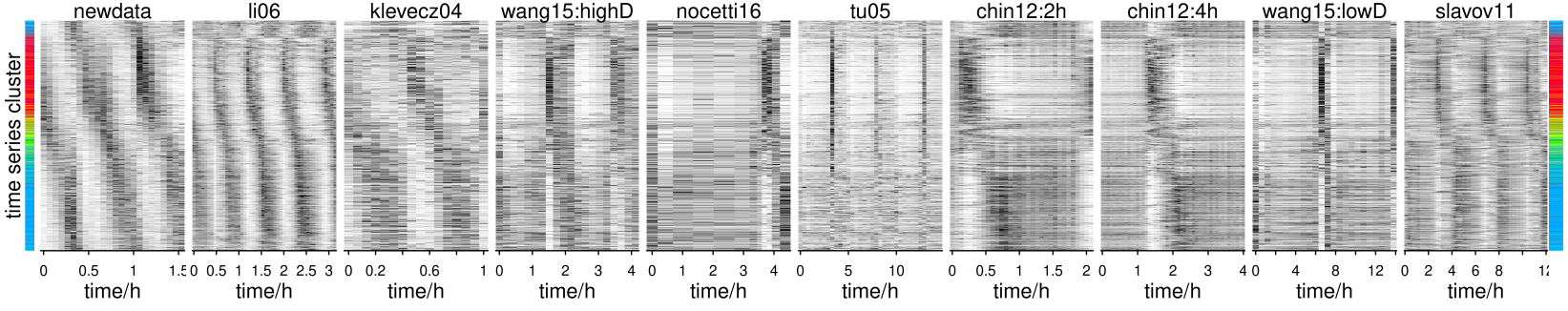
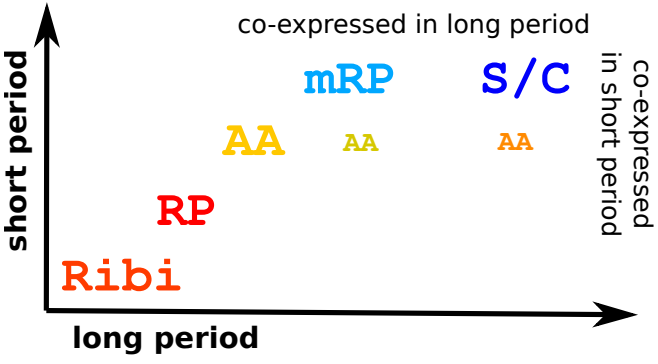




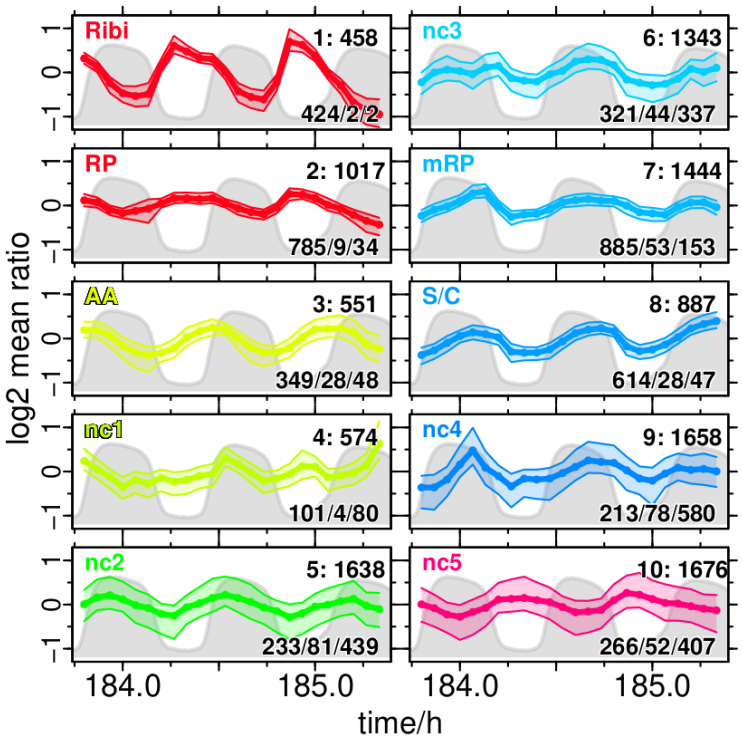
A conserved temporal transcription program:

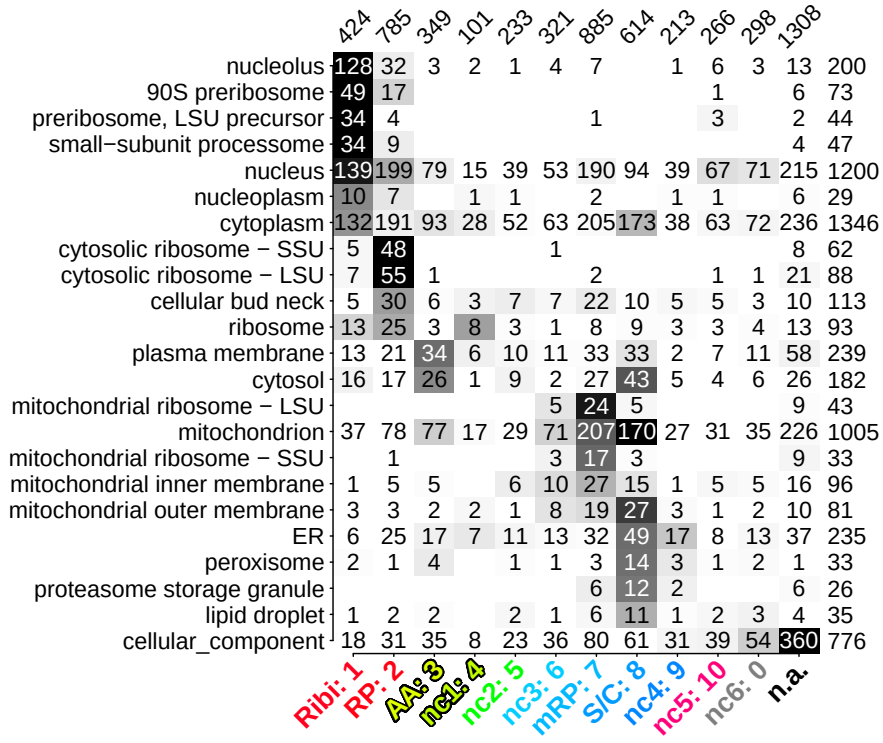
Ribi → RP → AA → mRP → S/C

unspooled at periods τ_{osc} from 0.6 h to 120 h!

A Conserved Temporal Program of Gene Expression

Lectures I & II - Noncoding transcription in LOC phase:

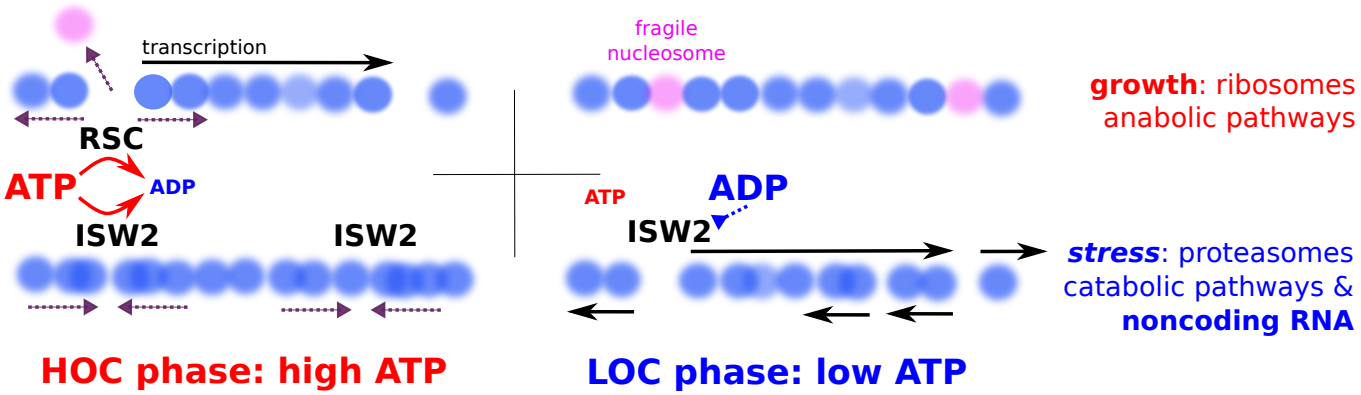




- **Unbiased** RNAseq segmentation and time series clustering,
- Five protein-coding clusters: Ribi → RP → AA → mRP → S/C,
- Noncoding clusters nc2-nc4 span LOC phase, include antisense transcripts, and transcripts emanating from genomically unstable sites, e.g. long-terminal-repeats of retrotransposons.

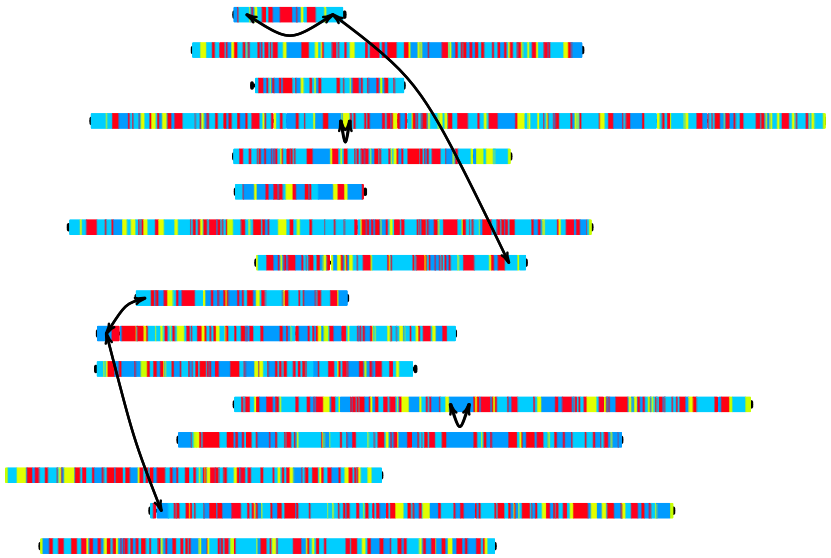
A Simple Mechanism for Global Gene Regulation

Lecture III - DNA as a metabolic sensor:

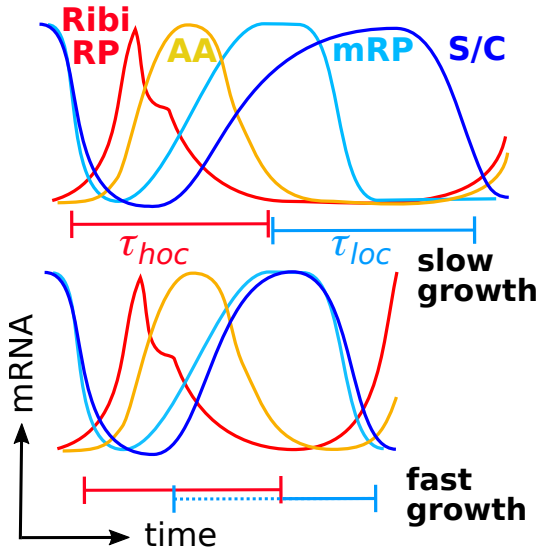


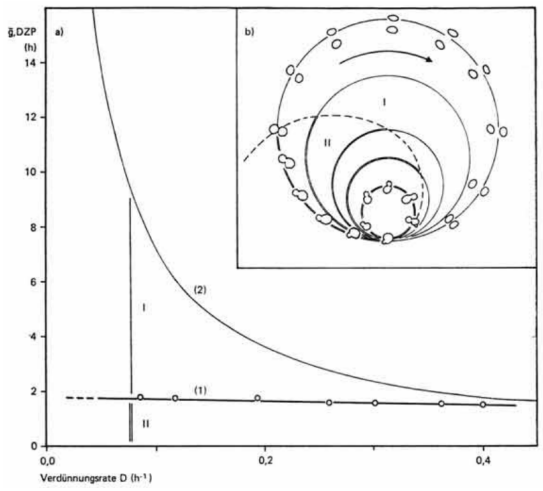
Machné and Murray (2012), Amariei et al. (2014)

Lecture IV - Chromosomal domains and genome evolution:



A Conserved Temporal Program of Gene Expression?





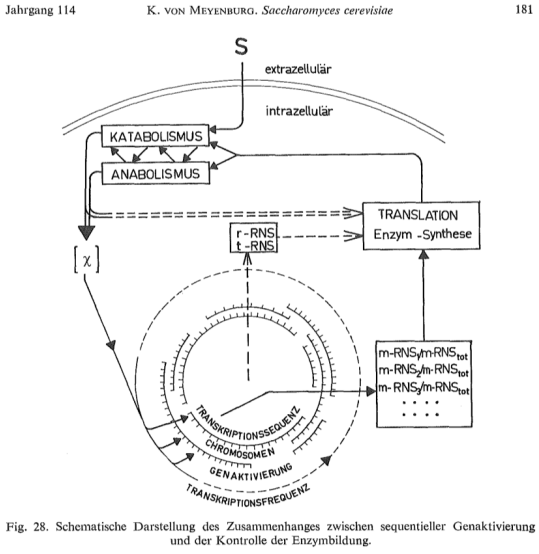


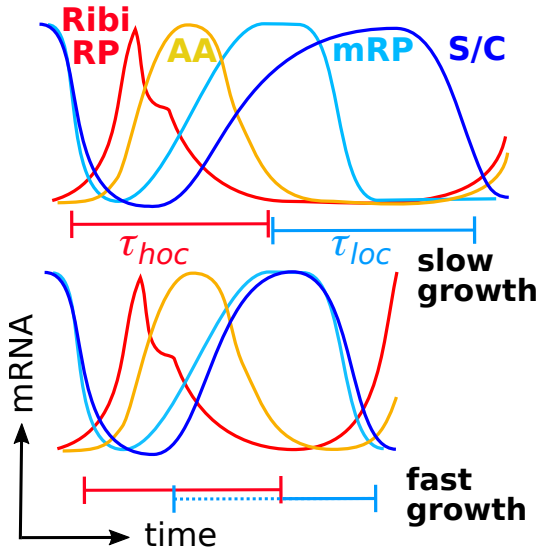
Fig. 28. Schematische Darstellung des Zusammenhanges zwischen sequentieller Genaktivierung und der Kontrolle der Enzymbildung.

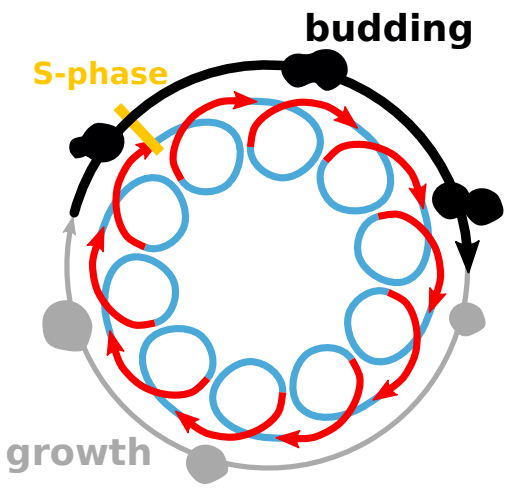
- Temporal program 👋 *makes sense* 👋:

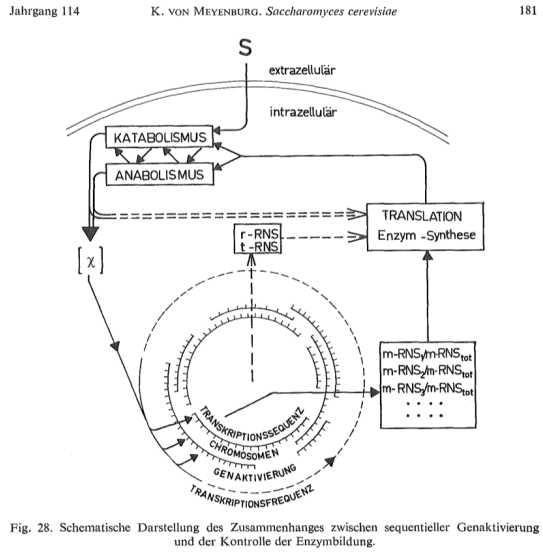
  $\rightarrow$ ribosomes $\rightarrow$ amino acids $\rightarrow$ energy $\rightarrow$ ribosomes $\rightarrow$
- But **why oscillate?**
  1. Metabolism of cell cycle phases:

ETH Zürich: Küenzi and Fiechter (1969); H. von Meyenburg (1969); Münch, Sonnleitner, and Fiechter (1992),
Various groups, mostly Europe, 1980s-1990s.

A Conserved Temporal Program of Gene Expression?







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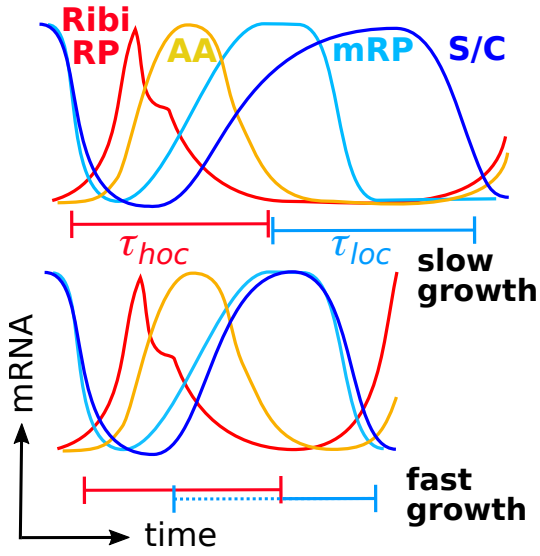
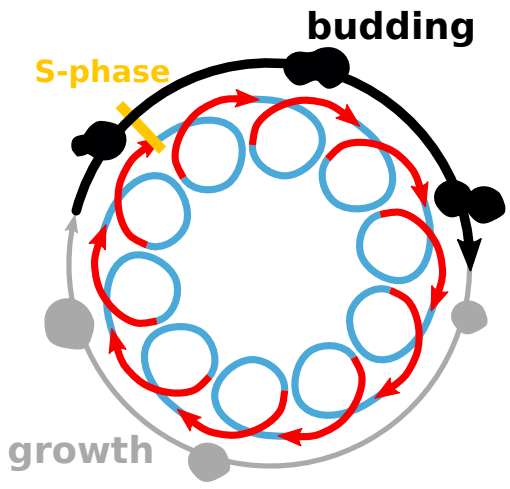
  2. Temporal compartmentalization of incompatible pathways:

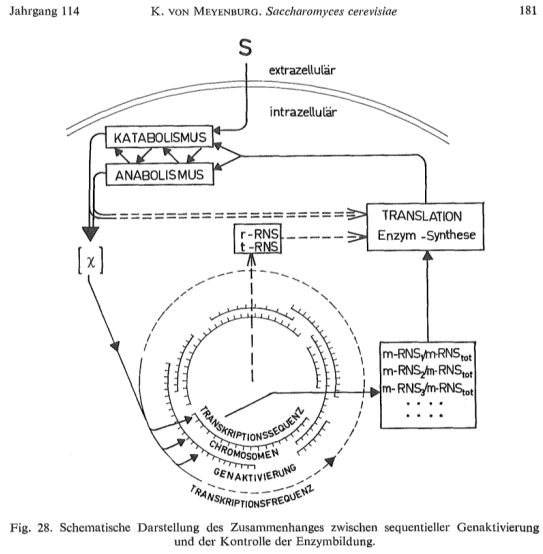
  DNA replication v. ROS from respiration (Klevecz et al. 2004),
  mechanism: H_2S v. respiration (Wolf et al. 2001).

variation of the *flight from light* conjecture by Pittendrigh (1993),

A Conserved Temporal Program of Gene Expression?







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- But **why oscillate?**
  1. Metabolism of cell cycle phases.
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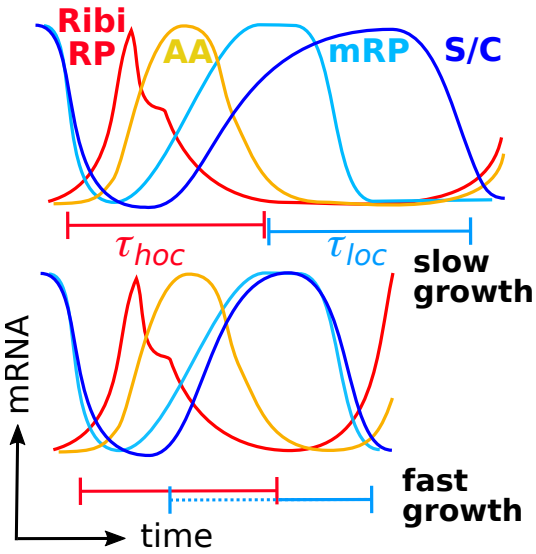
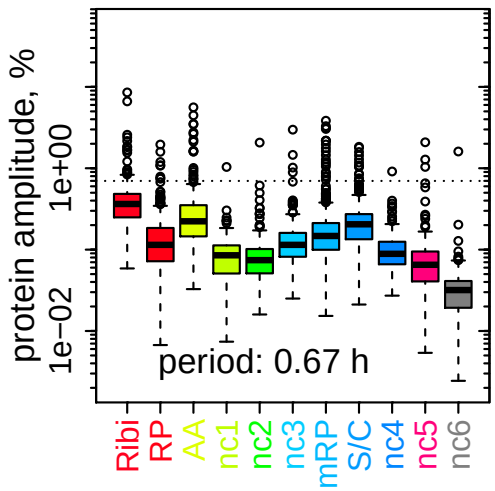
  3. Optimization of metabolic flux:

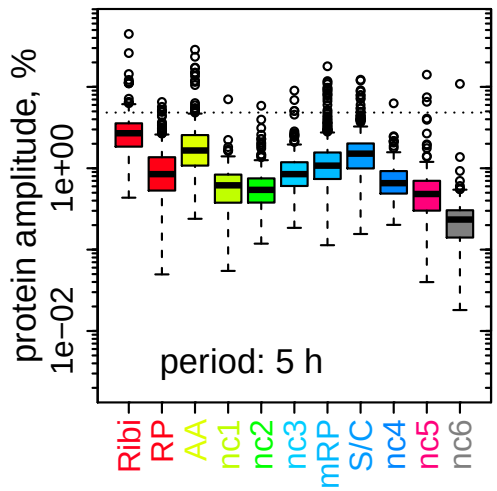
Oxidative (NAD^+) v. reductive (NADPH) metabolism (Lloyd and Murray 2007),
Catabolism v. anabolism (Machné and Murray 2012),
Minimization of leakage of futile metabolites (Liebermeister 2016) eg. H_2S, ethanol, CO_2.

just-in-time production of enzymes.

A Conserved Temporal Program of mRNA Abundance!







- Temporal program 👋 *makes sense* 👋:
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- **But** protein half-lives are too long:
  - Predicted (above) and measured (Feltham et al. 2019; O' Neill et al. 2020) protein amplitudes are **mostly** $< 1\,\%$ of mean abundance.

- A problem shared by circadian biology, shown in cyanobacteria (Waldbauer et al. 2012; Karlsen et al. 2021), plant (Krahmer et al. 2021), and mouse (Wang et al. 2018).

Protein amplitudes were predicted from measured relative transcript amplitudes and protein half-lives (Christiano et al. 2014) after Lück et al. (2014).

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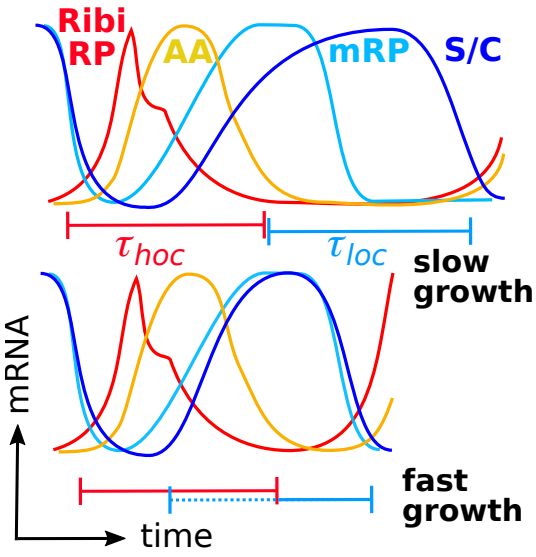

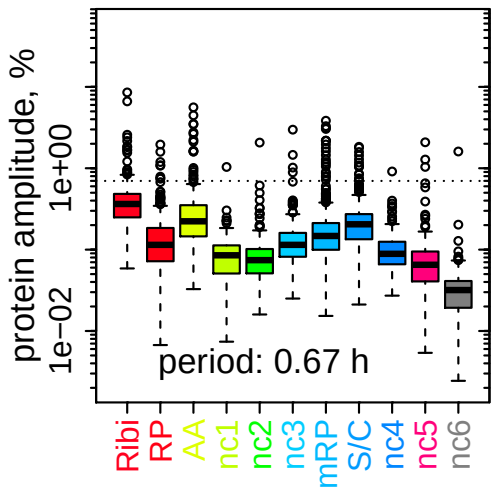

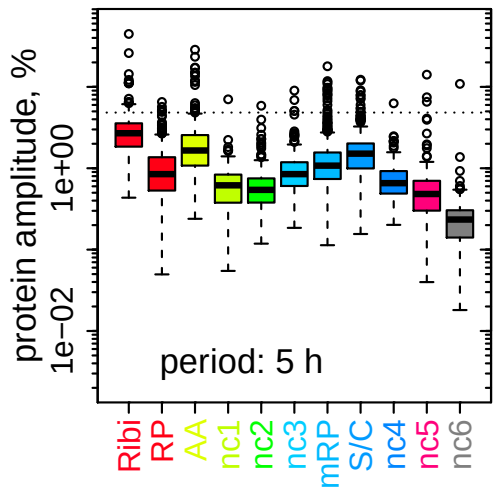

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- The few **unstable** proteins (TF, H_2S, CO_2) provide interesting candidates:
  $\Rightarrow$ **Lecture VII** on feedbacks and autocatalytic cycles.

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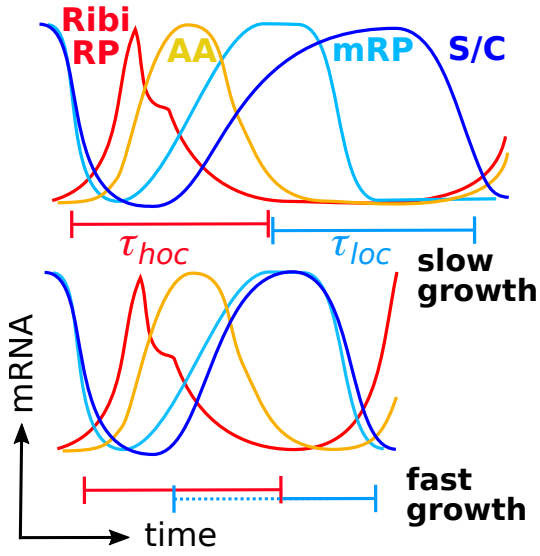
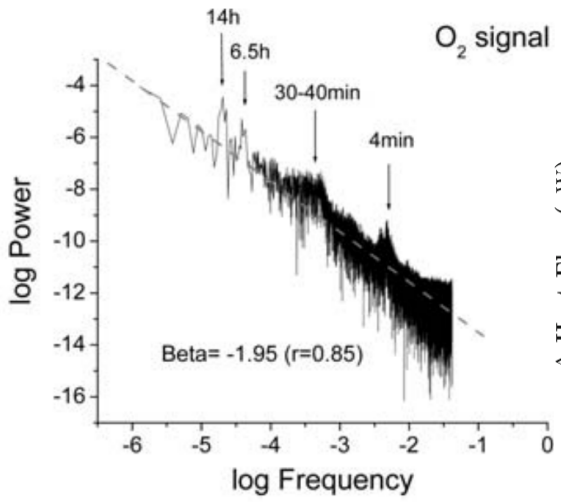
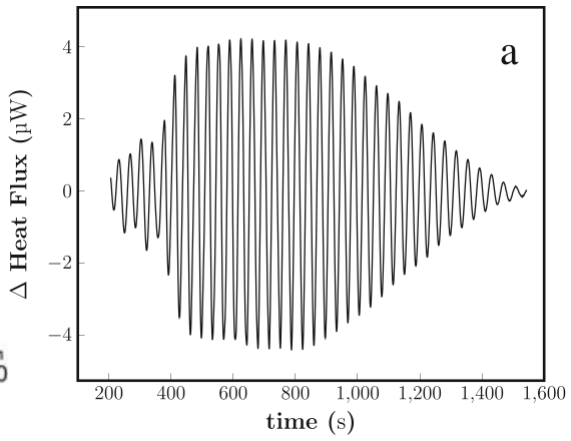
A Conserved Temporal Program of Gene Expression?







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  3. Optimization of metabolic flux.
  4. More fundamental reasons:

Multiscale coherence of cellular dynamics (Lloyd and Murray 2007; Aon et al. 2008),
Close-to-equilibrium thermodynamics (Thoke et al. 2018).

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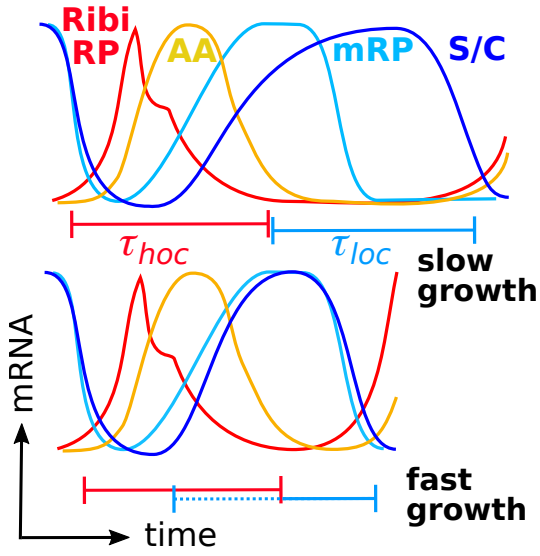

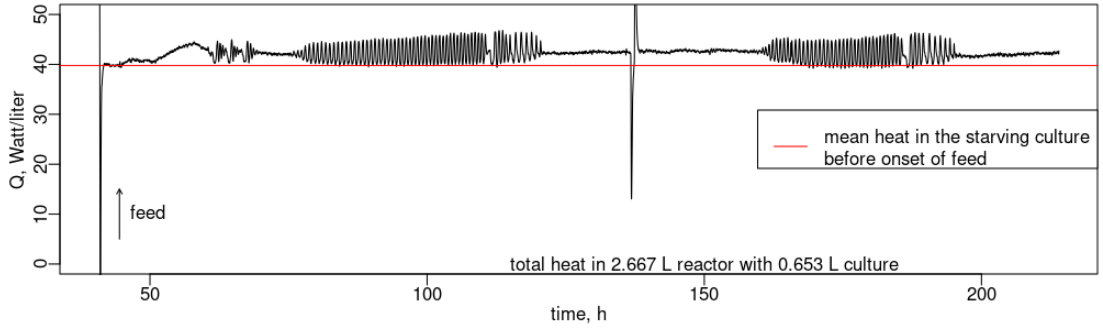

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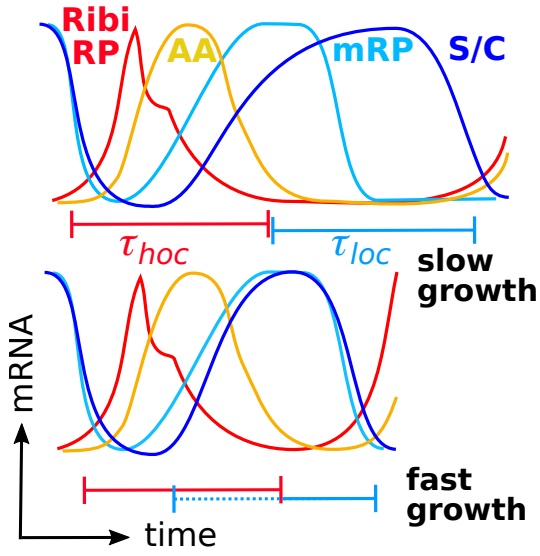

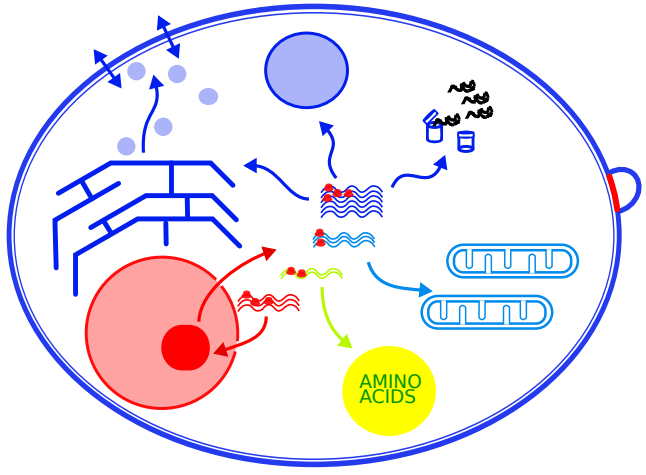

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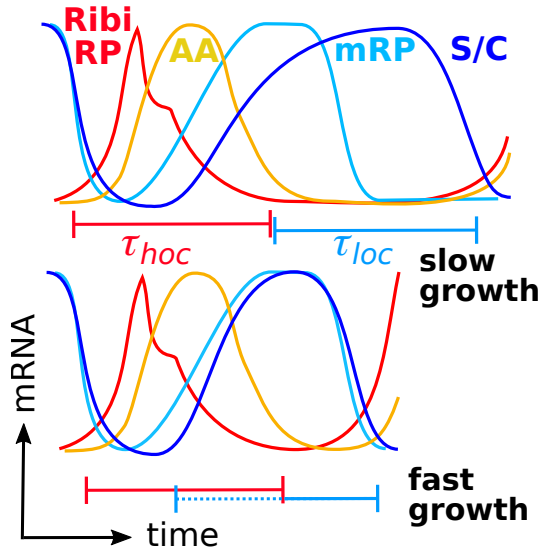
  4. Multiscale coherence and thermodynamics.
  5. Protein and genome homeostasis:

Pulses of protein translation and degradation (O' Neill et al. 2020),
Spatio-temporal pattern formation of the growing cell, and
Time constraints on macromolecular self-assembly. **Lectures VII–VIII**

A Conserved Temporal Program of Gene Expression?



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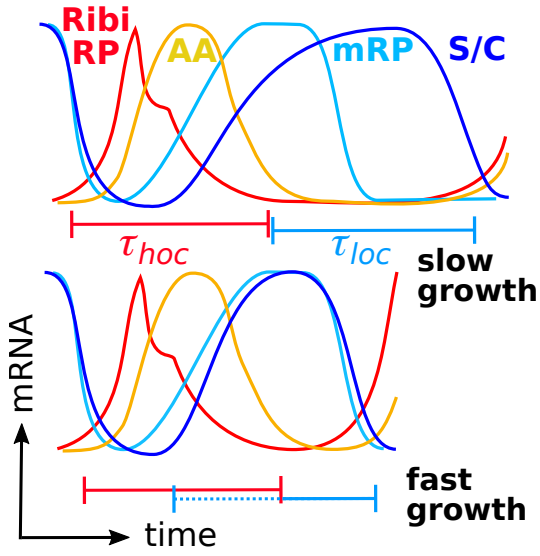
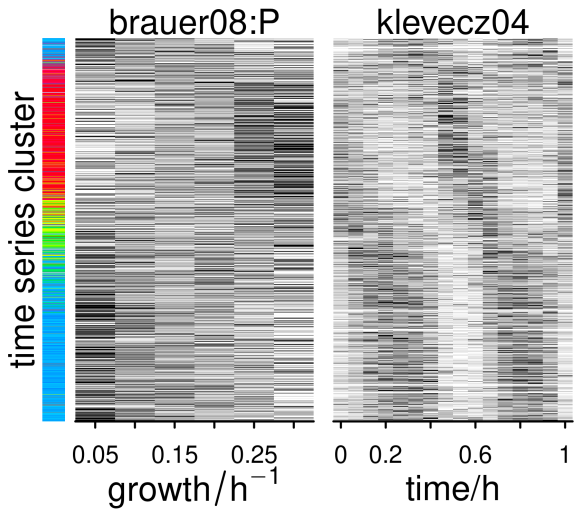
  5. Protein homeostasis:

  . . . by pulse width modulation of transcription: **this lecture**.

Growth Rate-Dependent Gene Expression

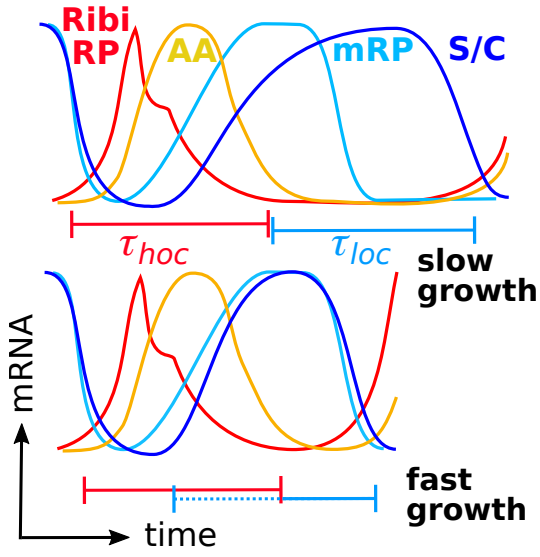


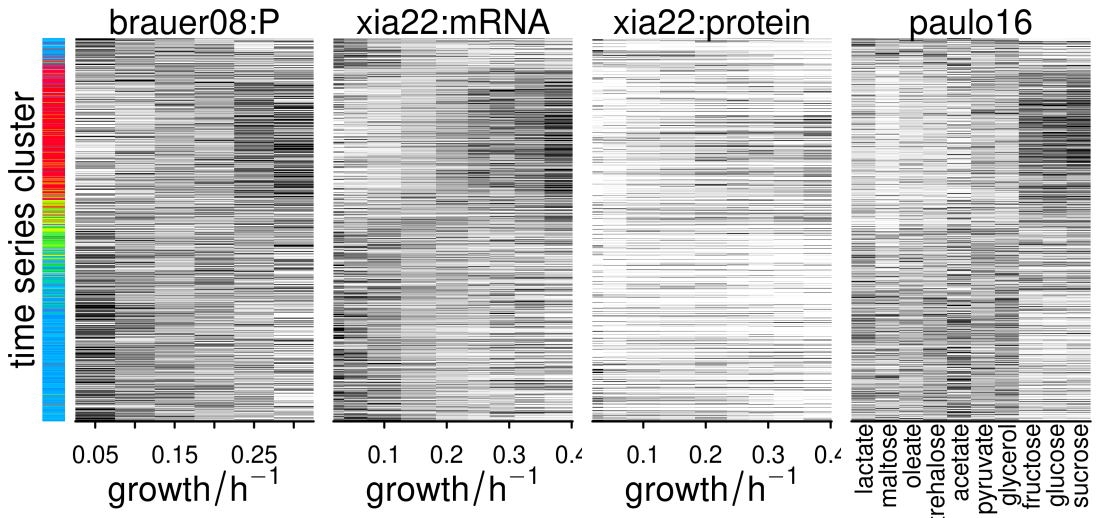


1. Brauer et al. (2005), Brauer et al. (2008):
   large fractions of the transcriptome scale with growth rate:

   $\leftrightarrow$ **relation to oscillation** as measured by Klevecz et al. (2004), Tu et al. (2005) and Li and Klevecz (2006).

Growth Rate-Dependent Gene Expression



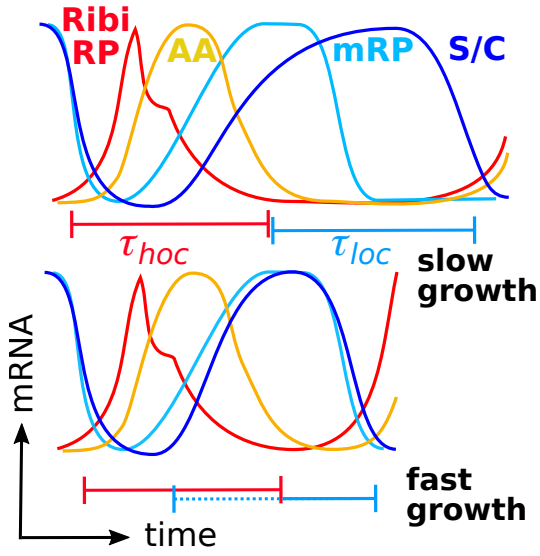


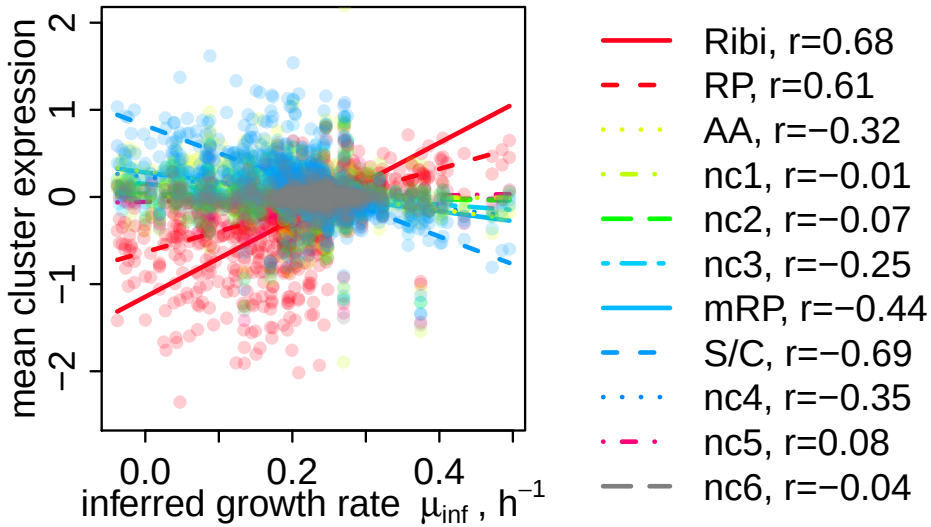
1. Brauer et al. (2005), Brauer et al. (2008): large fractions of the transcriptome scale with growth rate; **relation to oscillation**.

   here also on protein level!

Growth rate-dependent transcriptome and proteome data from continuous (chemostat) (Brauer et al. 2008; Xia et al. 2022) and batch cultures (Paulo et al. 2016).

Growth Rate-Dependent Gene Expression





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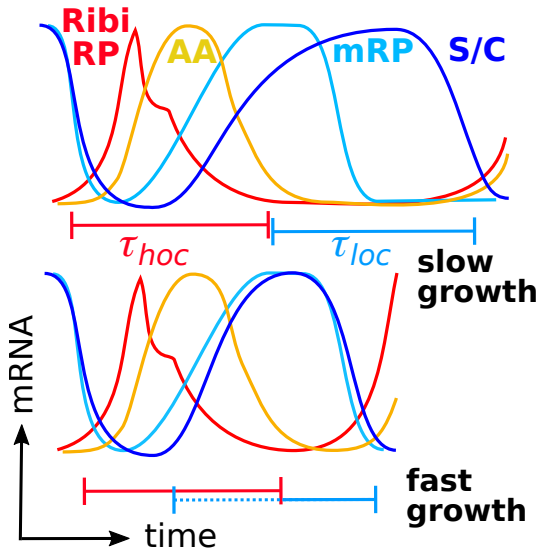
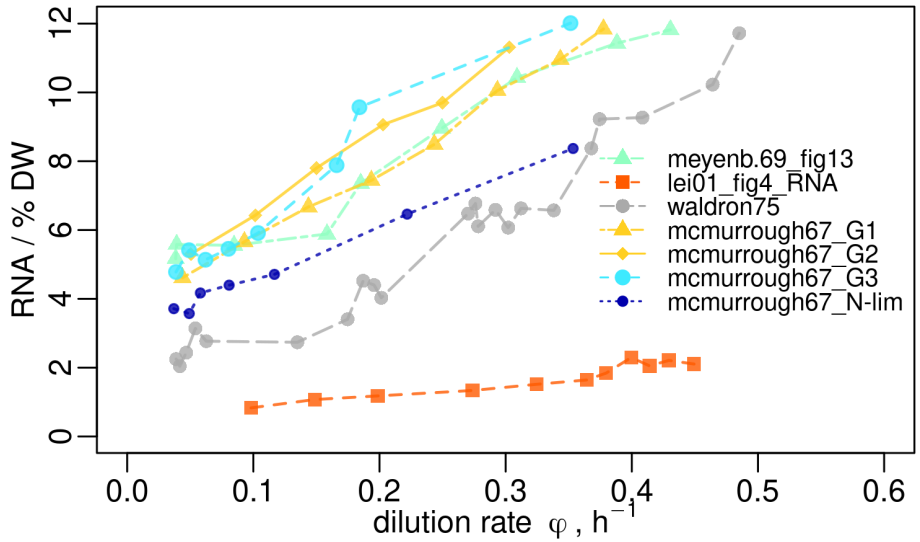
   Gradient descent implementation by Nikolai.

Growth rate was inferred (μ_{inf}) by gradient descent from the normalized expression values of **58 growth rate *signature genes* (Brauer et al. 2008)** for ~1.4k microarray experiments (McCord et al. 2007), and calibrated to experiments with known growth rates; and a linear regression and the Pearson correlation were calculated for the average expression of periodically co-expressed cohorts as a function of μ_{inf}.

Growth Rate-Dependent Gene Expression





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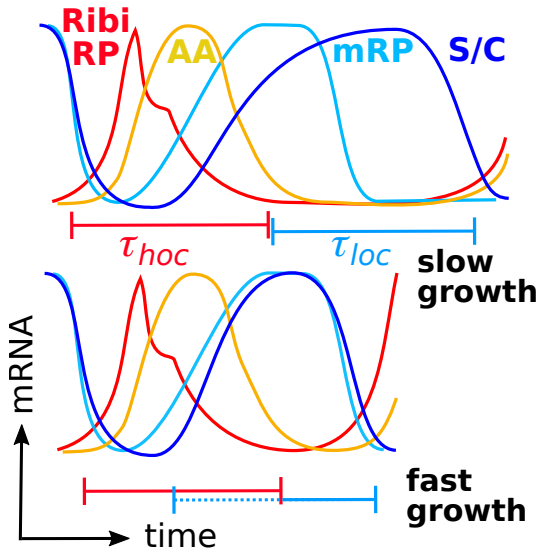
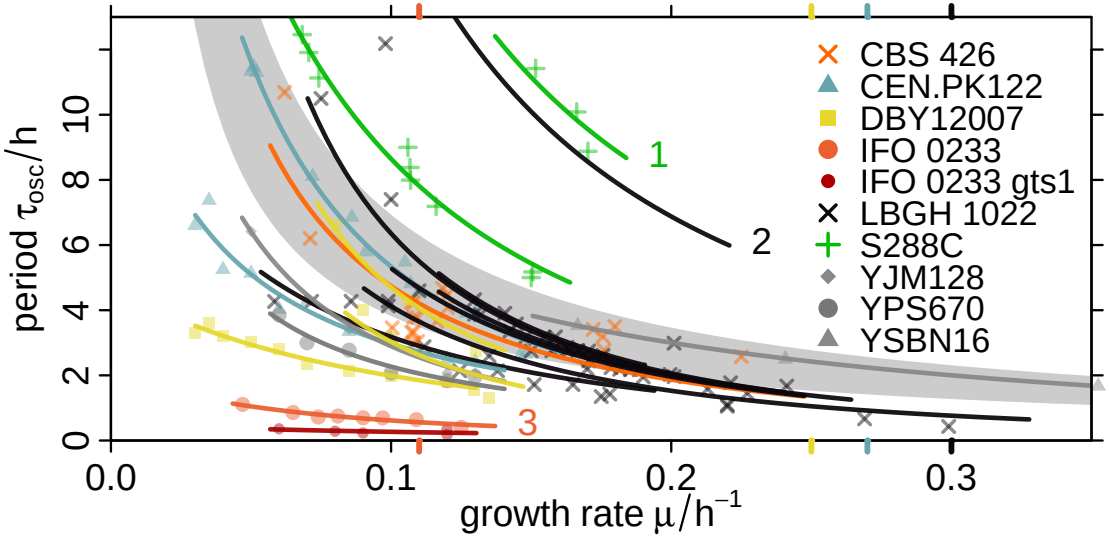
2. Schaechter, Maaløe, and Kjeldgaard (1958), Waldron and Lacroute (1975), Maaløe (1979), Scott et al. (2010): **ribosomes/biomass** $\propto$ **growth rate**.

The Copenhagen School of Microbiology:
ribosomes make ribosomes, and some other proteins,
currently still trending as *growth laws*.

RNA fraction of cell dry weight (% DW): mostly ribosomal RNA.
From the data collection at https://gitlab.com/raim/ChemostatData.

Growth Rate-Dependent Gene Expression





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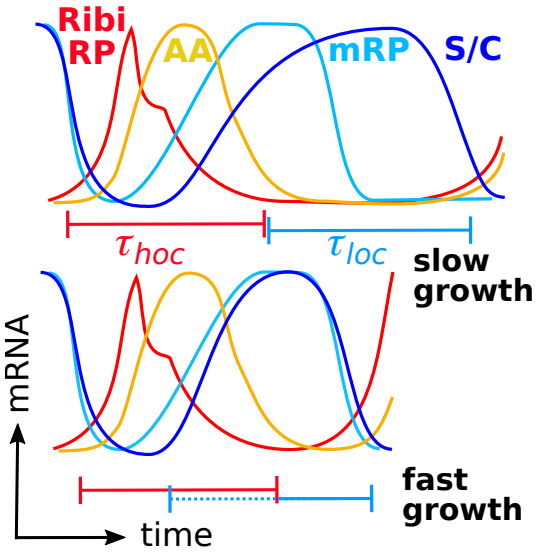
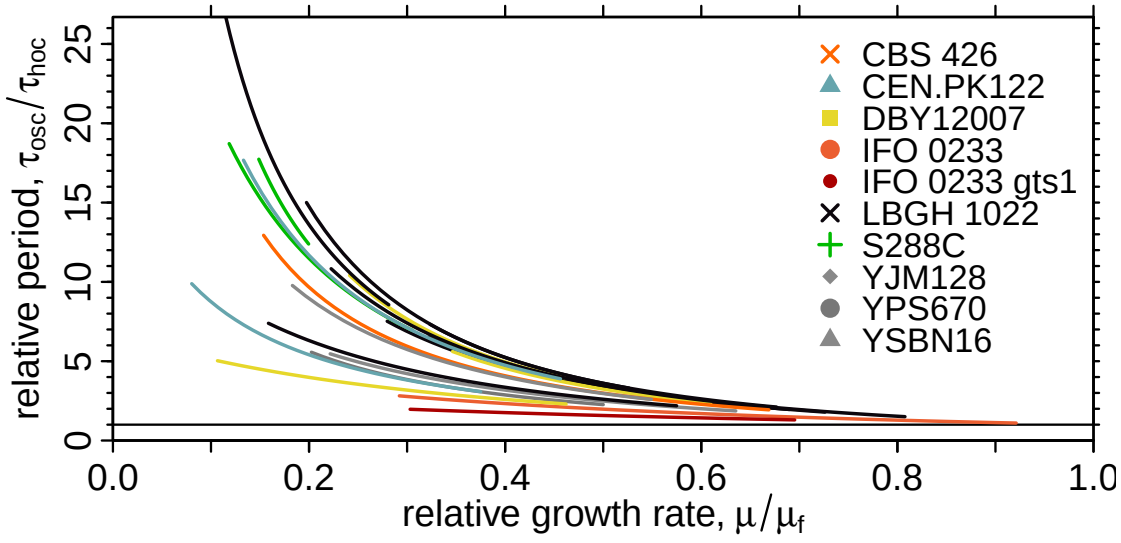
2. Schaechter, Maaløe, and Kjeldgaard (1958), Waldron and Lacroute (1975), Maaløe (1979), Scott et al. (2010): **ribosomes/biomass** $\propto$ **growth rate**.

3. Slavov and Botstein (2011), Machné and Murray (2012): HOC phase is a $\approx$**fixed temporal program**, 0.5 h to 2 h, and LOC phase duration **increases** with decreasing growth rate,
4. Burnetti, Aydin, and Buchler (2016), Machné (2017): minimal period, $\tau_{\mathrm{osc}} \to \tau_{\mathrm{hoc}}$, at strain-specific critical growth rate (fermentation), $\mu \to \mu_{\mathrm{f}}$.

Growth Rate-Dependent Gene Expression





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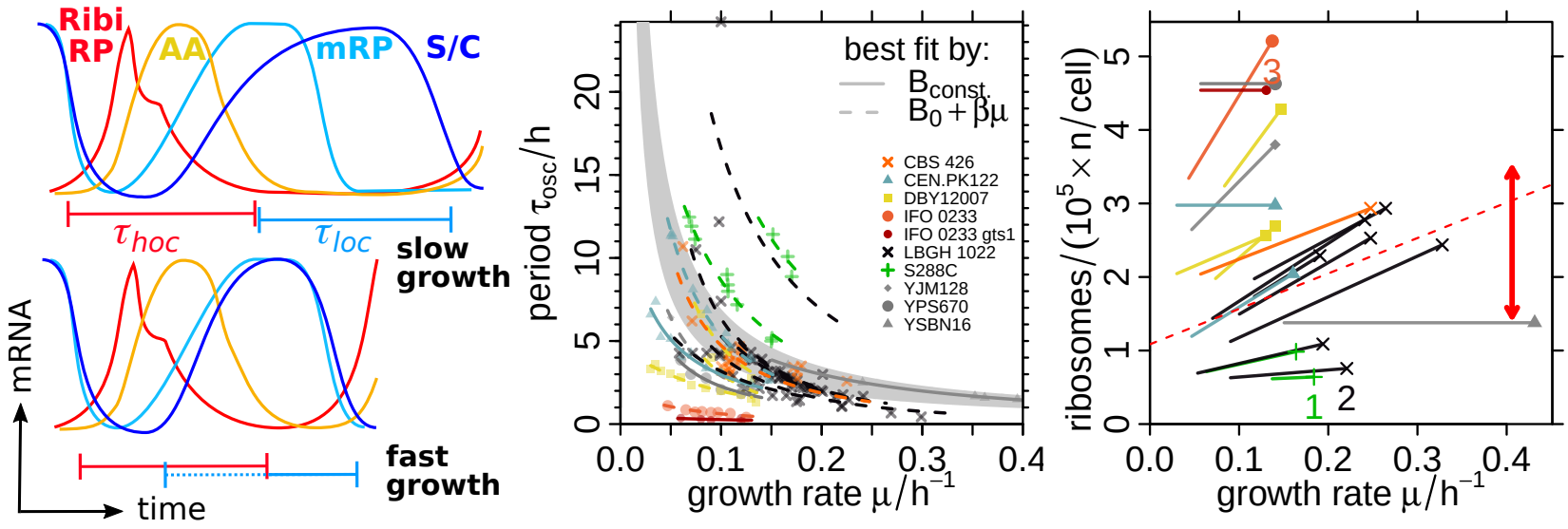
Growth Rate-Dependent Gene Expression



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5. The Pulse-Width Modulation Model: **Lecture VI**.

Growth Rate-Dependent Gene Expression

The hypothesis:

1. HOC phase duration is $\approx$ constant, and
2. LOC phase duration scales with the doubling time.
3. This **pulse-width modulation of gene expression** adjusts protein abundances of HOC phase and LOC phase-specific genes to growth rates.

This model is a generalization of:

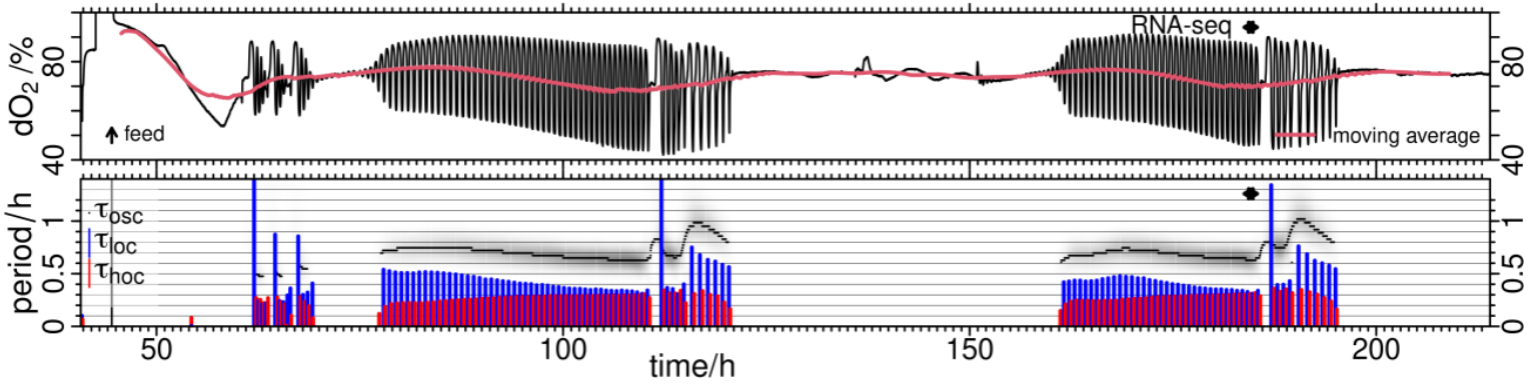
1. Budding phase (S/G2/M) duration is $\approx$ constant,
2. Growth phase (G1) duration scales with the doubling time.

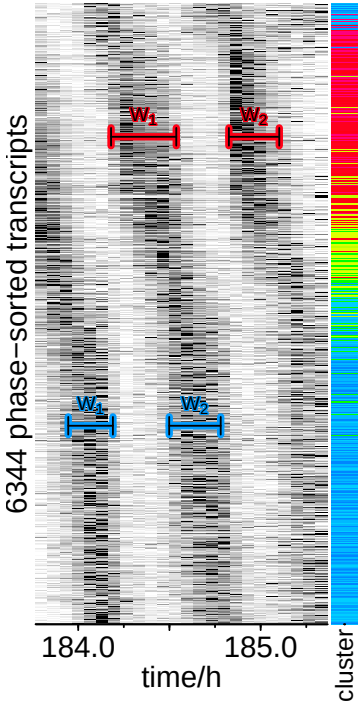
. . . necessitated by the observations of oscillations . . .

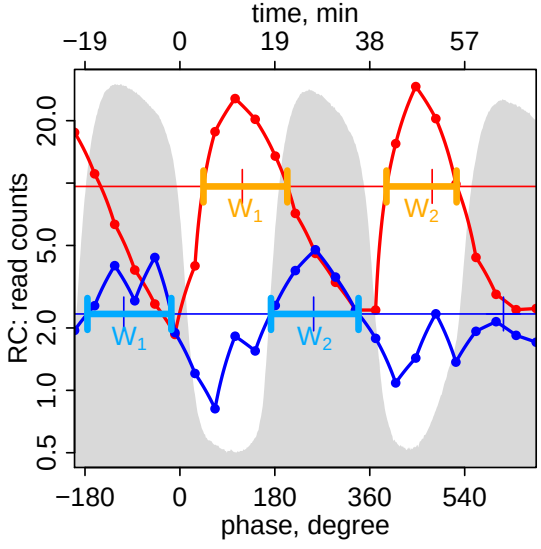
1. at periods significantly shorter or longer than cell division times,
2. on non-fermentable carbon substrates, w/o glycogen, and
3. in the absence of cell division.

$\rightarrow$ **let's ask the data!**

Peak Width Analysis: Transient Dynamics





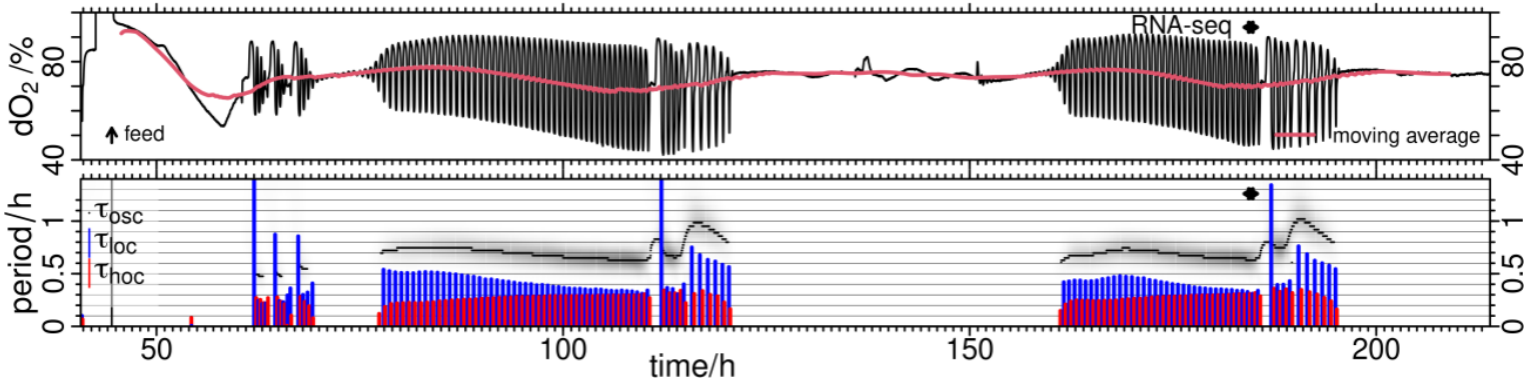


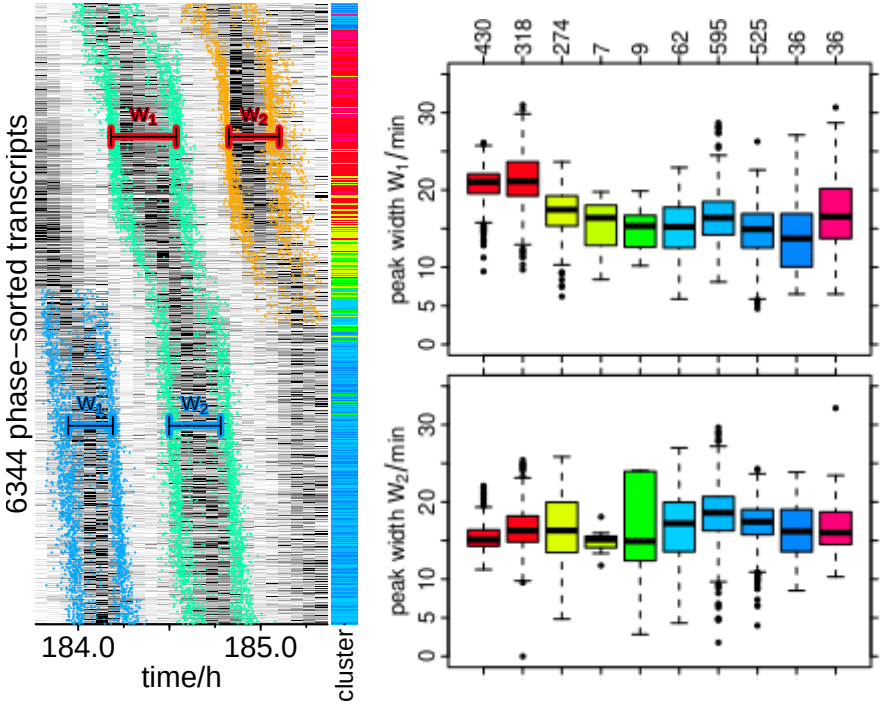
Transcript abundance peak widths
W:= time (or phase) spent above temporal mean.

W_1 and W_2:
first and second full cycle.

- Idea: $W \propto$ time of translational activity.

Peak Width Analysis: Transient Dynamics





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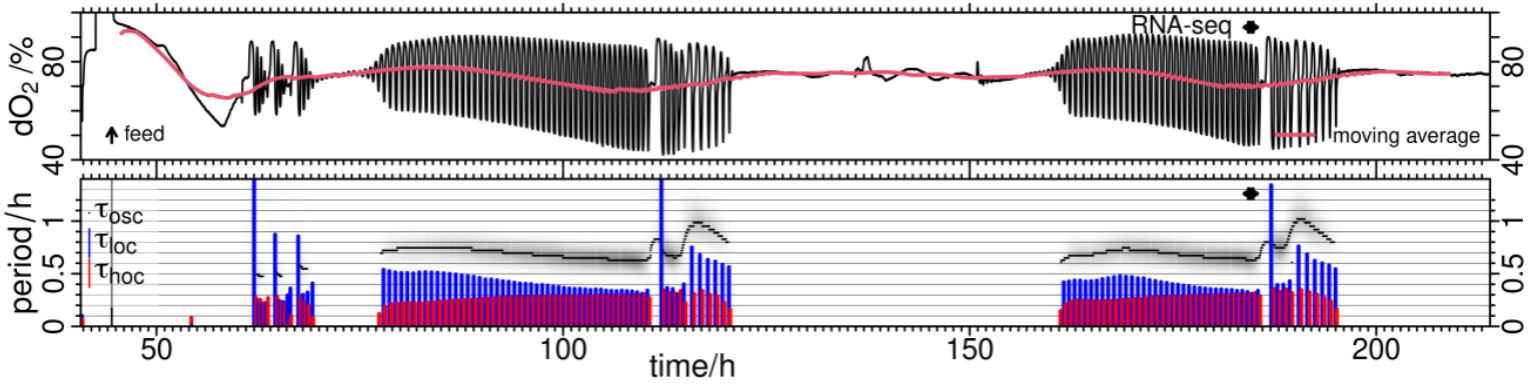
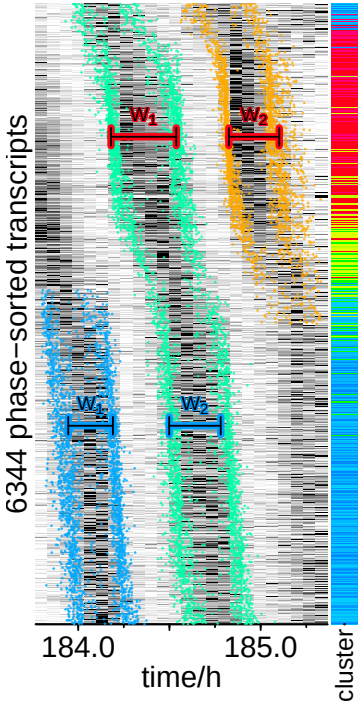
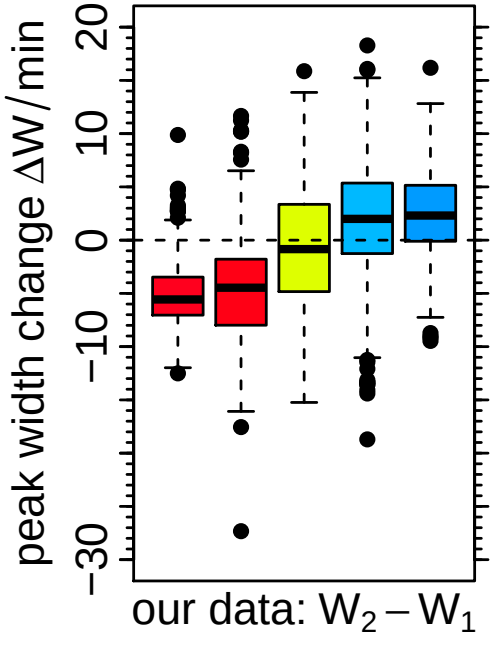






Shift to LOC phase:

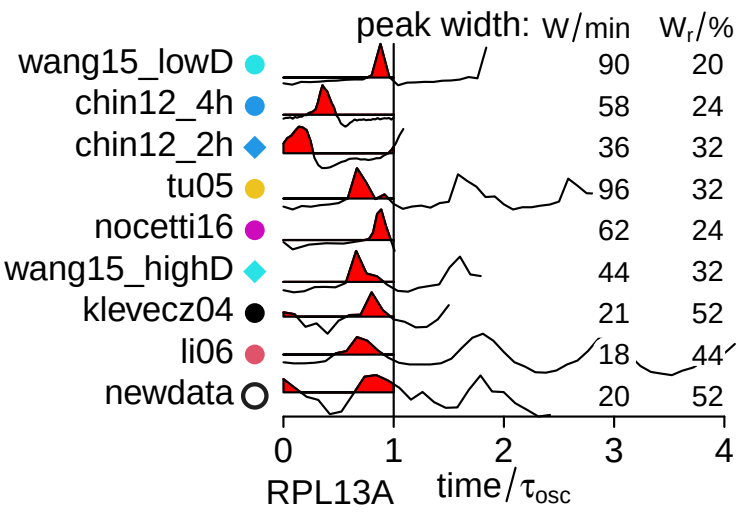
$\Delta W = W_2 - W_1$.

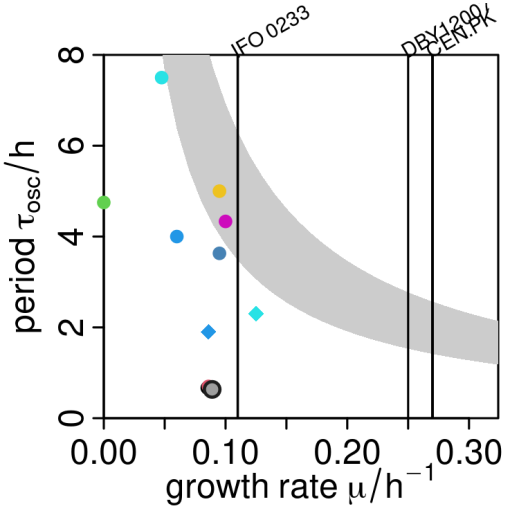
$\Delta W_{\mathtt{Ribi}} = -5\,\text{min}$ and
$\Delta W_{\mathtt{S/C}} = +3\,\text{min}$ at $\tau_{\text{osc}} \approx 36\,\text{min}$.

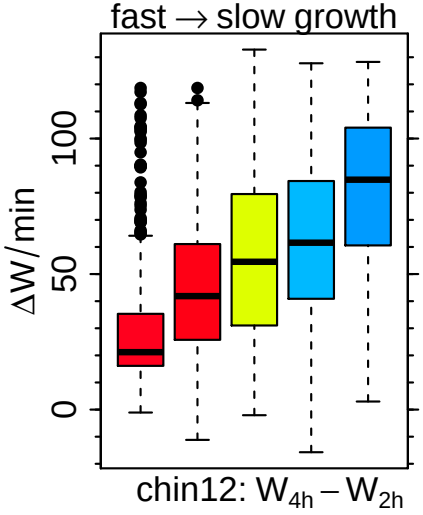
... **within just 2 cycles**, and **preceding** a pronounced shift of dynamics to a longer LOC phase.

- Idea: $W \propto$ time of translational activity.

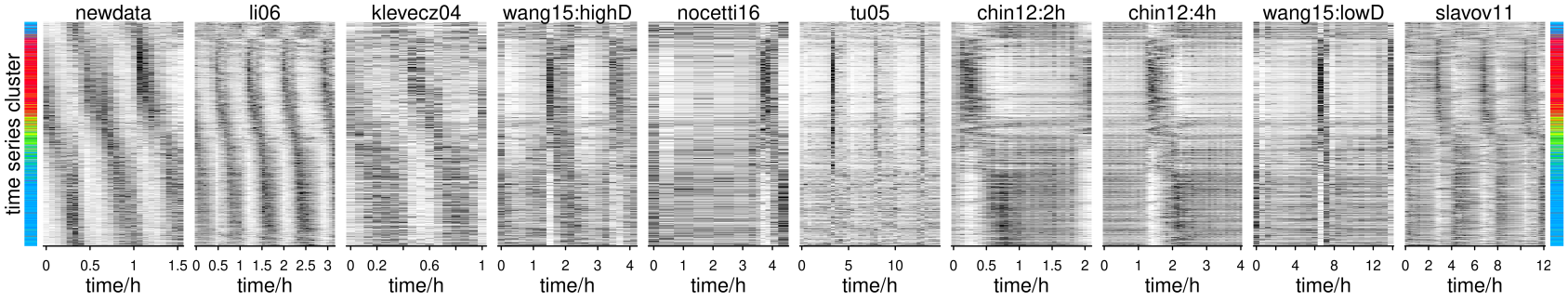
Peak Width Analysis across Transcriptomes



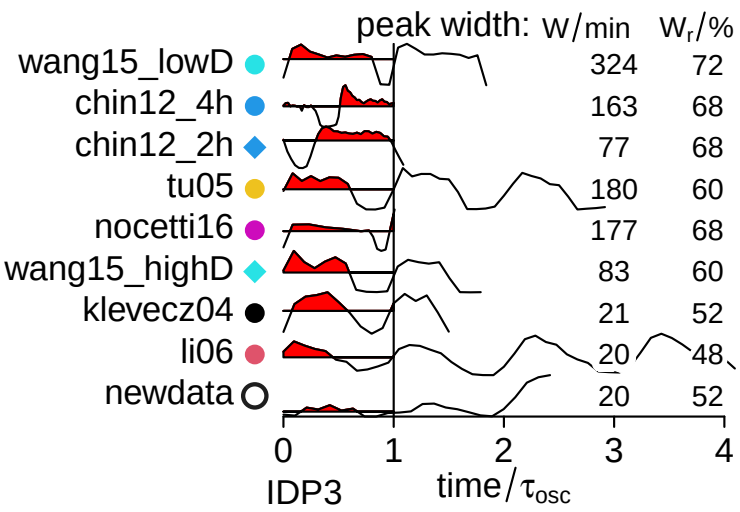


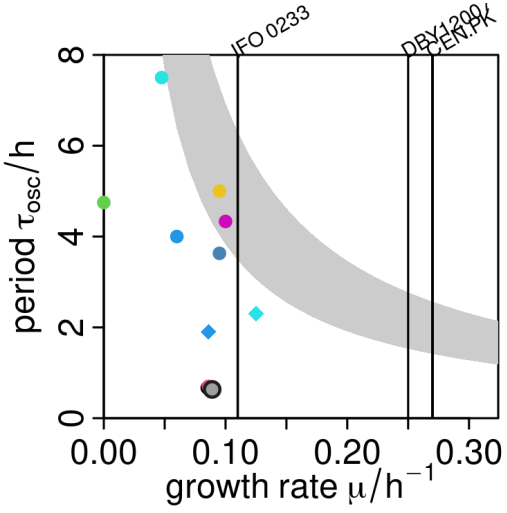


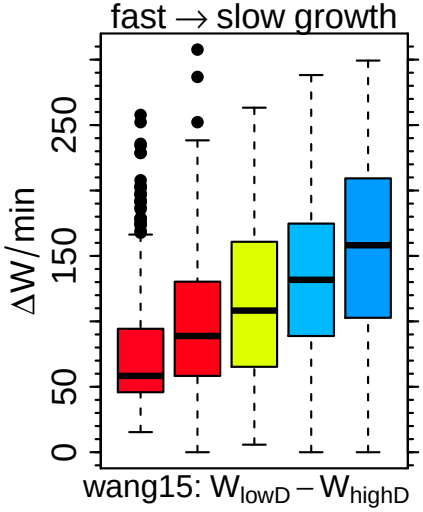
- Calculate peak widths W over the first cycle of each experiment.
- Compare data from same conditions at two different growth rates: **slower growth → longer period → longer `LOC` phase**.



Peak Width Analysis across Transcriptomes







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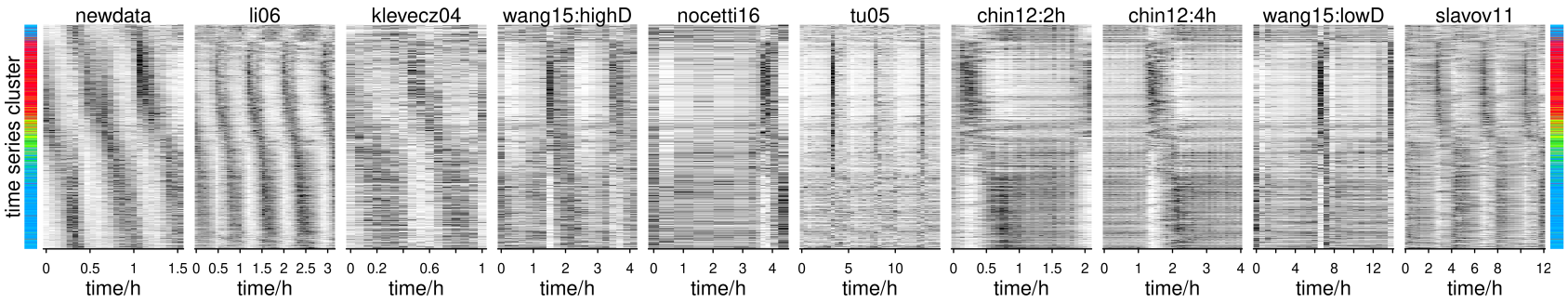

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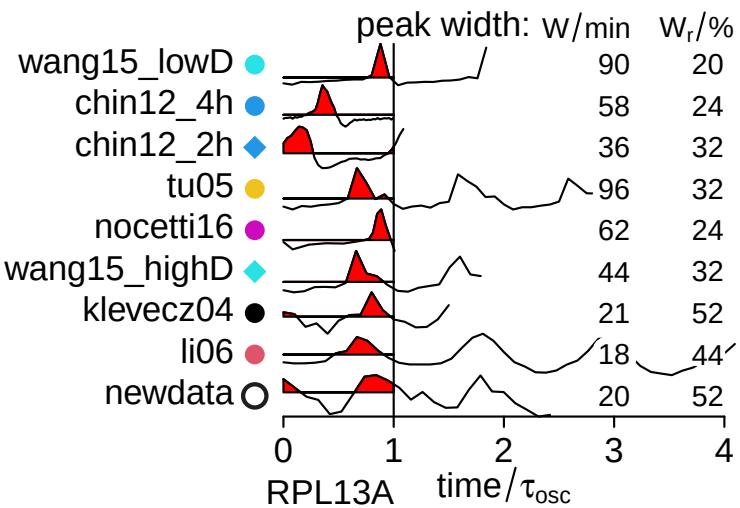
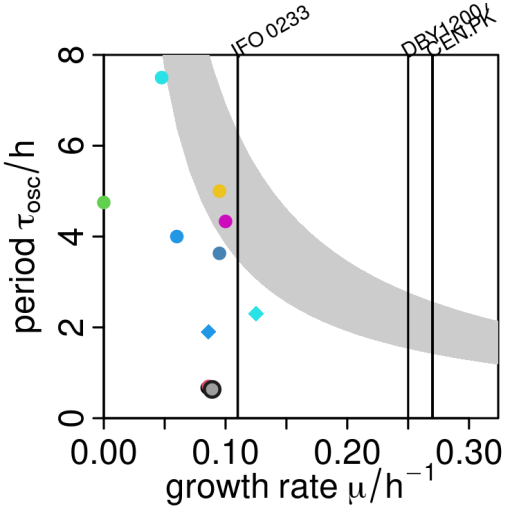
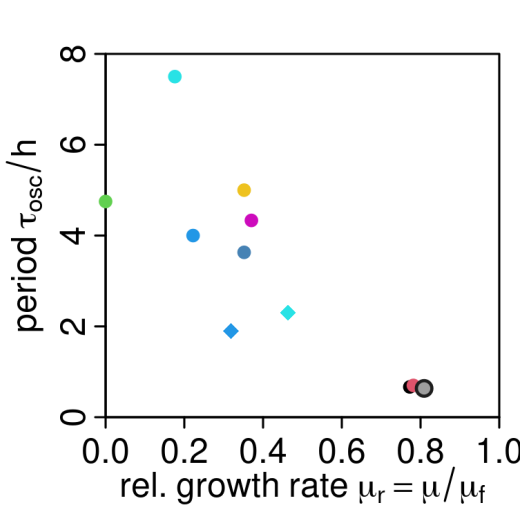




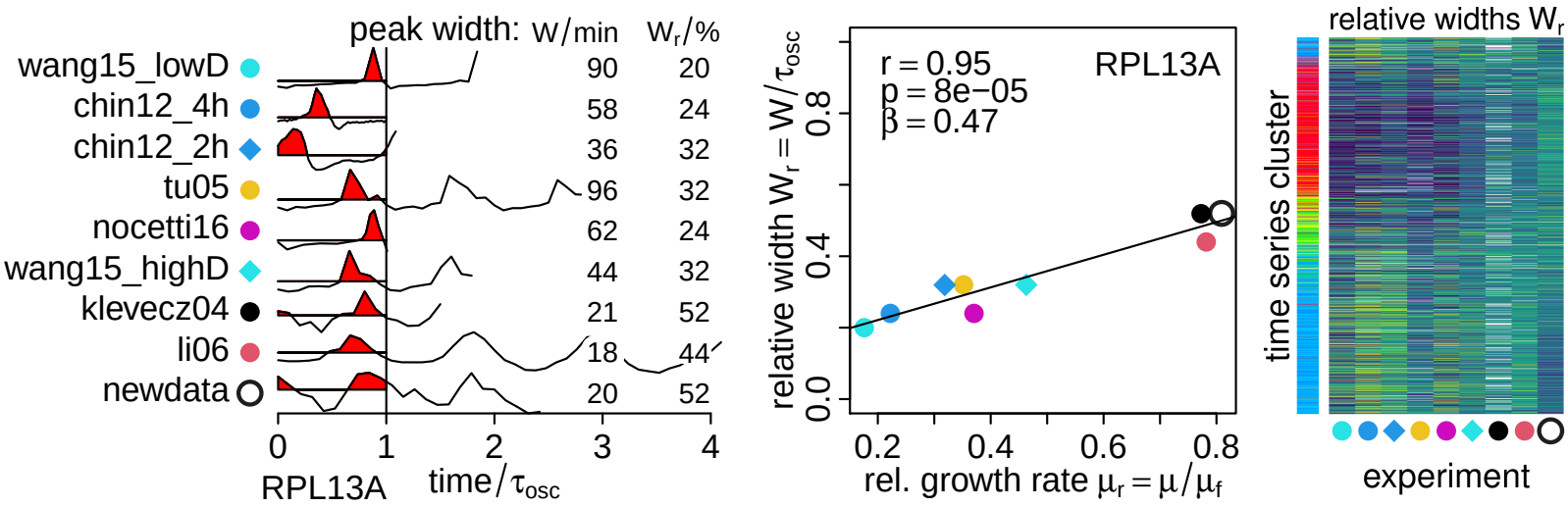


- Calculate relative peak widths $W_r = \frac{W}{\tau_{osc}}$,
- Calculate relative growth rate $\mu_r = \frac{\mu}{\mu_f}$:

strain	μ_f/h^{-1}	sources[1]
IFO 0233	0.11	Lindner (1919), Hansson and Häggström (1983) Satroutdinov, Kuriyama, and Kobayashi (1992)
CEN.PK122	0.27	van Dijken et al. (2000)
CEN.PK113-7D	0.27	assumed

[1] μ_f is a condition-specific and strain-specific growth rate where respiro-fermentative metabolism of glucose sets in.

Peak Widths Scale with the Relative Growth Rate

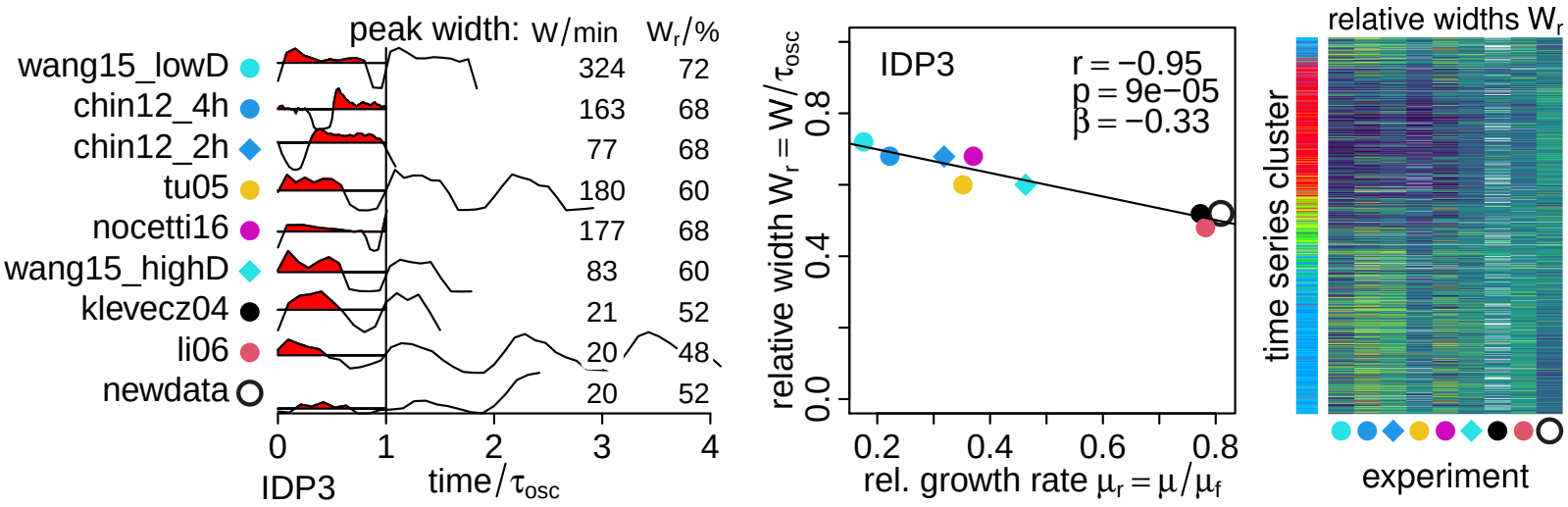


- Calculate relative peak widths $W_r = \frac{W}{\tau_{osc}}$,
- Calculate relative growth rate $\mu_r = \frac{\mu}{\mu_f}$.
- Linear regression $W_r = \alpha + \beta\mu_r$, and record slope $\beta = \frac{\Delta W_r}{\Delta \mu_r}$ and p-value.

- IFO 0233 short period data acts as a hook at high μ_r,
- Other data provide slopes.

- Idea: $W \propto$ time of translational activity.

Peak Widths Scale with the Relative Growth Rate



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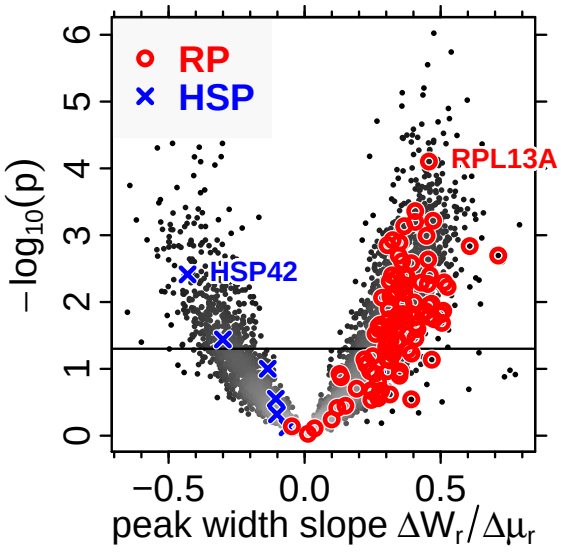
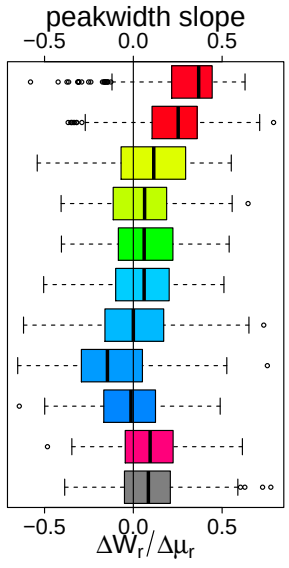
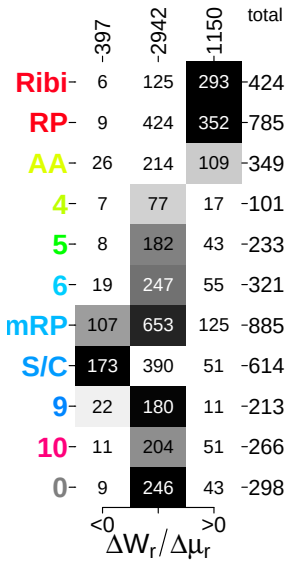


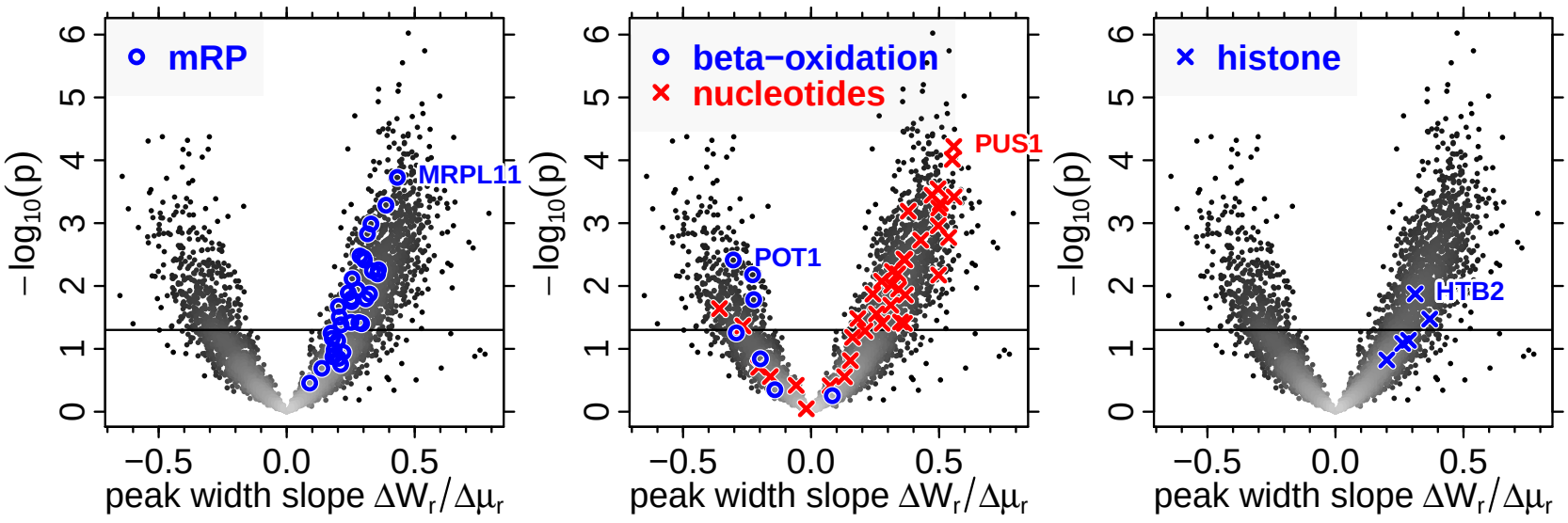




- Volcano plot:
  - slopes β of linear regression *v* p-values of Pearson correlation.
- Cohort distributions:
  - continuous,
  - discrete: slope $\beta > 0$ or $\beta < 0$ with $p < 0.5$.

$\Rightarrow$ **as expected**: HOC phase-specific genes show positive correlation and LOC phase-specific genes show negative correlation to the **relative** growth rate.

Peak Widths Scale with the Relative Growth Rate



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- Cohort distributions:
  - continuous,
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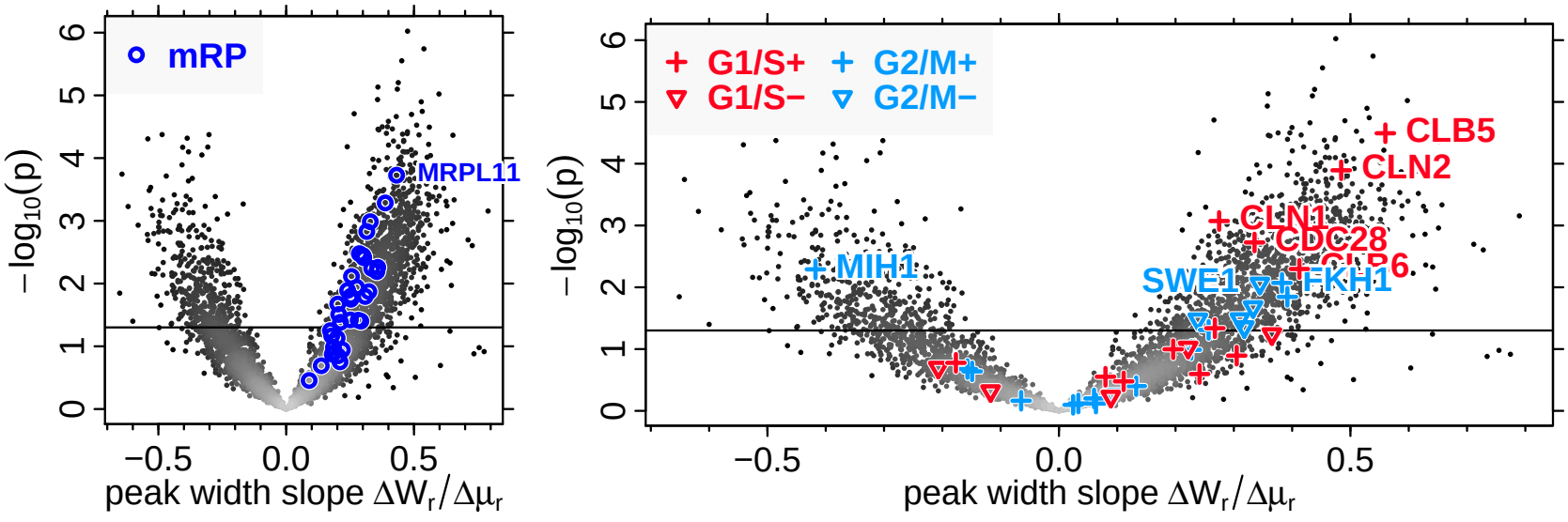
$\Rightarrow$ **as ~~expected~~**: HOC phase-specific genes show positive correlation and LOC phase-specific mitochondrial mRP show positive correlation!

Peak Widths Scale with the Relative Growth Rate



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- Cohort distributions:
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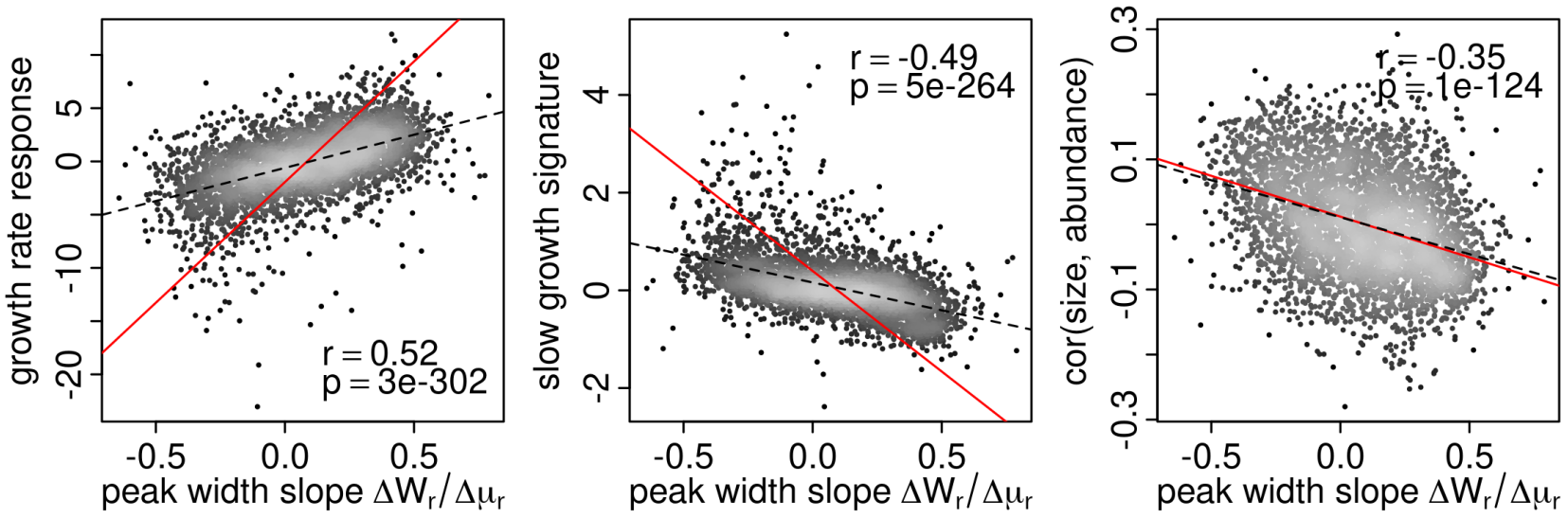
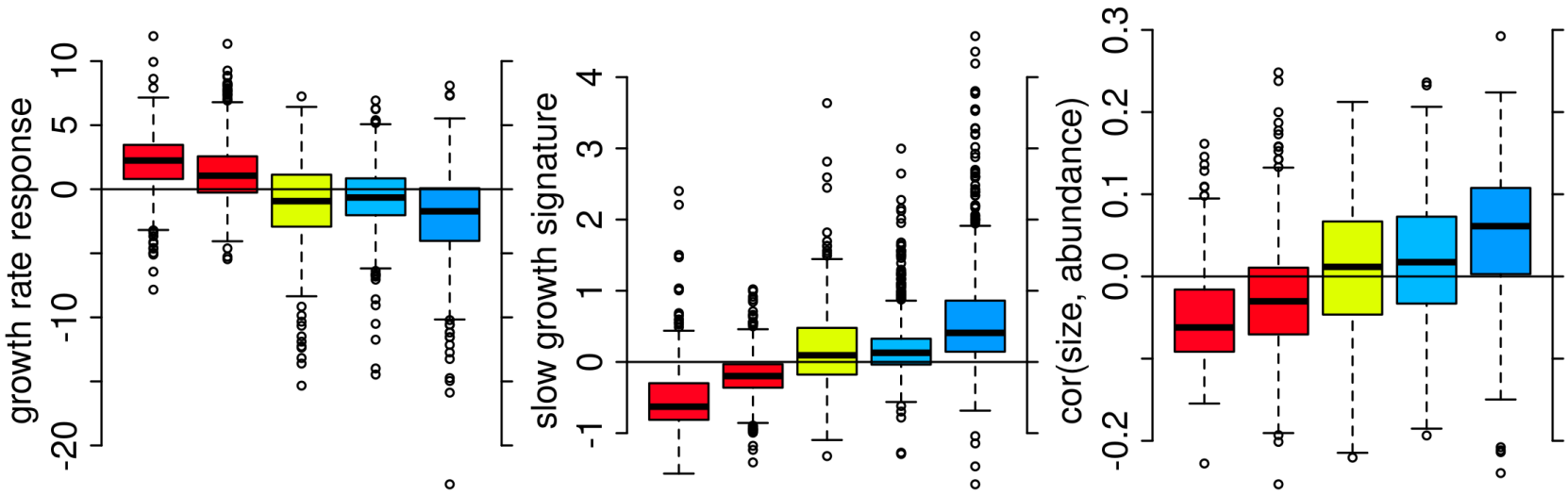
$\Rightarrow$ **bonus track**: analysis of cyclins.

Peak Widths Scale with the Relative Growth Rate



Good correlation to growth rate response (Brauer et al. 2008), slow growth signature (O'Duibhir et al. 2014), and size-abundance correlation (Swaffer et al. 2021).



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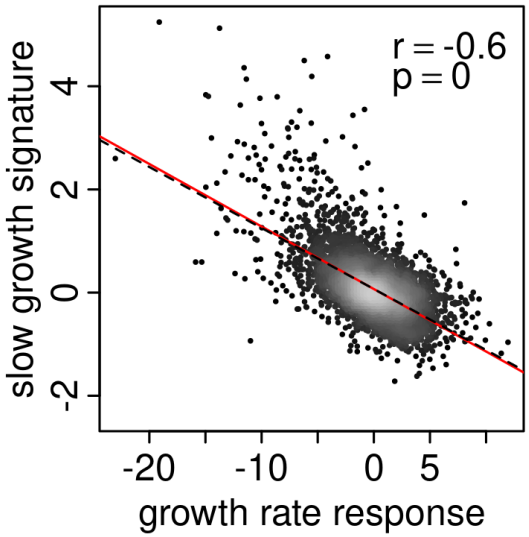
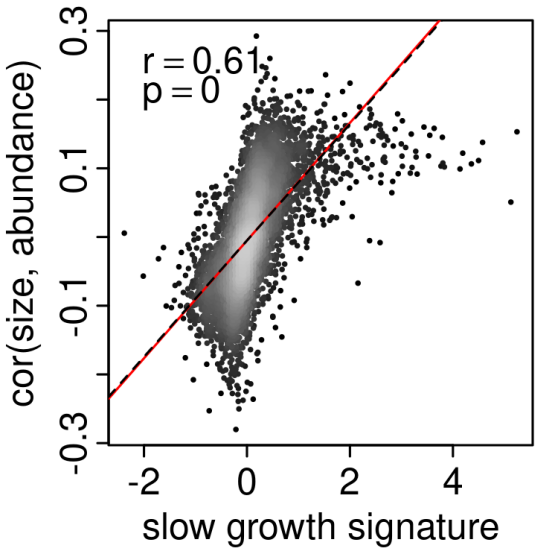
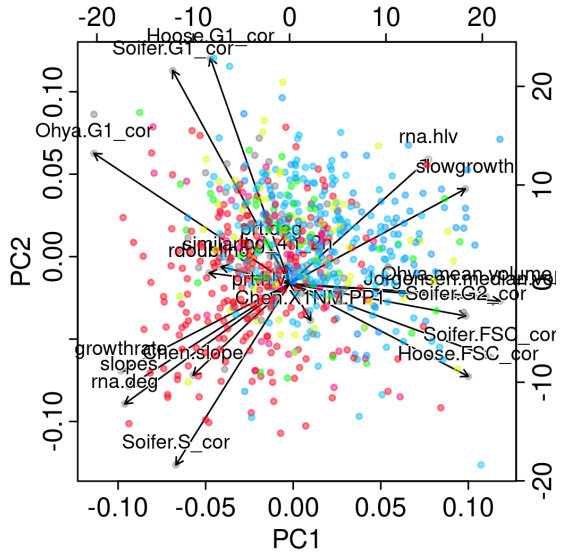
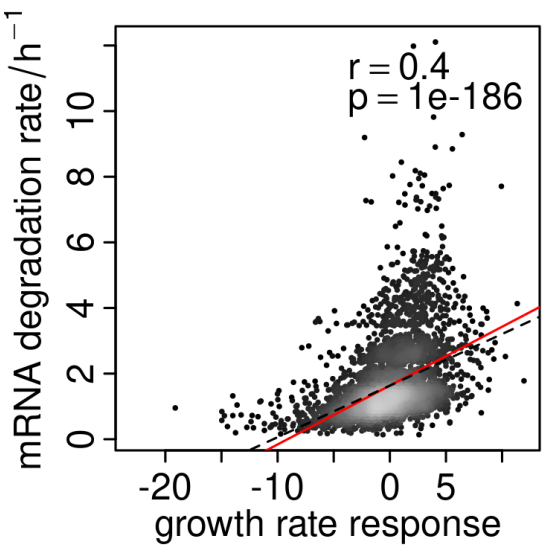
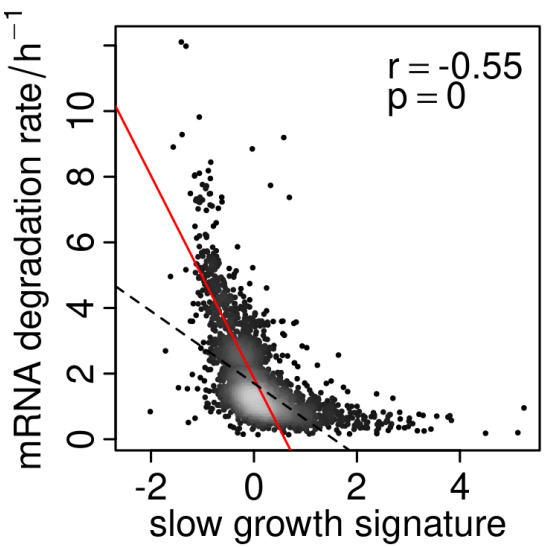


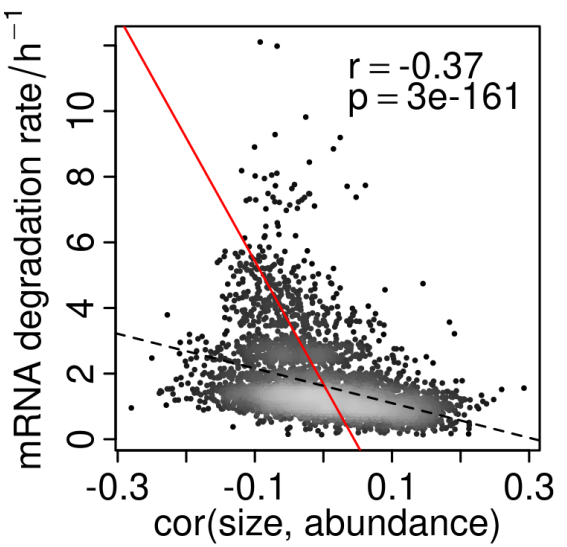




Good correlation of all to measures to RNA half-lives by Geisberg et al. (2014).
TODO: a consensus measure?







Summary & Outlook

Lecture V: Pulse-Width Modulation - Data Analysis

1. The periodic program affects the same gene groups as growth rate.
2. The relative durations of the program, `HOC` v `LOC` phase, change with growth rate — a **pulse-width modulation (PWM)** of gene expression.
3. This change of relative durations could underlie a fundamental observation in biology: In affluent conditions, ribosomes, the auto-catalytic core of life, can focus on producing more ribosomes.

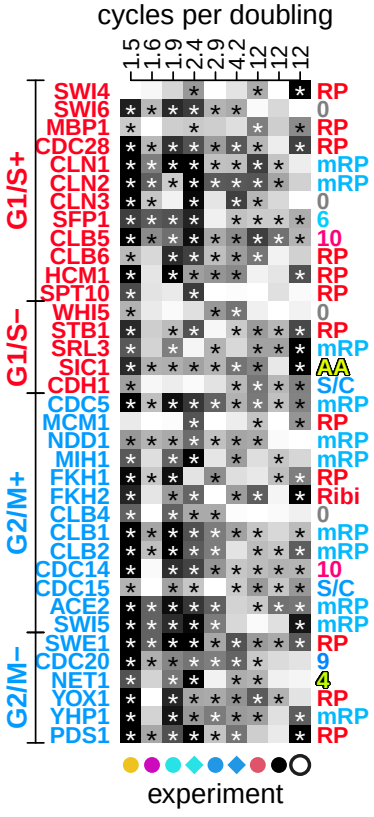
PWM: a mechanism for the **ribosome growth law** in eukaryotes!

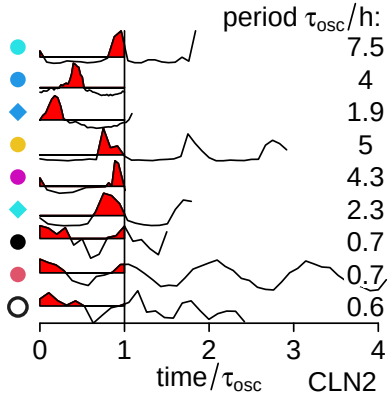
Next: **Bonus track on PWM of cyclins.**

Next Lectures:

VI. The PWM ODE Model: predicting ribosome and proteome concentrations, and oscillation periods.
VII. Metabolism: the feedbacks and auto-catalytic cycles of life.

Peak Width Change in Cell Cycle Regulators

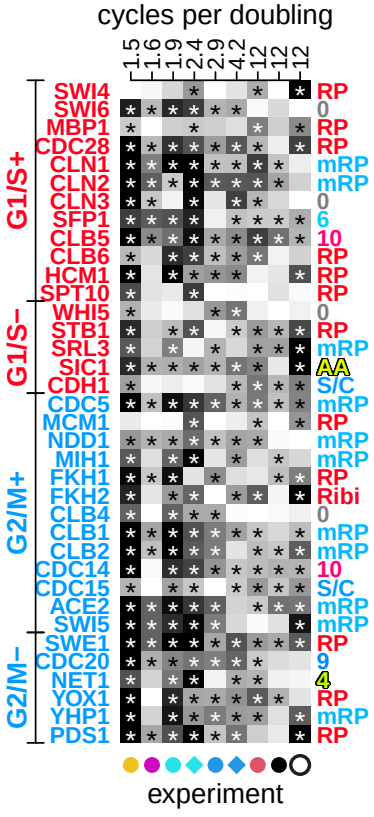


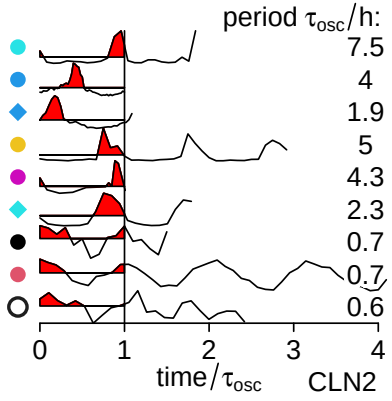


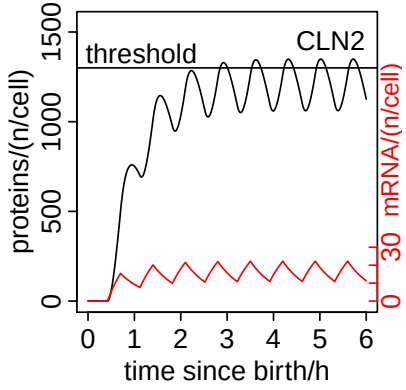
1. Custom collection of cell cycle regulators, classified into G1/S or G2/M, and activators (+) or inhibitors (-), and
2. Tested transcript oscillations in each data set (R package `rain`, Thaben and Westermark (2014)).
3.

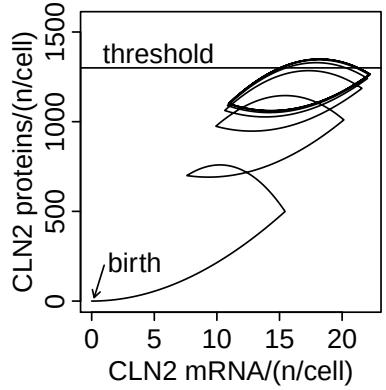
- Transcripts of cell cycle regulators oscillate even in short period oscillations with ~**12 cycles per division**; mostly with the RP and the mRP cohorts!

Peak Width Change in Cell Cycle Regulators





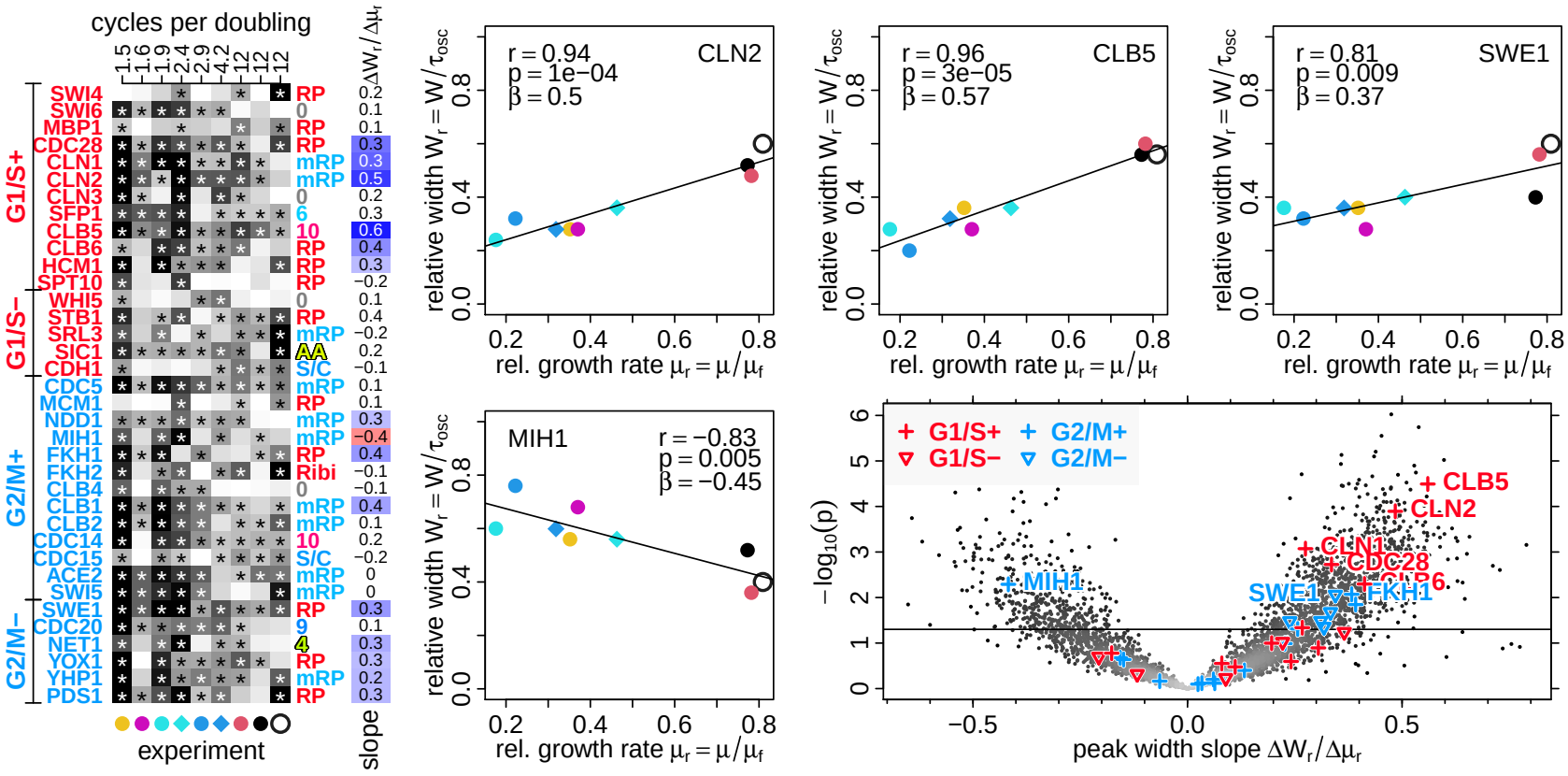




1. Custom collection of cell cycle regulators, classified into G1/S or G2/M, and activators (+) or inhibitors (-), and
2. Tested transcript oscillations in each data set (R package `rain`, Thaben and Westermark (2014)).
3. Simulated (ODE) with known parameters for CLN2: **long mRNA and short protein half-life** (**Lectures VI-VII**).

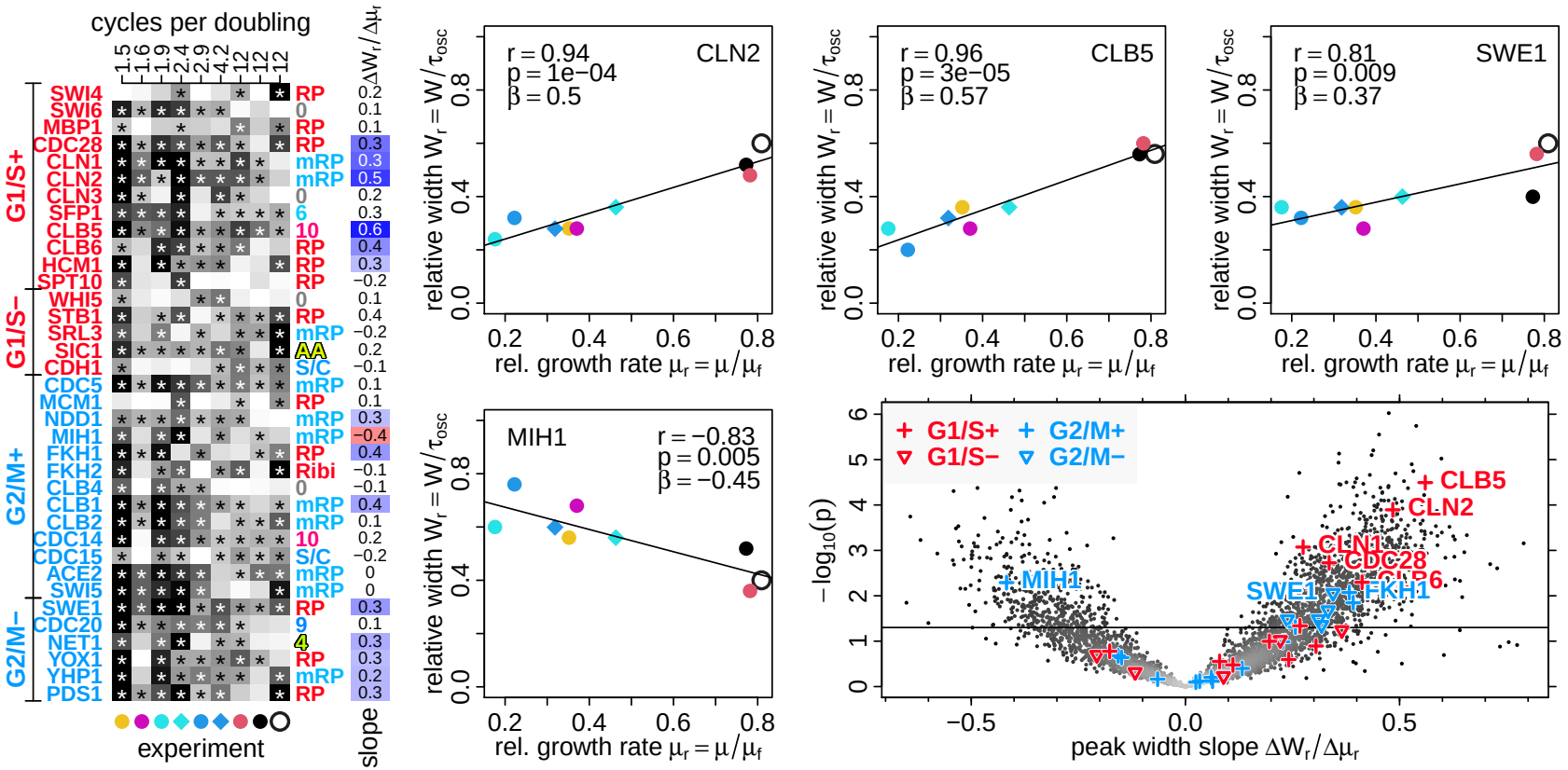
- Transcripts of cell cycle regulators oscillate even in short period oscillations with ~**12 cycles per division**; mostly with the RP and the mRP cohorts!
- This is **NOT consistent** with the standard model of cell cycle regulation (gradual increase), and instead predicts a
- **cyclic increase to the checkpoint threshold concentration**.

Peak Width Change in Cell Cycle Regulators



1. **Activators** of the G1/S transition (CLN1/2) and S phase initiation (CLB5/6) and the cyclin-dependent kinase (CDC28) positively correlate with the growth rate.

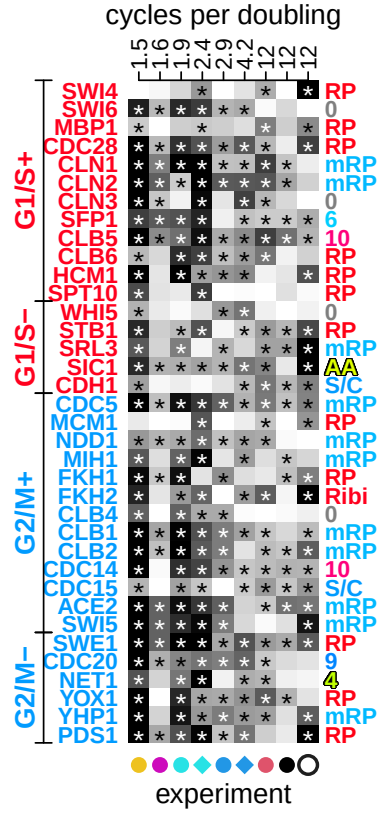
Peak Width Change in Cell Cycle Regulators

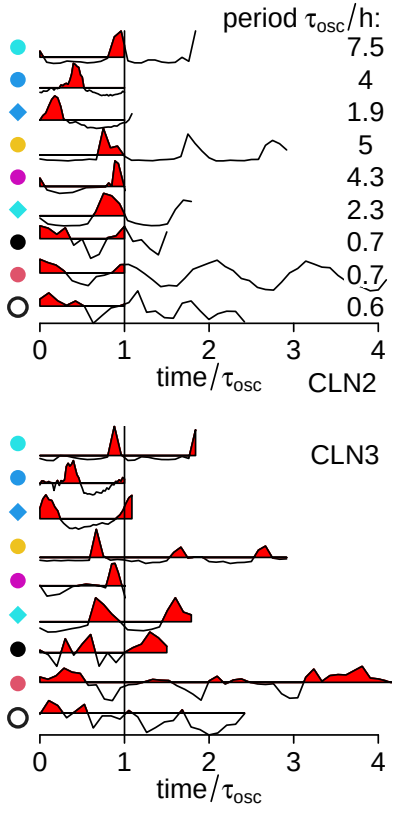


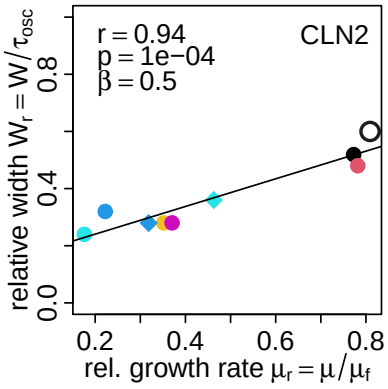
1. **Activators** of G1/S positively correlate with the growth rate, and
2. The **activator** of the **morphogenesis checkpoint** (MIH1) negatively, and its **inhibitor** (SWE1) positively correlate with the growth rate.

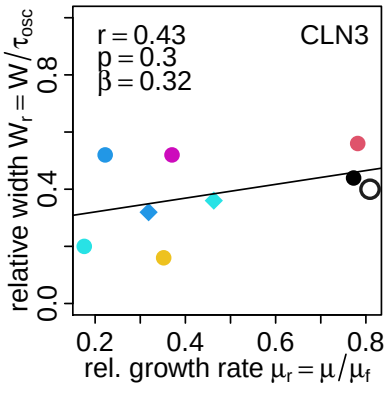
- 👋 At fast growth: S phase is not *gated* (Klevecz et al. 2004), but cells are pushed into S phase, while G2+M phase is prolonged, 4 h in IFO 0233. All integrity checks occur AFTER DNA replication. 👋

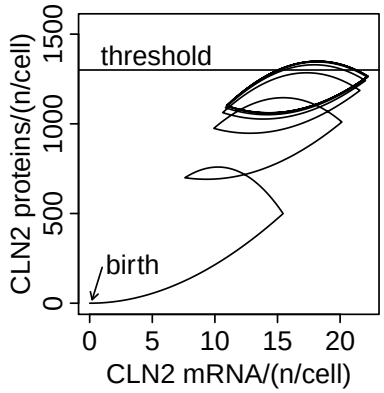
Peak Width Change in Cell Cycle Regulators







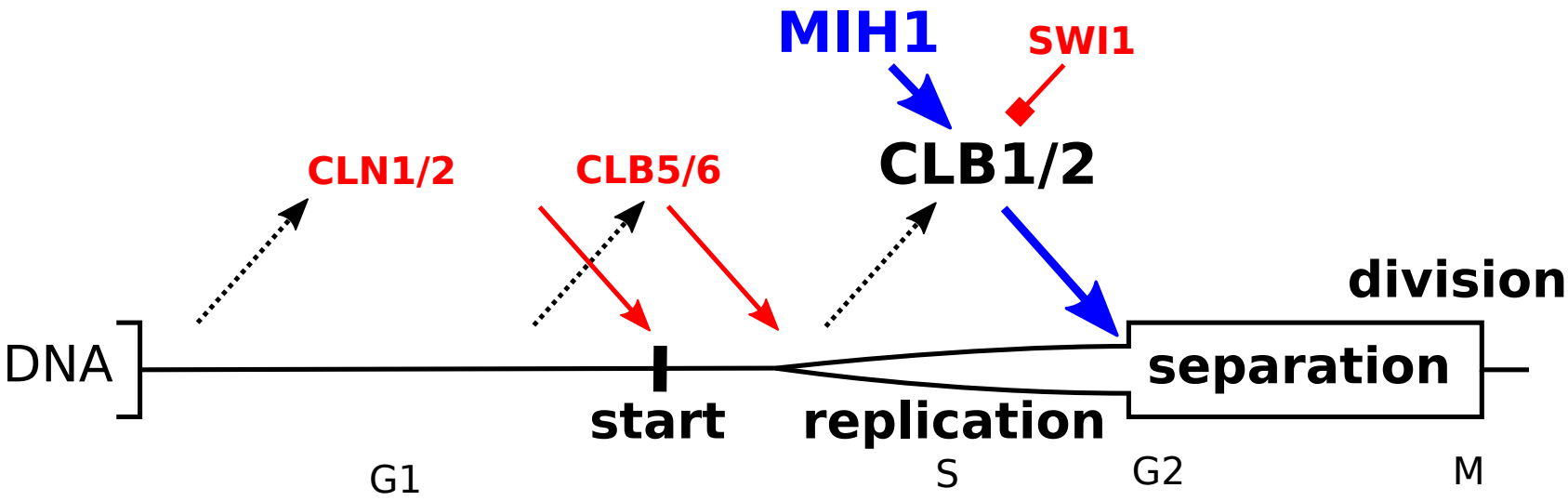


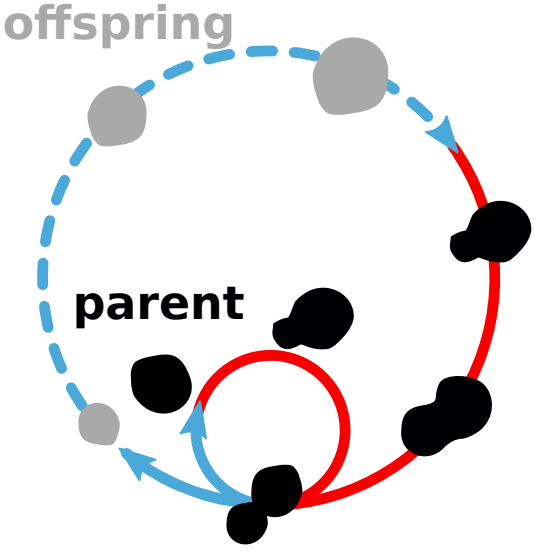


false negative?

- Transcripts of cell cycle regulators oscillate even in short period oscillations with ~**12 cycles per division**; mostly with the RP and the mRP cohorts!
- This is **NOT consistent** with the standard model of cell cycle regulation (gradual increase), and instead predicts a
- **cyclic increase to the checkpoint threshold concentration**, **also true for CLN3**, known as cyclin D, e.g. in Sabrina's data.

Peak Width Change in Cell Cycle Regulators

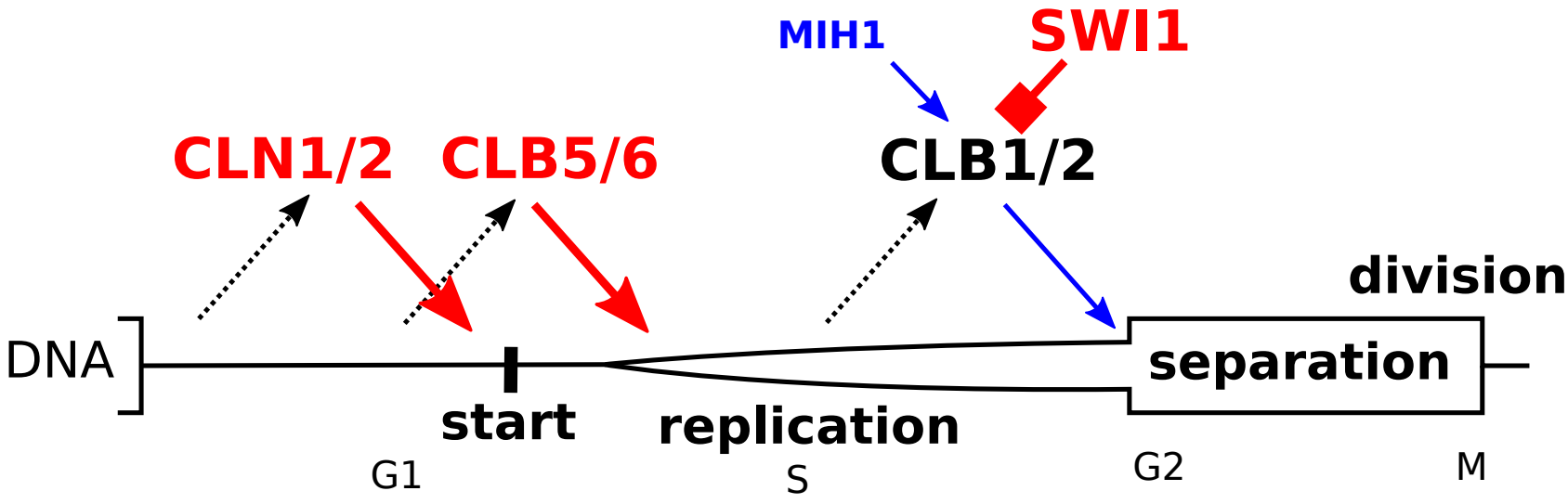


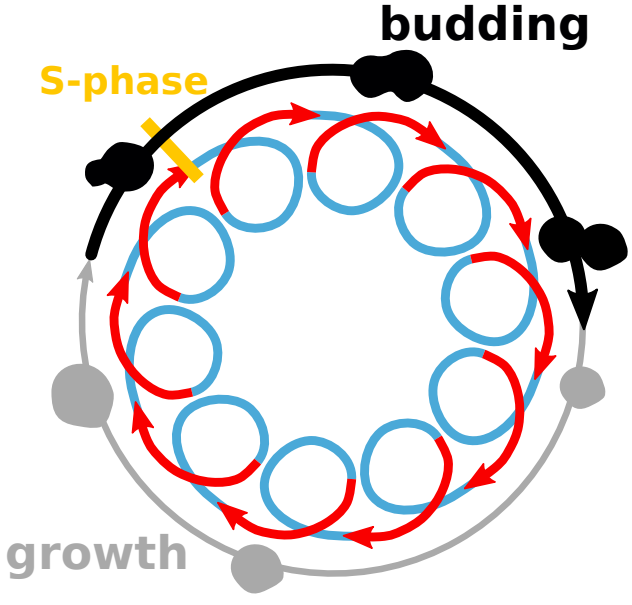


Conventional view:

- $\tau_{\text{bud}} \approx$ const. from 1 h to 2 h,
- HOC phase $\approx$ budding (S/G2/M), LOC phase $\approx$ growth (G1) $\propto$ doubling.
- Both, S phase (mid HOC phase) and mitosis (early LOC phase) are synchronized in continuous culture.

Peak Width Change in Cell Cycle Regulators





Strain IFO 0233 and/or $\mu \to \mu_f$:

- Budding index of 40 % to 45 % implies $\tau_{bud} \approx 4\,h$ (Satroutdinov et al. 1992).
- Cells are pushed into S phase at the HOC $\to$ LOC phase transition.
- Mitosis is delayed, for genome and cell integrity checks?

Summary & Outlook

Lecture V: Pulse-Width Modulation - Data Analysis

1. The periodic program affects the same gene groups as growth rate.
2. The relative durations of the program, `HOC` v `LOC` phase, change with growth rate — a **pulse-width modulation (PWM)** of gene expression.
3. This change of relative durations could underlie a fundamental observation in biology: In affluent conditions, ribosomes, the auto-catalytic core of life, can focus on producing more ribosomes.

PWM: a mechanism for the **ribosome growth law** in eukaryotes!

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4. The transcripts of many cell cycle regulators oscillate, even at $\tau_{\text{osc}} \ll \tau_{\text{bud}} < \tau_{doubling}$. This requires to modify the standard model of cell cycle regulation.
5. Some cell cycle regulators are differentially affected by PWM:
   👋At $\mu \to \mu_{\text{f}}$, DNA replication is initiated readily (short G1 phase), while mitosis is delayed (long budding (S/G2/M) phase) 👋.

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PWM: a mechanism for the **ribosome growth law** in eukaryotes!

Next Lectures:

VI. The PWM ODE Model: predicting ribosome and proteome concentrations, and oscillation periods.
VII. Metabolism: the feedbacks and auto-catalytic cycles of life.

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