Growth dynamics, the growth-division transition, and cell size in yeast

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Outline

1. Cell cycle control
2. Growth dynamics
3. Transitioning from growth to division
4. The growth phenotype
5. Previous research examples
6. Research goals
7. Cell size measurements
Cell cycle control

Cell proliferation is regulated by a mechanism that exerts control throughout a cell’s division cycle (CDC) to maintain order and prevent the propagation of defective cells.

Hence the “checkpoint” perspective of the cell cycle (e.g. cell size and DNA quality checkpoints)

Millar, 2002
Cell cycle history

Much of the 44 yrs of research* on the yeast cell cycle can be summarized in a directed graph, where edges indicate activation and repression for many genes and proteins. Such a graph provides a qualitative understanding of this systemic behavior. Although most “parts” are known, a molecular scale graph of the CDC remains incomplete (i.e. functional roles are unknown).

*pubmed Millar, 2002
Size dependence

Concomitant with this regulatory process is the "developmental" process of cell growth (critical cell size, wee mutants)

Growth without division leads to abnormally large cells and an inability to reproduce

Division without growth leads to small cells

Correlation exists between growth and division, and molecular variants exist on either side of the baseline

Millar, 2002
Growth dynamics

Interested in the correlation between growth and division, which is manifest at the G1/S transition.

- Does the transition depend on a single protein (cln3?) or several factors (ratios, concentrations, state assessment)?
- How does the environment (i.e. nutrient levels) alter the timing of the passage into S phase?

Measure phenotype of interest through the cell cycle.

- How do cell size and shape change over time; what are their rates of change?
- What are the temporal relationships between size/shape/growth rate and index in the cell cycle?
Growth dynamics

- Measure phenotype of interest through the cell cycle
- How do cell size and shape change over time; what are their rates of change?
- What are the temporal relationships between size/shape/growth rate and index in the cell cycle?
The growth-division transition

Current model suggests that a critical cell size threshold is set by translation rate (which increases over time).

Threshold is then modulated by nutrient availability via ribosome biogenesis (which provides an instantaneous assessment of environment).

The growth-division transition

- Alberghina et al. (2004) suggest there are two checkpoints that maintain the growth-division relationship:
  - CLN3-CDC28 / Far1 CKI
  - CLNb5,6-CDC28 / Sic1 CKI
Single cell growth phenotype

- The phenotype of interest is the growth of a single cell.
- This includes the size and shape of a cell, its doubling time, and its growth kinetics (rate of change in size over time).

\[ \frac{dr}{dt} \]

\text{doubling time}
Why is single cell growth an interesting or relevant phenotype?

- Heritable genotypic variation is manifested as a growth and division strategy (strains show different growth phenotypes)
- Corresponds to the “development” of a single cell
- Can be used to study how cells compete for resources and alter the environment
- Affects the downstream evolution of the population (life history trait)
Physiological characterization

Adams et al. (1985) grew diploid SC populations in glucose limited continuous culture for 260+ generations.
Geometry elongated (surface to volume ratio increased)

Glucose uptake rate and time until maximum uptake rate doubled
Division time remained the same
Single cell growth is a complex phenotype

Growth, notably at the G1/S transition, is regulated not only by
- teams of transcription factors (MBF/SBF)
- and cyclin-cdk pairs (cln3-cdc28),
- but also by over a dozen ribosomal proteins, transcription and translational capacity, and environment (via nutrient sensing pathways, e.g. Gpcr-Ras, TOR)
Single cell growth is a complex phenotype

Attempts at placing growth and cell cycle regulation into a detailed quantitative biochemical framework

without inclusion of environmental modulation
Single cell growth is a complex phenotype...

...this can require dozens of parameters whose values are not necessarily well founded.

Chen et al., 2004
Single cell growth is also a potentially troublesome phenotype

- The mapping from genotype to cell growth phenotype is highly dependent on environmental context
  - growth media
  - population density
  - temperature
  - mating pheromones
- No invariant or global phenotype for a strain
- Requires a lot of data
A quantitative phenotype

We want to make several measurements, corresponding to observations of cell cycle length, size, and geometry, as well as cell and population growth kinetics.

The growth phenotype for a particular population or strain is defined as this set of measurements.

A given phenotype will be valid under a particular set of environmental criteria.

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<th>size</th>
<th>CDC length</th>
<th>geometry</th>
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A quantitative phenotype

- Compare phenotypes of natural strains instead of genetic knockouts
- 8 strains collected from PA and NJ
- Natural variants typically exhibit “softer” phenotypes, which provides a wider range of values and more quantitative information

<table>
<thead>
<tr>
<th></th>
<th>YPS183</th>
<th>YPS2060</th>
<th>YPS2079</th>
<th>YPS3137</th>
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| YPS2060 | YPD30°C, 45°F | CDC length | geometry | gluc/s | kinetics |...
| YPS2079 | YPD30°C, 45°F | CDC length | geometry | gluc/s | kinetics |...
| YPS3137 | YPD30°C, 45°F | CDC length | geometry | gluc/s | kinetics |...
Recap

So we’ve defined the following

- **Subject**: the yeast growth-division transition
- **Observations**: set of measurements corresponding to the growth phenotype
- **Samples**: natural strains

What are the goals of this study?
Major goals

1. Survey phenotypic and genetic variability pertaining to the G1/S transition using natural yeast strains
2. Construct a dynamical model of single cell growth
3. Simulate long term evolutionary competitions between "genotypes" in different environments using above model
4. Validate simulation predictions with long term batch culture evolution experiments
1. Survey phenotypic and genetic variability in natural yeast strains

What are the phenotypes of interest and how can they be measured accurately in a repeatable fashion?

- Cycle length, size, geometry, cell/population growth kinetics (viz. phenotype matrix)
- Measure for each natural strain in different environmental conditions
1. Survey phenotypic and genetic variability in natural yeast strains

What are the heritable variations that generate these cell growth phenotypes?

- High level: metabolic parameters such as resource uptake efficiency, biosynthetic efficiency, size threshold

- Molecular level: genetic and regulatory components
  - microarrays - compare transcriptomes to identify heterochronic differences
  - transcription factor network - determine which may be altered by regulatory elements
  - genotype candidate sequences to look for coding variation (CDF) and regulatory variation (promoter, UTRs)
2. Construct a dynamical model of single cell growth

Formulate a high level metabolic model of the growth-division transition, incorporating environmental resource modulation

- Possible models: timing mechanism, sizing mechanism, ratio of activator/inhibitor, combination thereof

- Estimated parameters will be used to represent a particular strain/genotype

- Key is in modeling how nutrient effects modulate cell size and the G1/S transition
3. Simulate long term evolutionary consequences of particular "genotypes" using above model

- Predict the evolutionary consequences of two given genotypes competing in a variety of environments in a batch culture setting
- Is it possible to evolve large or small cells predictably?
- Test the predictions using a particular strain and environmental regime
1. Survey phenotypic and genetic variability in natural yeast strains

What are the phenotypes of interest and how can they be measured accurately in a repeatable fashion?

- Cycle length, size, geometry, cell/population growth kinetics (viz. phenotype matrix)
- Measure for each natural strain in different environmental conditions
Phenotypic measurements of natural strains

- Cell cycle length: time between peaks on a plot of percent budding cells vs time

![Graph showing 8 natural strains plus one lab strain](image)

**Repeat 1:**
- 2073 < 2079 < 2060 < 3137 < 3060 < 183

**Repeat 2:**
- 2073 < 3137 < 2079 < 2060 < 3060 = 183
Phenotypic measurements of natural strains

- Cell size: diameter of parent cell before budding at the G1/S transition
- Microscopic measurements performed on single cells that you select
- Time consuming to collect large sample of measurements
- Need to make certain assumptions about cell shape
- Coulter counter measurements provide a size distribution for a sample of cells
- Evaluating accuracy and precision
Replicating size distributions

- **Settling effect**: yeast cells will settle in a tube over time.

- **Cell cycle effect**: cells will continue to progress through the cell cycle, making multiple cross-sample comparisons difficult.

- **Technical replication effect**: If you run the same sample through the Coulter counter twice, do you get the same distribution and number of cells?

- **Biological replication effect**: If you measure the size distributions of the same strain grown in separate flasks, do you get the same distribution and number of cells?
Settling effect

-1e3/5m

-.02/5m

-.01/5m
Cell cycle effect
Replication effects
Replication effects

![Graph showing cell diameter distribution with different replicates.](image-url)
Strain comparison

Differential Number (Average)

- A2-6s183 #av
- a2-6s2055 #av
- a2-6s2060 #av
- a2-6s2066 #av
- a2-6s2067 #av
- a2-6s2073 #av
- a2-6s2079 #av
- a2-6s3060 #av
- a2-6s3137 #av

Number

Cell Diameter (µm)

LC = 2.189 µm  UC = 6.071 µm {68163}
Strain comparison

Differential Number (Average) (Smoothing=7)

Number (%)

Cell Diameter (μm)
Need more precision

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</table>

YPS2055: 2.857 ± .434 (1sd) => [2.423, 3.291]
YPS3137: 3.302 ± .14 (1sd) => [3.162, 3.442]

1sd range for YPS2055 spans all natural strains!
Decoupling growth and division

- Jorgensen et al. systematically measured median size and doubling time of all 6000 single cell deletion mutants in SC lab strain
- Identified 400 extreme size mutants (top/bottom 5% of combined size distribution)
Decoupling growth and division

No strong correlation between growth and division
Short term direction

- Continue evaluating phenotypic variability and improve measurement precision
- Sequence candidate loci for polymorphisms (e.g. cln3)
Additional ideas

- Literature mining algorithm to construct current yeast growth/cell cycle network from the literature
- Network scaling algorithm to view/simulate network at various levels of abstraction
FINIS