# Quantitative Microbiology: Exponential Growth is Rarely Balanced

Rainer Machné

May 15, 2024

# 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?*

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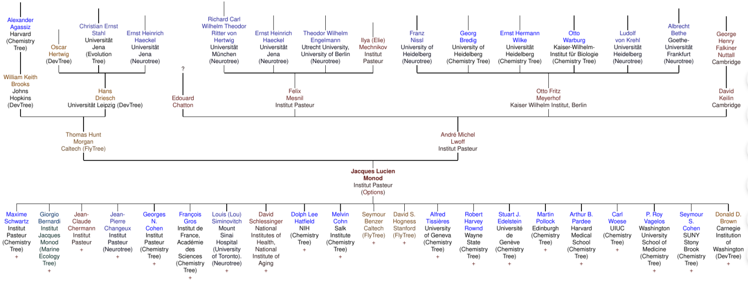


https://academictree.org (2023-09-26) of Jacques Monod

In this lecture series, we will trace a few specific paths through the vast directed acyclic[1] graph of scientific progress. Is it a linear and logical process (Karl Popper) or a historical development, shaped by traditions, fashions and big egos (Ludwik Fleck)?

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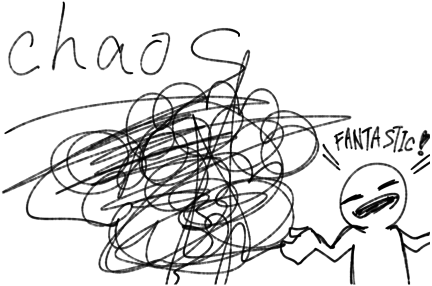




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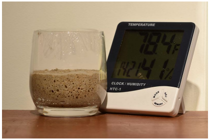
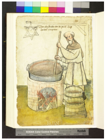
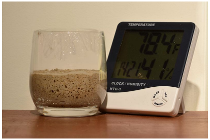


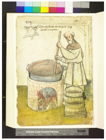




Quelle: Deutsche Fotothek

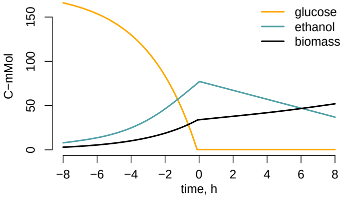

## *Saccharomyces cerevisiae*: sugar-fungus of beer

- Cultivated by and co-evolving with humans since $\geq$ 5000 years.
- Plinius the Elder: **sugar + yeast** $\Rightarrow$ **alcohol + more yeast**.
- Greek: $\zeta\nu\mu\eta$ ~ dough, Latin: *fermentum* (hot, boiling),
  German/English: Hefe ~ raiser, Gischt/Gest ~ yeast,
  Austrian: Germ ~ gären or *germen* (Latin) ~ germ? 👋






'*The alcohol level is [...] about 14%. Aroma has a great spicy orange edge to it. Review coming.*'
http://beerobsessed.com/blog/?p=1044 (2017)

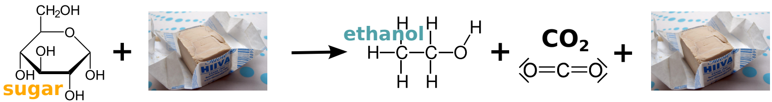


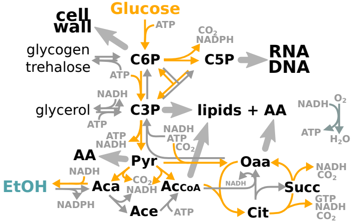

Zampar et al. (2013) Mol Syst Biol

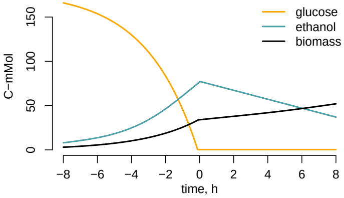
## *Saccharomyces cerevisiae*: sugar-fungus of beer

- Leeuwenhoek: contains cells, Schwann (1837): a single celled fungus!
- Louis Pasteur vs. Justus von Liebig: Is **alcoholic fermentation** performed by a **life force** or by a **chemical catalyst**?
- Development of minimal and defined growth media (minerals+sugar), and discovery of vitamins (Lindner (1919): the search for `bios`).

https://hopsmore.co.uk/blogs/beer-blog/the-yeast-story https://en.wikipedia.org/wiki/Liebig–Pasteur_dispute




Zampar et al. (2013) Mol Syst Biol
mammals: various from Rabinowitz Lab

## *en-zyme*: 'in yeast' also in cancer (lactate)!

- Both Pasteur and Liebig were right: **Microbiology and Biochemistry**.
- Pasteur effect: more alcohol **without oxygen**,
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- At **high sugar** concentration, yeast first ferments sugar to alcohol,
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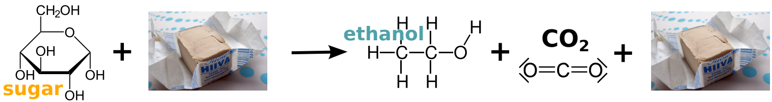


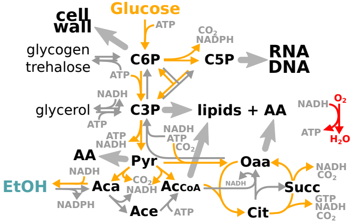


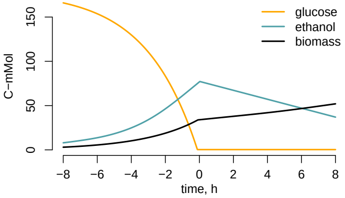


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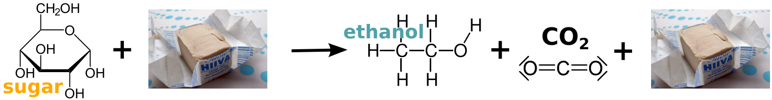


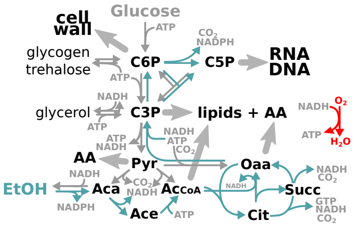


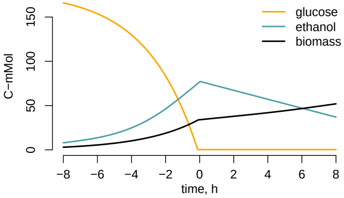


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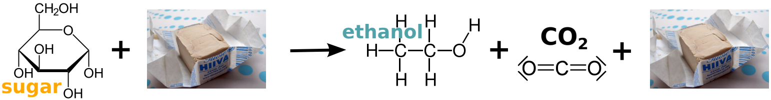
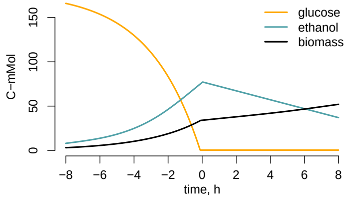



$$\frac{dX}{dt} = \mu X$$
$$\frac{dS}{dt} = S_0 - \frac{1}{y}\mu X \ ,$$

with growth rate $\mu = f(S)$
and yield $y = \frac{\Delta X}{-\Delta S}$.

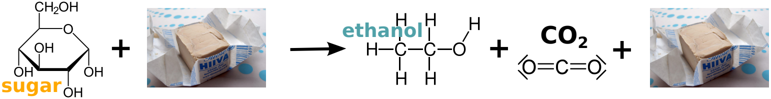



Zampar et al. (2013) Mol Syst Biol
Andrea Weisse et al. (2015) PNAS

## Quantitative Microbiology

Cells as auto-catalytic enzymes.

- Jacques Monod at the Institut Pasteur: **from Physiology to Genetics**.
- Diauxic Growth: *E. coli* and *B. subtilis* **first** use glucose, **then** other sugars, with lower yield $y$ and/or lower growth rate $\mu$.
- Discovery of gene regulation: the ***lac* operon** (with Francois Jacob)!




$$\frac{dX}{dt} = \mu X$$
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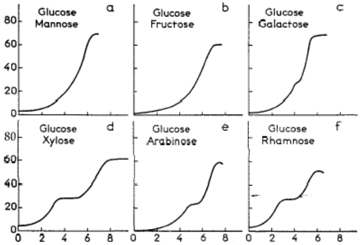


Fig.1. Growth of *Esherichia coli* in the presence of different carbohydrate pairs serving as the only source of carbon in a synthetic medium[30].

Monod (1942) Doctoral Thesis

## Cells as auto-catalytic enzymes. Quantitative Microbiology

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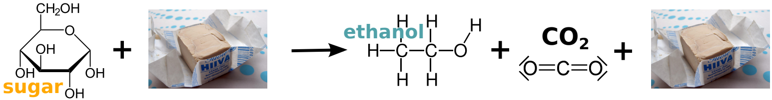
Two steps forward, one back (y-axis labels!): absorbance $\propto$ biomass $\propto$ cell number?

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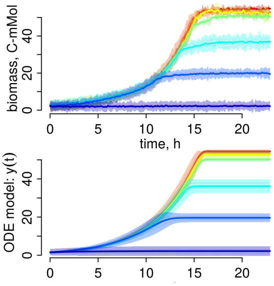


*E. coli* at different glucose concentrations; BIQ980, 2019.

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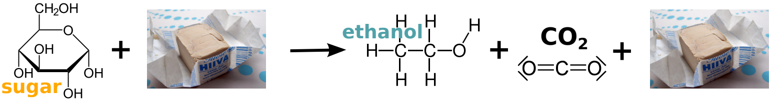


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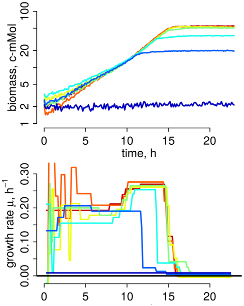


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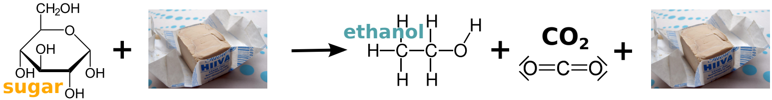
**Nice algorithm**: piecewise linear segmentation by `dpseg`, Machné and Stadler (2019) CRAN.

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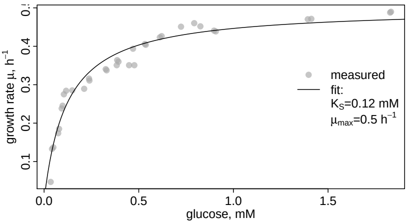


Snoep et al. (2009) Microbiology

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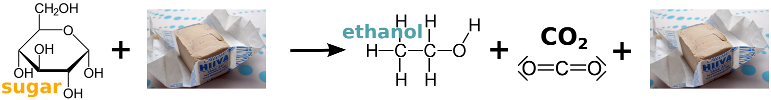


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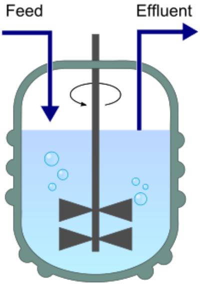


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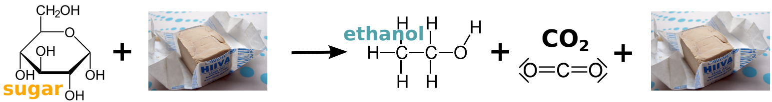
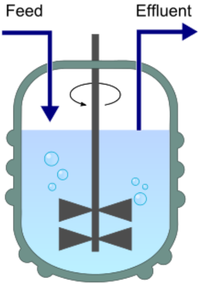


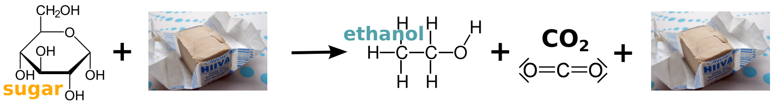

At $\frac{dX}{dt} = 0$, the dilution rate sets the growth rate:

$$\phi = \frac{\text{flow rate}}{\text{volume}} = \mu = \frac{\ln 2}{\tau_{\text{doubling}}}$$




## Continuous Culture: **Quantitative Microbiology**

- Stable states of growth can be achieved over a large range of $\phi = \mu$.
- Cell growth is then limited by a single nutrient in the growth medium.




Dilution rate is 1/retention time:

$$\phi = 0.303\,\mathrm{h}^{-1}$$

$$\tau_{\mathrm{doubling}} = \frac{\ln 2}{\phi} = 2.3\,\mathrm{h} \approx \tau_{\mathrm{osc}}$$

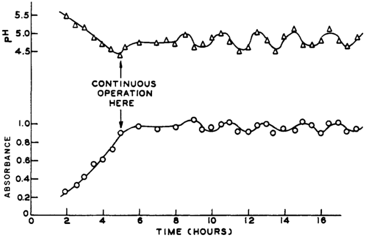



Figure 4. Example of steady cycling observed in continuous yeast propagator

*S. carlsbergensis*
Retention time 3.3 hours

## Continuous Culture: **Quantitative Microbiology**

- Finn and Wilson (1954), Finn (1954):
  *Saccharomyces carlsbergensis* → *Saccharomyces pastorianus*[3], at **2% glucose**.
- **Spontaneous synchronization of cell division**,
  with a period $\tau_{\mathrm{osc}}$ close to the doubling time $\tau_{\mathrm{doubling}}$.
  ⇒ **(i) cells "communicate" and (ii) metabolic differences of cell cycle phases**.

[3] old (~1k years) **bottom-fermenting hybrid** of *S. cerevisiae* and *S. eubayanus* (Libkind et al. 2011)

## Continuous Culture: Quantitative Microbiology

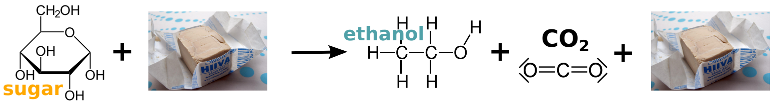



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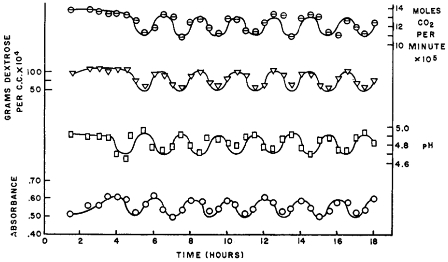
Figure 6. Growth of *S. carlsbergensis* in batch vessel at constant pH levels

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- **Spontaneous synchronization of cell division**,
  with a period $\tau_{\mathrm{osc}}$ close to the doubling time $\tau_{\mathrm{doubling}}$, and
- with dynamic changes to culture pH and $CO_2$ production.

**Exponential growth is rarely balanced ...**

# Exponential growth is rarely balanced . . .

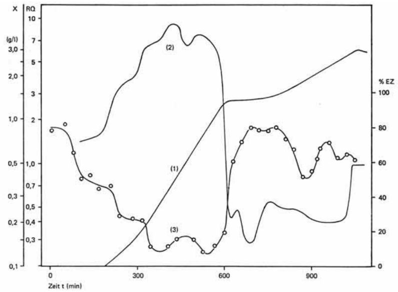
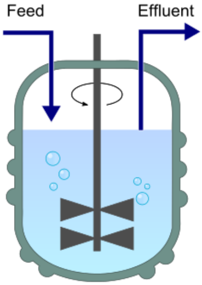


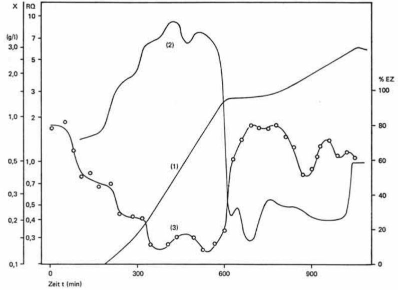

*Fig. 1.* Wachstum von *Saccharomyces cer.* H 1022 in statischer Kultur (batch) in Medium NL 10 (0.92% Glucose) unter aeroben Bedingungen. Änderungen der Trockensubstanz (X), des Respirationsquotienten (RQ) und der Populationsverteilung in Funktion der Zeit. Kurve (1): Trockensubstanz X, g/l, logarithmisch aufgetragen. Kurve (2): Respirationsquotient RQ $= Q_{CO_2}/Q_{O_2}$. Kurve (3): Prozentualer Anteil der Einzelzellen (nicht sprossend) in der Population. % EZ = 100% — % DZ.

Küenzi, Meyenburg, Fiechter, Sonnleitner at ETH Zürich




- **Reproducible Biology**: bioreactors with dissolved $O_2$ and pH measurement, and constant pH and temperature by PID controllers.

# Exponential growth is rarely balanced . . .

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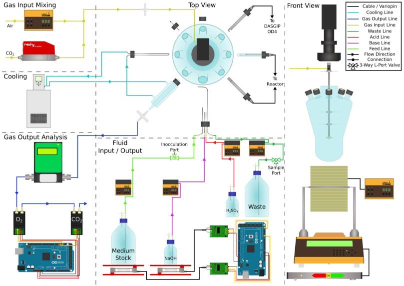


Behle, Dietsch et al. (2022) Nucl Acids Res
Arduino-based offgas module (500 €),
and weigh module (100 €).

- **Reproducible Biology**: bioreactors with dissolved $O_2$ and pH measurement, and constant pH and temperature by PID controllers.
- **Quantitative Biology**: with controlled aeration (ingas) and offgas measurements ($CO_2$, $O_2$, $H_2S$) we have a quantitative picture of growth. We know what goes in (defined medium and gas) and what comes out.
- Including metabolic heat production: Calorimetry via $T_{\text{culture}} - T_{\text{bath}}$.

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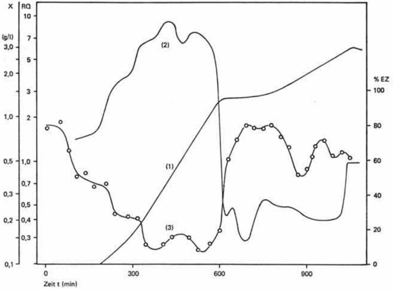
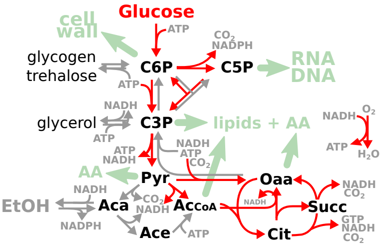



*Fig. 1.* Wachstum von *Saccharomyces cer.* H 1022 in statischer Kultur (batch) in Medium NL 10 (0.92% Glucose) unter aeroben Bedingungen. Änderungen der Trockensubstanz (X), des Respirationsquotienten (RQ) und der Populationsverteilung in Funktion der Zeit. Kurve (1): Trockensubstanz X, g/l, logarithmisch aufgetragen. Kurve (2): Respirationsquotient RQ $= Q_{CO_2}/Q_{O_2}$. Kurve (3): Prozentualer Anteil der Einzelzellen (nicht sprossend) in der Population. % EZ = 100% — % DZ.

Küenzi, Meyenburg, Fiechter, Sonnleitner at ETH Zürich




- Von Meyenburg (1968): dynamic RQ at **constant biomass growth rate**!
- The Respiratory Quotient: $\mathrm{RQ} = \frac{q_{CO_2}}{-q_{O_2}}$:
  1. RQ $\approx 1$: complete oxidation of glucose to $CO_2$,
  2. RQ $> 1$: additional $CO_2$ by fermentation,
  3. RQ $\approx \frac{2}{3}$: complete oxidation of ethanol.
- EZ %: percent of non-budding cells.

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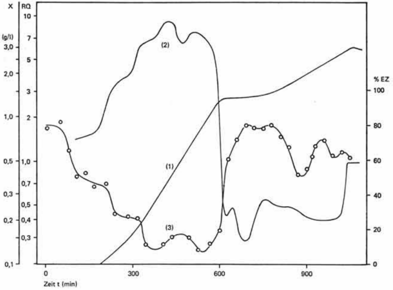


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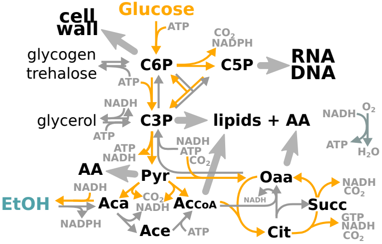


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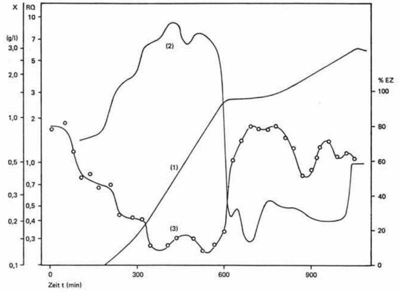


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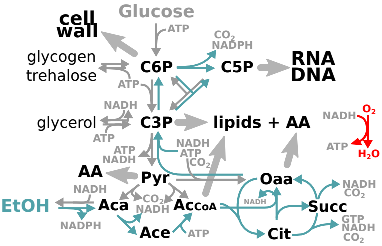


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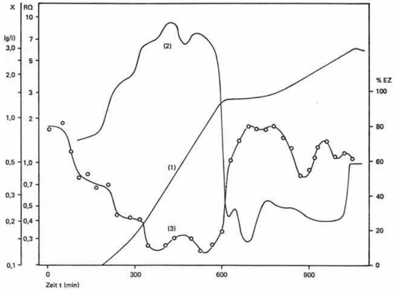
- EZ %: percent of non-budding cells: **change of phase durations!**

# Exponential growth is rarely balanced . . .

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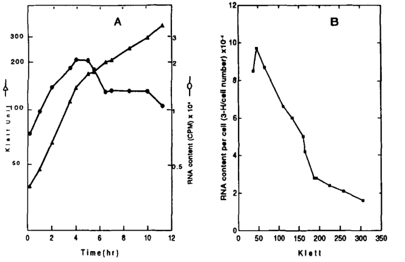


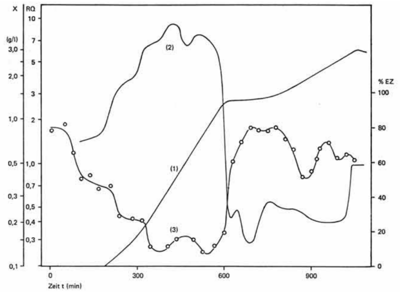

Figure 1. Ribosomal RNA content of growing yeast cell. A culture of W303a

Ju and Warner (1994) Yeast

- Von Meyenburg (1968): dynamic RQ at **constant biomass growth rate**!
- Ju and Warner (1994): ribosomal RNA per cell continuously decreases,

  **cells sense the *potential* for growth?**

# Exponential growth is rarely balanced . . .

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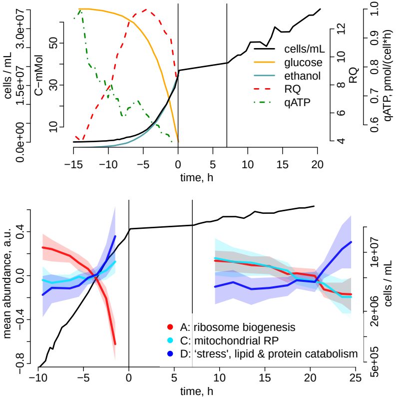
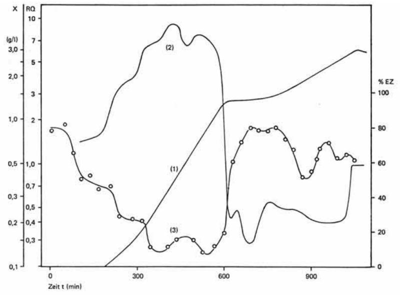



Slavov et al. (2014) Cell Rep - transcripts

- Von Meyenburg (1968): dynamic RQ at **constant biomass growth rate**!
- Ju and Warner (1994): **ribosomal RNA per cell continuously decreases**.
- Slavov et al. (2014): dynamic RQ as above, qATP decreases
  + `omics`: **large transcript groups continuously change accordingly**.

**Lectures I-VI**

# Exponential growth is rarely balanced . . .

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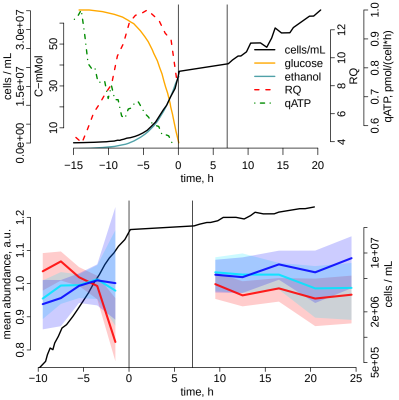


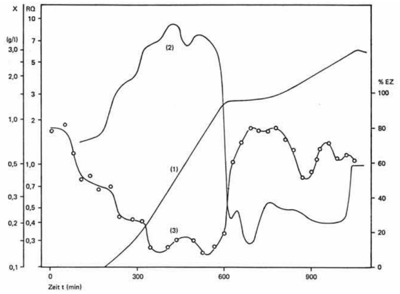

Slavov et al. (2014) Cell Rep - proteins

## . . . which didn't matter for Molecular Biology and Genetics:

- An intersecting path through the DAG of scientific progress, incl.
  Lamarck/Darwin/Mendel, Morgan (PostDoc mentor of Monod), Schroedinger, Luria/Bertani (CSHL), Chargaff/Franklin, Watson/Crick, McClintock, Doudna/Charpentier, . . .
- Ignore the complex growth on LB medium, and just "harvest at OD=0.3".

**Lectures II–IV**

# Exponential growth is rarely balanced . . .

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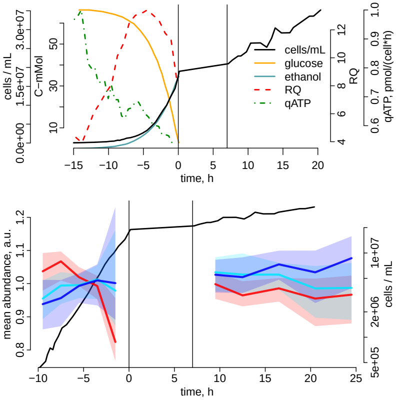


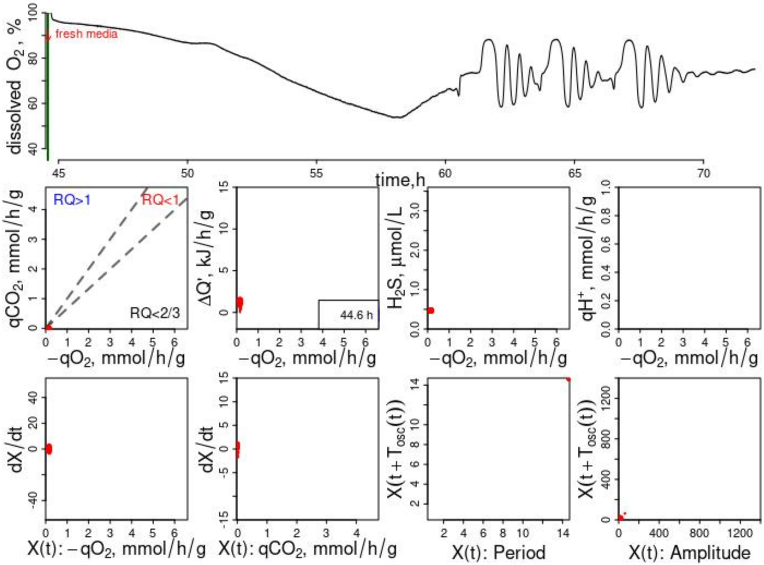

Slavov et al. (2014) Cell Rep - proteins

## . . . which didn't matter for the Copenhagen School:

- An intersecting path through the DAG of scientific progress, incl. Monod → Maaløe, Warner, Travers, Gourse, Terry Hwa, Teusink/Bruggeman, Andrea Weisse, the EPCP forum, . . .
- Growth laws: ribosomes as the auto-catalytic core of life,
derivation of growth behaviour and optimal gene regulation from first principles.

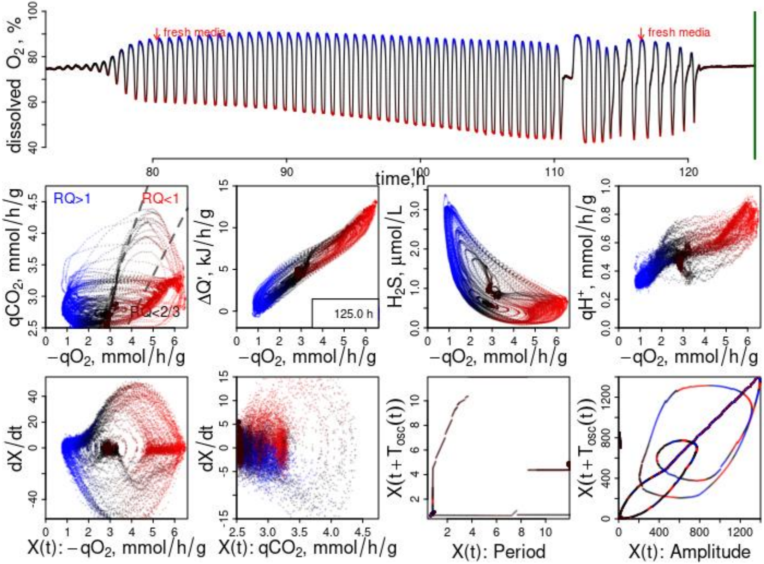
**Lectures V-VII**

# Synchronization at High Cell Density



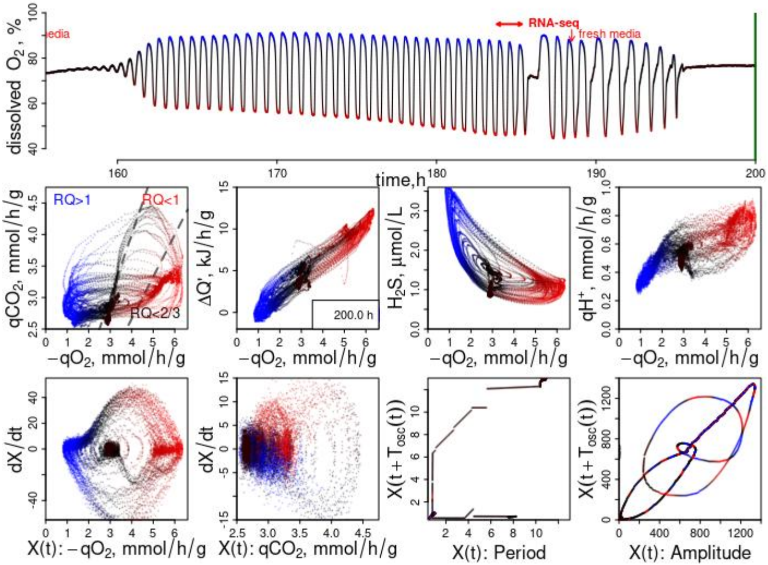

Experiment by Dougie Murray, Jan 2013, Music by Die Goldenen Zitronen.

# Synchronization at High Cell Density




Experiment by Dougie Murray, Jan 2013, Music by Tocotronic.

# Synchronization at High Cell Density




Experiment by Dougie Murray, Jan 2013, Music by Superorganism.

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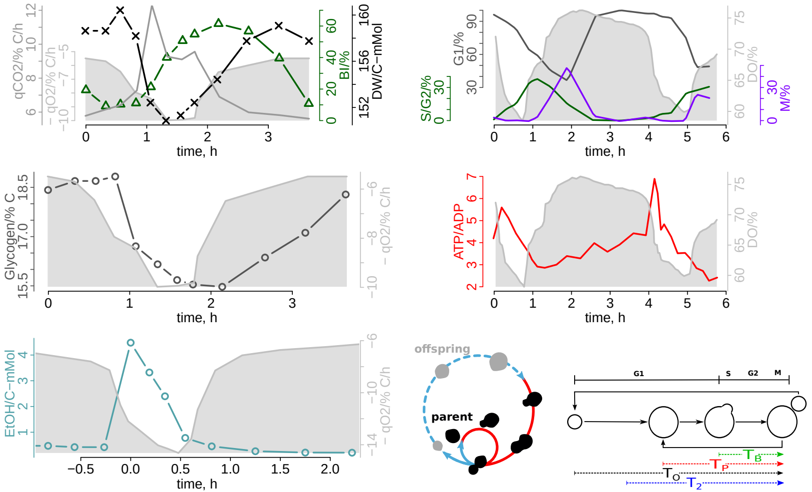
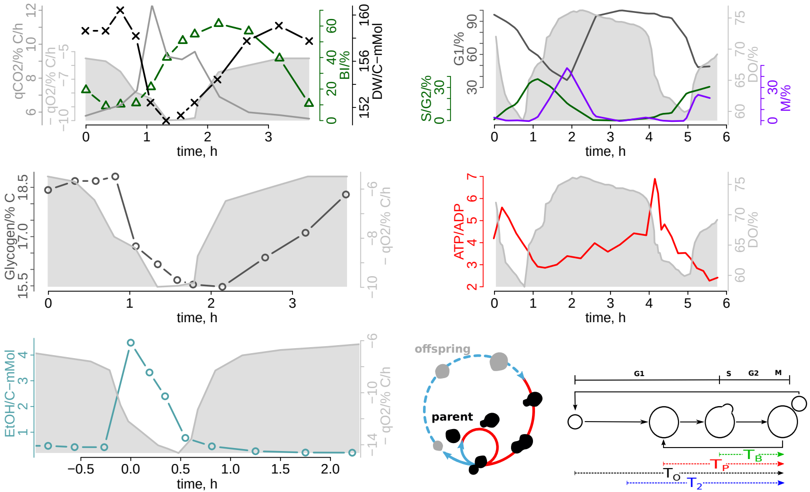


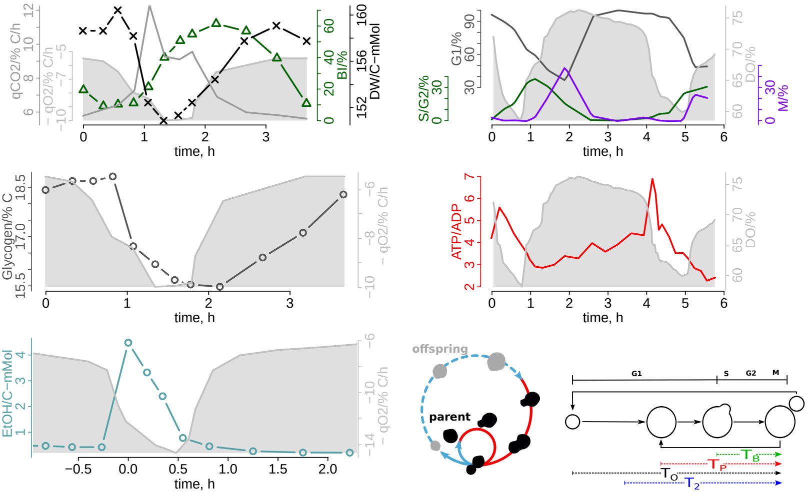

- Fiechter lab (1960s) at ETH Zürich, esp. Kaspar von Meyenburg: the metabolism and energetics of the **asymmetric** yeast division cycle: ***budding***.
- Hartwell and Unger (1977): "*particularly pertinent prior studies*" for clarification of the cell division cycle.

# Synchronization at High Cell Density



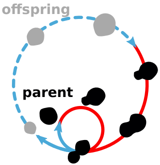

- Budding: S, G2 and M phase; requires glycogen mobilization, and a maximal rate of respiration, with additional overflow metabolism ∼ HOC phase.
- Biomass growth occurs in G1, including build-up of glycogen reserves, at purely respiratory RQ ≈ 1 ∼ LOC phase.

# Synchronization at High Cell Density



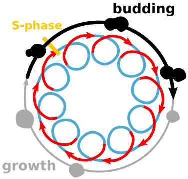

- The culture medium and conditions established by v. Meyenburg are still used in most studies of respiratory/metabolic oscillations.
- An absurd story of **scientific regress** occurred with the unfortunate Tu et al. (2005), distorting prior knowledge; we're still recovering.

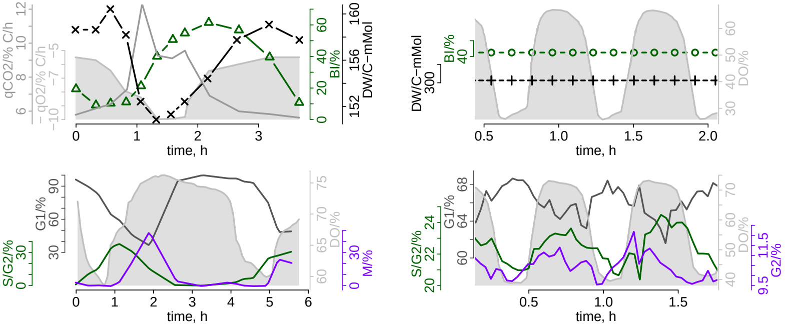
# Not Just Division: A Cell Growth Cycle?




LBGH 1022

IFO 0233



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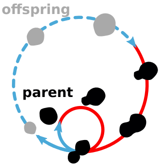


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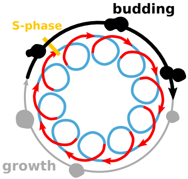


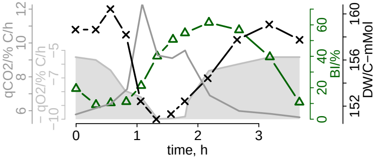


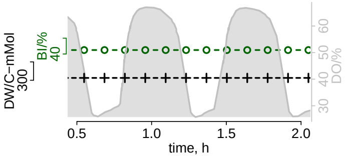


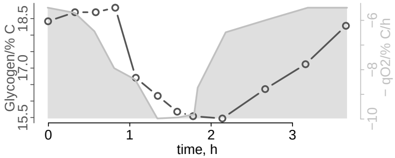


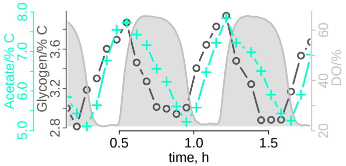

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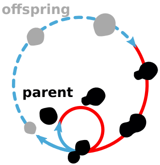


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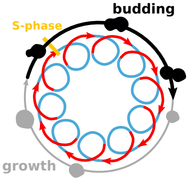


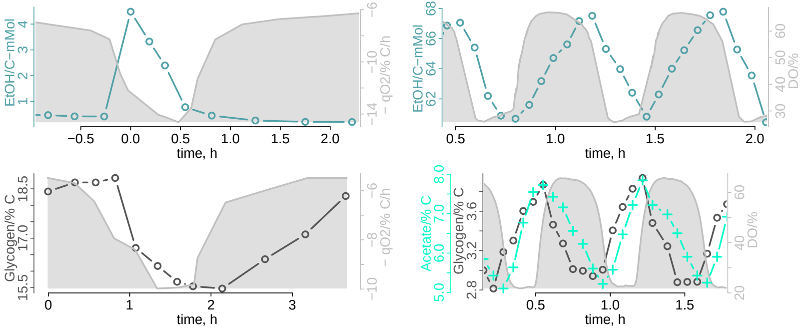

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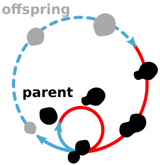


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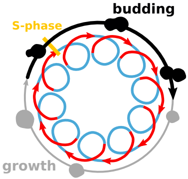


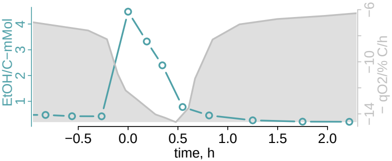


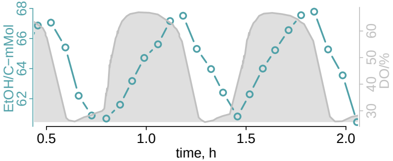


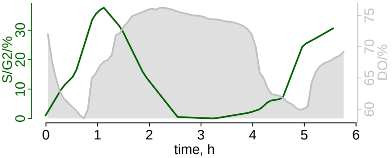


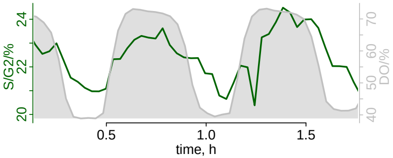

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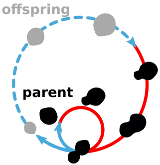


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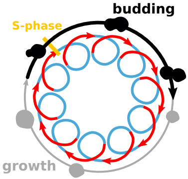
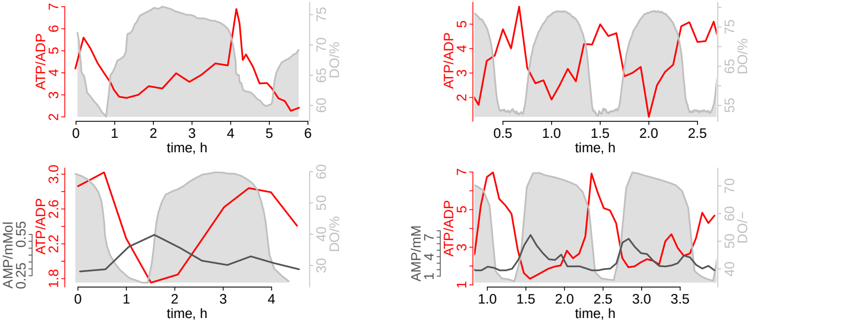
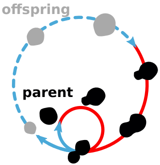


# A Cell Growth Cycle: cell-structural proof-reading?

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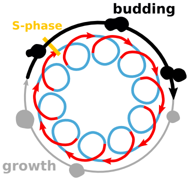
IFO 0233

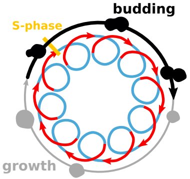



- nitrogen uptake, AA synthesis,
- translation pulses, high sensitivity to rapamycin (**Tor**),
- soluble protein peak, autophagy and degradation
- pH, ions, heat dissipation, ...

**Lectures I-IV**

# Finding IFO

IFO 0233

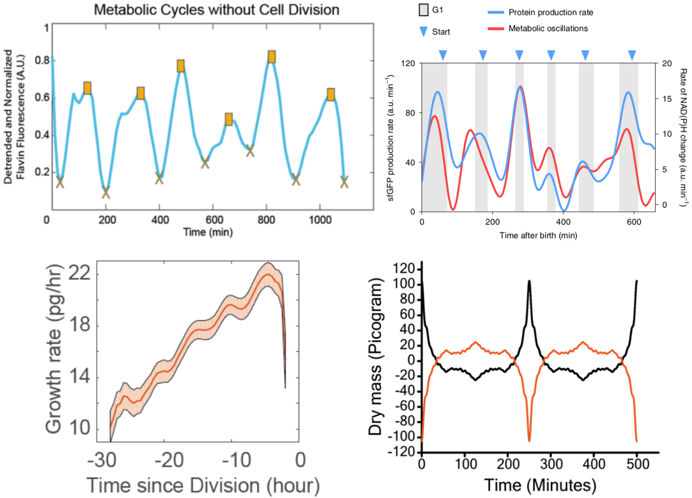



$q_{CO_2}$ and yield vs. growth rate: **normal**!

- Periods and glycogen v growth rate,
- Contraction of phases,
- IFO 0233 - Rasse II:
  - large lipid inclusions: lipid instead of glycogen?
  - concentric colonies: regular and fast oscillations?

**Lectures V-VII**

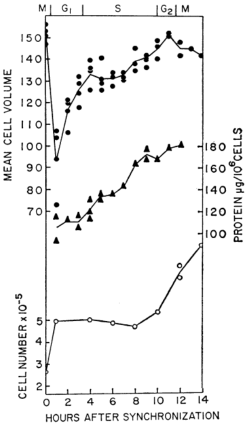
# A Cell Growth Cycle in Single Cells

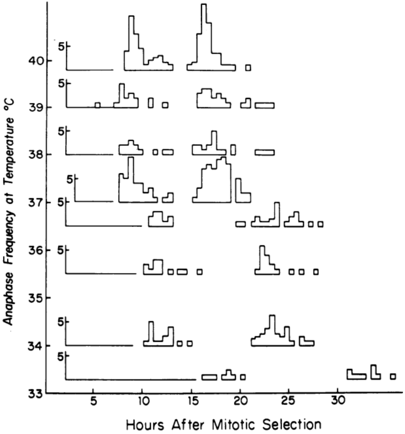



**yeast**: Cookson et al. (2010), Bryan et al. (2010), Litsios et al. (2019): **translation pulses around** `Start`, Baumgartner et al. (2018), Papagiannakis et al. (2017): metabolic oscillations w/o division.

**mammalian cells**:
Liu et al. (2020), Ghenim et al. (2021): mass density and translation/degradation with $\tau_{osc}$ = **4–5 h periods.**

# A Cell Growth Cycle in Mammals?

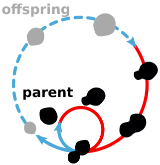



Klevecz and Ruddle (1968), **Science**: Cyclic changes in enzyme activity in synchronized mammalian cell cultures.
Klevecz (1976), PNAS: Quantized generation time in mammalian cells as an expression of the *cellular clock*. ⇒ *cell growth cycle* ∼ growth rate.
Klevecz et al. (2004), PNAS: A genomewide oscillation in *transcription gates* DNA replication and cell cycle. ⇒ *pulse-width modulation*.

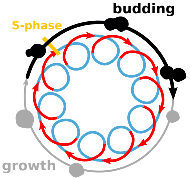
TODO: Brodsky (1975)

# A Cell Growth Cycle in Mammals?




LBGH 1022

IFO 0233




- Another path through the DAG: biological clocks, circadian biology, developmental clocks,
- Bob Klevecz and growth pulses in mammalian cells

**Lectures VIII-X**

# Quantitative Cell Biology

- Anabolic vs. catabolic split of Monod model,
  - couple to full metabolic model, FBA to get overall stoichiometries.
- PWM Model of ribosome production.

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