# Thesis Title

## Humboldt Universität Berlin

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Day Month Year

# Contents

# Chapter 1

# Introduction

Growth is a fundamental property of life. External resources are absorbed, transformed and incorporated in a continuous process of expansion. Individual entities compete for these resources in a constant struggle for survival and proliferation.

Just as fundamental as growth itself, is its regulation. Smaller entities assemble in larger units by coordination. Prime examples of failed regulation are overexpression of cell components, cancerous cell growth in multicellular lifeforms or the current economic and political system.

## 1.1 Aim Of This Work

This project has two parents, both models that originate from the same research group. Even though sharing a common theme- the growth of *s.cerevisiae*- they look at it from two very different angels. To merge these approaches in one model is the aim of this work.

- **Osmolyte homeostasis controls single-cell growth rate and maximum cell size of Saccharomyces cerevisiae** (published 2019 in npj Systems Biology and Applications by T.Altenburg, B.Goldenbogen, J.Uhlendorf and E.Klipp). For reasons explained later it will be called VGM (Volume Growth Model)

- **Size homeostasis can be intrinsic to growing cell populations and explained without size sensing or signalling** (published 2012 in the FEBS Journal by T.W.Spiesser, C.Müller, G.Schreiber, M.Krantz and E.Klipp). It will be called BGM (Biomass Growth Model)

The VGM models the volume changes of a yeast cell over time, as driven by water influx due to pressure differences outside and inside the cell and a resulting plastic deformation of the cell wall. It accurately reproduces experimental single

cell data for unperturbed and perturbed growth (osmotic-shock), as well as bud growth. Two current limitations of the simulation with bud are the preset lengths of the cell phases, where at first the mother grows alone, then the bud emerges and the coverage of only one cell cycle. Expanding the model to several generations and introducing a more elaborate cell cycle control are possible next steps.

The BGM relies on a so called self-replicator to simulate growth. An abstract biomass produces cell volume and more biomass. It incorporates a simplified version of the signalling network that controls the cell cycle: so called cyclins are produced with rates dependent on the amount of available biomass. Upon reaching a certain threshold level the cell cycle progresses to the next phase. Similar to the VGM it comprises mainly of two phases: a first without bud growth and a second with bud growth. At the end of the latter the mother cell and the bud separate and the newborn daughter cell starts producing buds herself. As generation after generation is being simulated, this leads to an ever growing number of cells, allowing interesting observations on the population level. The self-replicator approach, though conceptually useful, is inaccurate when compared to experimental single cell data. Therefor replacing it with the VGM while keeping the biomass dependent cyclin network could improve the existing model.

## 1.2   Biological Background

Yeast, in particular *Saccharomyces cerevisiae*, is a common model organism. Reason be its easy cultivation, relative small genome and yet distinctive eukaryotic architecture. A feature of widespread interest is the cycle of cell division, worthwhile to study on yeast, as it is conserved throughout eukaryotic organisms up to mammalians. While the sequence of events in that cycle is well understood, their regulation leaves open questions. Two intriguing observations are the rather stable size of newborn cells at cell division and an apparent critical size of daughter cells, before they start budding. Both phenomena point to a kind of size control, to ensure stable cell cycle progression. Various implementations of this size control have been suggested, with no definitive answer yet.

### 1.2.1   The Cycle Of Cell Division In Yeast

New cells descent from existing cells by replication of all necessary components and subsequent division. Needless to say, precise coordination is crucial.

Even though very similar to others the cell cycle in *s.cerevisiae* has some particularities, namely the formation of a bud and a following asymmetrical division, resulting in a big mother cell and a smaller daughter cell, as opposed to the more common equal division with two indistinguishable daughter cells. It is divided in interphase (I) and mitotic phase (M), where in the former, much longer phase all growth related processes take place and in the latter the actual divi-

sion happens. The interphase is again subdivided into a first gap phase between division and DNA-replication (G1) where only the mother cell grows, followed by a synthesis phase (S) where after passing the start signal (START) at the end of G1 DNA-replication and bud formation begin and a second gap phase (G2) where mother and bud continue growing and the duplicated nucleous is transferred to the bud, before division in the mitotic phase.

## 1.2.2   Cell Cycle Control

The progression from one phase to another is controlled by a vast network of signalling proteins, culminating in the expression of a cascade of genes at each transition. This network foremost consists of two classes of proteins: cyclins and cyclin-dependent-kinase (CDK). A kinase is an enzyme that transfers a phosphate from a high energy molecule, such as ATP, to a substrate, hence drastically changing its biochemical properties (source wikipedia). CDK's need a cyclin to bind to them, in order to be active. Depending on the binding cyclin they target different substrates.
Another import class of regulatory proteins is called transcription factors (TF). Located in the nucleous they bind to strands of DNA and activate or deactivate genes, by changing its morphology and blocking/exposing binding sites for polymerases. Once a binding site, called promoter, is prepared, a RNA-polymerase is recruited and starts transcribing the corresponding gene into mRNA, which is translated into polypeptid chains by ribosomes, to then form active proteins. The activity of many TFs is regulated by phosphorylation and dephosphorylation. When a cyclin is abundant in sufficient amount and binding to CDKs, cyclin-specific TFs are targeted and the genes of another cyclin are expressed. This chain ends, when the genes for transition to the next cell phase are expressed.

# Chapter 2

# The biomass growth model

The BGM simulates cell populations with independent single cells growing and dividing over several generations. This allows some interesting in silico experiments, regarding the effect of changes of the single cell properties on the resulting population.

## 2.1 Idea and assumptions

The heart of the BGM is the simulation of single cells. As they grow in size they go through a recurring cycle of phases. Most important is the first phase, where the cell simply grows until it is mature enough to grow daugther cells. Once this point is reached for the rest of the cycle the cell itself stops growing and a bud grows instead. Again when the bud is mature enough, it separates from the mother and becomes an independent cell.

The growth dynamics are modeled using a self-replicator approach. Two kinds of biomass are defined, one called *structual biomass* being directly proportional to the cells' surface and another called *internal biomass*. The internal biomass produces both the structural biomass and itself, resulting in exponential growth. This is dampened by the next assumption. The efficiency of the cells biosynthetic machinery, in this model the internal biomass, is assumed to be inversely proportional to the cell volume. Another assumption is a proportional relationship between the biosynthetic efficiency and cell surface, to account for greater capacities to take up nutrients as the cells' surface increases.

The progress of the cell cycle is regulated by the signalling network described in the introduction. Cyclins are produced at rates depending on the cells' biosynthetic capacity (just like the biomass) and the availability of their respective mRNA. This mRNA is produced in stochastic bursts, independent of biosynthetic capacities or cell volume. Although in this thesis and the original publication by T.Spießer only minimal versions of the cyclin network are implemented, one could make the transcription of mRNA dependent on the abundance of cyclins or other regulatory components. This would be the starting point to

implement more elaborate schemes of the network. Cyclins themselve do not exhibit catalytic activity, but program free CDKs. The CDKs are assumed to be abundant in sufficient and stable amount and are not explicitly modeled to reduce the number of involved species. The point of phase transition is defined by a threshold amount of cyclin molecules, the transition itself as an instantaneous event.

## 2.2  Equations and parameters

In the tables below all species, parameters and equations used in the original BGM are listed. As mentioned before cell surface is defined as proportional to the structural biomass. A spherical cell shape is assumed and total surface and volume result from adding those of the mother and the growing bud.

| Species | description |
|---------|-------------|
| $mCLN$ | mRNA of the G1 proxy cyclin $Cln$ |
| $mCLB$ | mRNA of the S/G2 proxy cyclin $Clb$ |
| $Cln$ | proxy cyclin regulating length of the G1-phase |
| $Clb$ | proxy cyclin regulating length of the S/G2-phase |
| $B_r$ | internal biomass shared by mother and bud |
| $B_{Am}$ | structural biomass of mother |
| $B_{Ad}$ | structural biomass of bud |

| parameter | specification | G1 | S/G2/M |
|---|---|---|---|
| $k_{growth}$ | growth rate (arb.unit) | 0.029 | 0.029 |
| $k_{pCln}$ | production rate Cln ($\mathrm{min}^{-1} \cdot \mathrm{mol}^{-1}$) | 0.589 | 0 |
| $k_{pCln}$ | production rate Cln ($\mathrm{min}^{-1} \cdot \mathrm{mol}^{-1}$) | 0 | 1.606 |
| $k_R$ | synthesis coefficient for internal biomass (arb.unit) | 4.089 | 1.04 |
| $k_{Am}$ | synthesis coefficient for structural biomass of mother (arb.unit) | 1 | 0 |
| $k_{Ad}$ | synthesis coefficient for structural biomass of daughter (arb.unit) | 0 | 1 |
| $k_{deg}$ | degradation rate of cyclins and mRNA ($\mathrm{min}^{-1}$) | 0.1 | 0.1 |
| $P_x$ | probability of mRNA transcription ($\mathrm{min}^{-1}$) | 0.4 | 0.4 |
| $threshold$ | amount of Cln (G1)/ Clb (G2) needed to trigger phase transition (mol) | 150 | 150 |

**ODE**

$$\frac{d}{dt}mCLN = f\left(P_x\right) - k_{deg} \cdot mCLN$$

$$\frac{d}{dt}mCLB = f\left(P_x\right) - k_{deg} \cdot mCLB$$

$$\frac{d}{dt}Cln = k_{pCln} \cdot mCLN \cdot B_R \cdot \frac{A}{V} - k_{deg} \cdot Cln$$

$$\frac{d}{dt}Clb = k_{pClb} \cdot mCLB \cdot B_R \cdot \frac{A}{V} - k_{deg} \cdot Clb$$

$$\frac{d}{dt}B_R = k_{growth} \cdot \left(\frac{k_R}{k_R + kAm + k_{Ad}}\right) \cdot B_R \cdot \frac{A}{V}$$

$$\frac{d}{dt}B_{Am} = k_{growth} \cdot \left(\frac{k_{Am}}{k_R + kAm + k_{Ad}}\right) \cdot B_R \cdot \frac{A}{V}$$

$$\frac{d}{dt}B_{Ad} = k_{growth} \cdot \left(\frac{k_{Ad}}{k_R + kAm + k_{Ad}}\right) \cdot B_R \cdot \frac{A}{V}$$

| $f\left(P_x\right)$ | Function executed in regular time intervals (implement as once per simulated minute), adds one molecule of mRNA with probability $P_x$. |
|---|---|

**Equation**

| $A_{m/d} = B_{Am/Ad}$ |
|---|
| $V_{m/d} = A_{m/d}^{\frac{3}{2}}$ |
| $A = A_m + A_d$ |
| $V = V_m + V_d$ |

## 2.3 Main results

In the following sections main results of the original study by T.Spießer are presented, intended to give an overview of its conclusions and to set stage for a

later comparison with the modified model.

### 2.3.1 G1 cyclin suffices to reproduce experimental G1 durations

This same early version also reproduced experimental findings, that G1 duration would decrease with genealogical age. While *in vivo* this effect would lessen as the cells grow older, the simulated G1 durations kept becoming shorter.
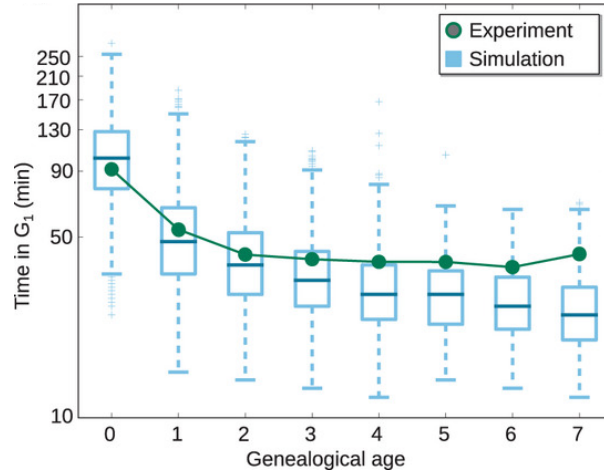


Figure 2.1: Figure taken from (cite spießer 2012 here!!!). Average time spend in G1 depending on genealogical age. Comparison of experimental data and a simulated population

### 2.3.2 Size homeostasis on the population level and growth rate dependent mean cell size

An early version of the BGM published in 2012 (T.Spießer et al.) had only one cyclin $Cln$ governing the length of the G1 phase and a fixed S/G2/M phase. Already this even simpler version produced populations with a quickly converging mean cell size. The interesting point here was, that the mean cell size converged, while the individual cells didnt stop growing, due to the assumption of the biosynthetic efficiency being inversely proportional to the cell volume.
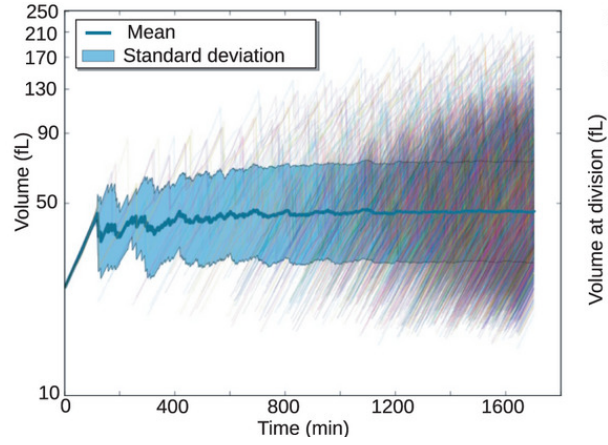
Figure 2.2: Figure taken from (cite spießer 2012 here!!!). A simulated population with cell volume plotted against simulation time. Population mean and standard deviation, single cell trajectories are represented by thin lines.

The growth rate of a population in a laboratory can be tuned adjusting the nutrient composition of the growth medium. It can be seen that fast growing populations have higher average cell sizes and shorter G1 phases. At the same time the variability of the size distribution in the population remains constant over a wide range of growth rates. The first effect can be reproduced with the mentioned G1 cyclin *Cln* and a fixed G2 duration already, the second not. With increasing growth rate the size variability of the simulated population decreased.
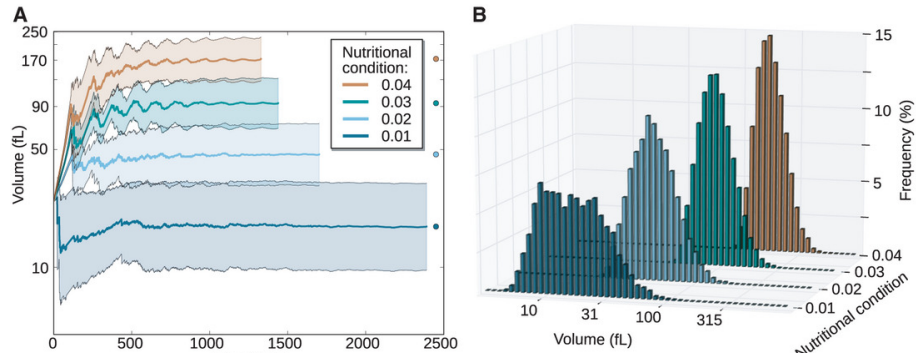


Figure 2.3: Figure taken from (cite spießer 2012 here!!!). (A) Cell volume vs. time(min) of cultures simulated for different parameter values of $k_{growth}$, mean cell size and standard deviation plotted. (B) Volume distribution at the end of simulations in (A). For high values of $k_{growth}$ average cell size increases and becomes less variable.

10

### 2.3.3 Growth rate dependent G2 durations and reduced size variability

The experimentally observed dependence of the S/G2/M phase duration on the growth medium/growth rate can be reproduced by introducing a G2 cyclin *Clb* equivalent to *Cln*. Still the populations' constant size variability over different growth rates can not be reproduced . The model also fails to predict the very similar G2 duration of mother and daughter cells. Only scaling the internal biomass in the production term of the G2 cyclin *Clb* with the buds' proportion of the total cell volume, therefor effectively localizing it in the bud, resolves this issue. This version of the model is shown in the equations and parameters section.
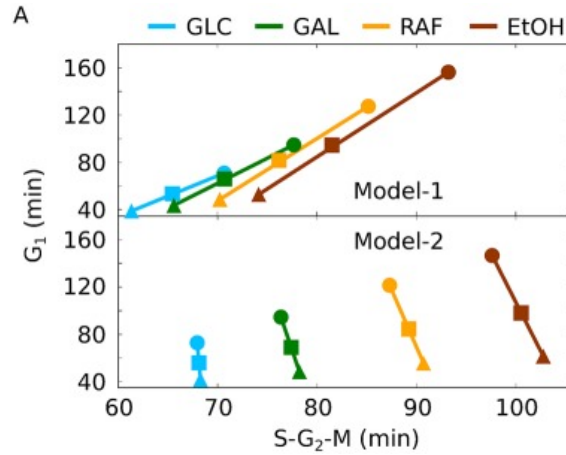


Figure 2.4: Figure taken from (cite spießer 2012 here!!!). Plot average G1 vs S/G2/M duration of mother cells (triangles), daughter cells (circles) and whole population (squares) in 4 different growth media/rates. Above results of simulation without localization of *Clb* (Model-1), beneath results of simulation with *Clb* localization (Model-2. In both models the G1 durations of daughters cells is much longer than that of mother cells. Only the second model shows a similar G2 duration, as is observed in expirements.
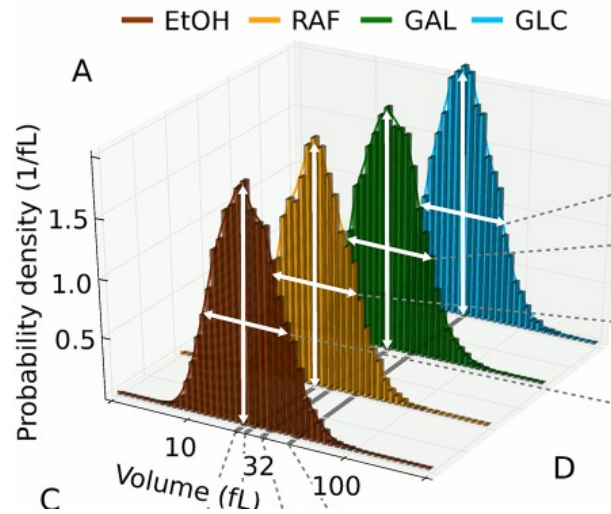
Figure 2.5: Figure taken from (cite spießer 2012 here!!!). Four 2D-plots distribution of cell volume for four growth media/rates. Simulation with bud localized *Clb* (Model-2). While the mean cell volume of the population increases with higher growth rates (from EtOH up to GLC), the size distribution remains rather constant.

# Chapter 3

# The volume growth model

## 3.1 Idea and Assumptions

The VGM links three basic quantities to describe the growth trajectory of single cells: volume, osmotic and tugor pressure. Based on a formalism established by Kedem-Katchalsky water flux across the cell membrane is proportional to the difference between pressures driving water out of the cell (outer osmotic pressure and tugor pressure) and pressures driving water into the cell (inner osmotic pressure). Using Van t'Hoff's law inner and outer osmotic pressure are proportional to inner and outer concentration of osmotically active molecules (osmolytes). The concentration of osmolytes outside of the cell is set constant. The concentration of osmolytes insde the cell increases with an uptake rate proportional to the cell's surface and decreases due to dilution of the growing cell and osmolyte consumption proportional to the cell's volume.
The tugor pressure acts on the cell wall and is increased by water influx- the cell wall gets elastically expanded. When a critical tugor pressure is reached, the cell wall expands plastically (permanently) and tugor pressure is released.
This circuit results in a stepwise increase in cell volume, until the final size is reached: When the inner osmotic pressure is greater than the sum of outer osmotic pressure and tugor pressure water flows in and the cell grows. The tugor pressure increases, ultimately leading to a growth stop, but upon reaching the critical value for plastic expansion drops again, allowing some more growth.

To simulate the growth of a bud during S/G2/M phase, after letting the mother cell grow in G1, a second cell is initialized with small starting volume. Both cells are coupled via exchange terms for water and osmolytes, depending on tugor pressure and osmolyte concentrations. Both mother and bud are approximated as spheres.

## 3.2    Equations and parameters

The tables below contain all species, parameters and equations used in the VGM.

| Species | description |
|---------|-------------|
| $V_{os}$ | Osmolitically active volume, increases with water influx |
| $V_b$ | Volume of solid components, proportional to reference volume |
| $V$ | Total cell volume |
| $V_{ref}$ | Reference volume, volume of relaxed cell without elastic expansion (grows by plastic expansion) |
| $\Pi_t$ | Tugor pressure |
| $c_i$ | Inner osmolyte concentration |

| parameter | decription | value | unit |
|-----------|------------|-------|------|
| $V_{os}^0$ | Initial volume of solid components | 10 | $\mu m^3$ |
| $V_b^0$ | Initial volume of solid components | 3 | $\mu m^3$ |
| $c_i^0$ | Initial inner osmolyte concentration | 322.2 | $mM$ |
| $\Pi_t^0$ | Initial tugor pressure | $2.0 \cdot 10^5$ | $Pa$ |
| $c_e$ | Outer osmolyte concentration | 240.0 | $mM$ |
| $R$ | Ideal gass constant | 8.314 | $\frac{J}{mol \cdot K}$ |
| $T$ | Temperature | 293.0 | $K$ |
| $L_p$ | Membrane water permeability | $1.19 \cdot 10^{-6}$ | $\frac{\mu m}{s \cdot Pa}$ |
| $\Pi_{tc}$ | Critical tugor pressure | $2.0 \cdot 10^5$ | $Pa$ |
| $d$ | Cell wall thickness | 0.115 | $\mu m$ |
| $\Phi$ | Cell wall extensibility | $1.0 \cdot 10^{-7}$ | $\frac{1}{s \cdot Pa}$ |
| $E$ | Young's modulus | $2.58 \cdot 10^6$ | $Pa$ |
| $k_{uptake}$ | Osmolyte uptake rate constant | $2.5 \cdot 10^{-16}$ | $\frac{mM}{s \cdot \mu m^2}$ |
| $k_{cons}$ | Osmolyte consumption rate constant | $3.0 \cdot 10^{-16}$ | $arb.unit$ |

14

| **ODE** |
|---|
| $\frac{d}{dt}V_{os} = -L_p \cdot A \cdot (\Pi_t + \Pi_e - \Pi_i)$ |
| $\frac{d}{dt}V_b = 0.2 \cdot V_{ref}$ |
| $\frac{d}{dt}V = \dot{V}_{os} + \dot{V}_b$ |
| $\frac{d}{dt}V_{ref} = \frac{\Phi \cdot r}{2 \cdot d} \cdot f(\Pi_t) \cdot V_{ref}$ |
| $\frac{d}{dt}\Pi_t = \frac{E \cdot 2d}{r} \cdot \frac{\dot{V}}{V_{ref}} - E \cdot \Phi \cdot f(\Pi_t) - \Pi_t \cdot \frac{\dot{V}}{V}$ |
| $\frac{d}{dt}c_i = k_{uptake} \cdot \frac{A}{V} - k_{cons} - c_i \cdot \frac{\dot{V}}{V}$ |

| |
|---|
| $f(\Pi_t) = max(\Pi_{tc} - \Pi_t, 0)$ |
| $A = (4\pi)^{\frac{1}{3}} \cdot (3V)^{\frac{2}{3}}$ |
| $r = \left(\frac{3}{4\pi}V\right)^{\frac{1}{3}}$ |

## 3.3 Main results

### 3.3.1 Single cell growth trajectories have rather sigmoidal shape

It is often assumed, that single cells grow exponentially. The VGM predicts another, rather sigmoidal growth trajectory. The model was fitted and tested with experimental single cell volume data from light microscopy.
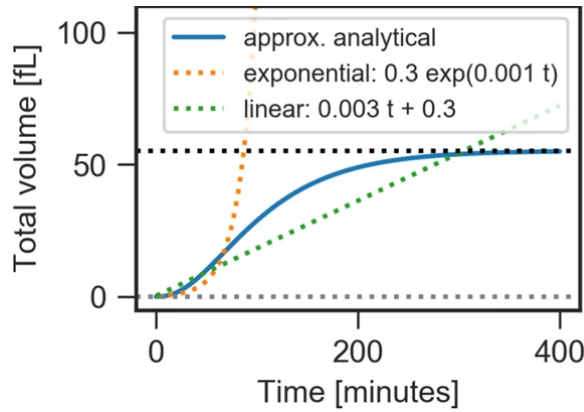


Figure 3.1: Figure taken from (cite Goldenbogen here!!). Plot total cell volume over time. The predicted single cell growth trajectory is neither an exponential function nor a linear function, but rather sigmoidal.

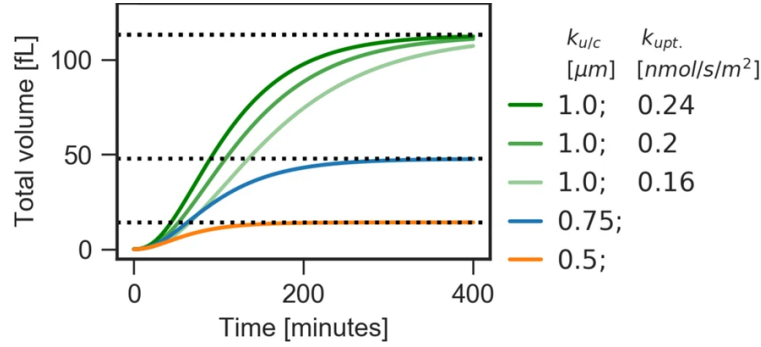### 3.3.2 Ratio of osmolyte uptake and consumption defines final cell volume



Figure 3.2: Figure taken from (cite Goldenbogen here!!). Plot total cell volume over time. The ratio between $k_{uptake}$ and $k_{consume}$ determines the maximum cell volume. If this ratio is kept constant but $k_{uptake}$ and $k_{consume}$ increased or decreased only the growth rate changes.

### 3.3.3 Cell wall extensibility of bud has to be orders of magnitude greater than mother in order to grow
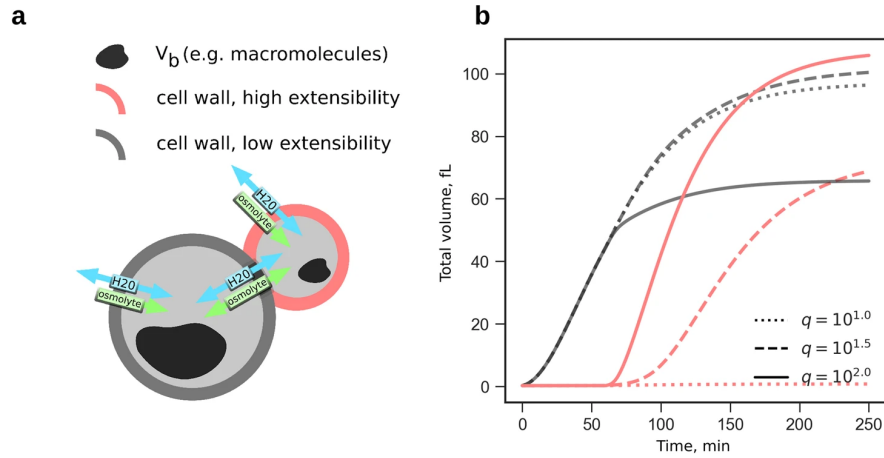


Figure 3.3: Figure take from (cite Goldenbogen here!!). (Left) Scheme of mother/bud coupling. (Right) Growth trajectories of mother and bud for different ratios of their cell wall extensibilities.

# Chapter 4

# Results

## 4.1 Biomass

Merging the two models difficulties arise concerning the biomass. In the BGM it is the biomass producing itself and cell volume. In the VGM cell growth is independent of biomass. Both approaches are conflicting, unless complicated (and mostly hypothetical) feedback between biomass and cell volume is introduced. A new volume dependent biomass is needed.

Experimental data suggest that the bouyant density of yeast cells varies by around 1% throughout the cell cycle (Baldwin,Kubitschek 1984). Still cell density is not really dependent on cell volume and approximately constant. This relationship allows the definition of a volume dependent biomass.

$$\rho_{cell} = \frac{B}{V} \tag{4.1}$$

Where $B$ is the weight of the cell's biomass, $V$ the cell volume and $\rho_{cell}$ their constant ratio. The volume $V_b$ directly taken up by $B$ depends on its own density $\rho_b$.

$$\rho_b = \frac{B}{V_b} \tag{4.2}$$

Since the cell has to be at least as big as the volume taken up by its biomass, it is clear that $\rho_b \geq \rho_{cell}$. As in the VGM the total cell volume $V$ is split in osmotically active and inactive volume $V_{os}$ and $V_b$.

$$V = V_{os} + V_b \tag{4.3}$$

Three types of biomass are defined.

- 'structural' biomass $B_A$

- 'metabolic' biomass $B_R$

- 'basic' biomass $B_N$

with a total biomass

$$B = B_N + B_A + B_R \tag{4.4}$$

The *structural biomass* and the *metabolic biomass* (formerly called *internal biomass*) are directly imported from the BGM. The *basic biomass* is new. It corresponds to the nucleous and only grows in buds.

The VGM differentiates between reversible elastic expansion and irreversible plastic expansion of the cell wall. Only the latter is equated with 'real' growth and the incorporation of new biomass. It is monitored through the reference volume $V_{ref}$. The *structural biomass* is therefor proportional to the relaxed cell surface $A_{ref}$:

$$\sigma_A = \frac{B_A}{A_{ref}} \tag{4.5}$$

Assuming all biomass has the same density, the parameter $\sigma_A$ can be estimated from the surface $A_{ref}$ and thickness $d$ of the cell wall:

$$\begin{aligned} B_A &= \rho_b \cdot V_{wall} \\ &= \rho_b \cdot d \cdot A_{ref} \end{aligned}$$

and consequently

$$\sigma_A = \rho_b * d \tag{4.6}$$

Next a function for the *metabolic biomass* is derived. Insert Eq. 4.3 in Eq. 4.1

$$B = \rho_{cell} \left( V_{os} + V_b \right) \tag{4.7}$$

with Eq. 4.2

$$B = \rho_{cell} V_{os} + \frac{\rho_{cell}}{\rho_b} \cdot B$$

Rearranging $B$ on the left side of the equation yields

$$B = \frac{\rho_{cell} \cdot \rho_b}{\rho_b - \rho_{cell}} \cdot V_{os} \tag{4.8}$$

This equation for the total biomass as a function of the osmotically active volume is now inserted in Eq. 4.4 and rearranged for $B_R$ and its time derivative

$$B_R = \frac{\rho_{cell} \cdot \rho_b}{\rho_b - \rho_{cell}} \cdot V_{os} - B_A - B_N \tag{4.9}$$

$$\dot{B}_R = \frac{\rho_{cell} \cdot \rho_b}{\rho_b - \rho_{cell}} \cdot \dot{V}_{os} - \dot{B}_A - \dot{B}_N \tag{4.10}$$

Next definitions of the cell volume $V$ and the cell radius $r$ as a function of $V_{os}$ are derived.

Together Eq. 4.7 and 4.8 yield the osmotically inactive volume $V_b$ as a funciton of $V_{os}$:

$$V_b = V_{os} \cdot \frac{\rho_{cell}}{\rho_b - \rho_{cell}} \tag{4.11}$$

According to Eq . 4.3 the total cell volume now reads as

$$V = \frac{\rho_b}{\rho_b - \rho_{cell}} \cdot V_{os} \tag{4.12}$$

If a spherical cell is assumed, the cell radius $r$ can be written as

$$r = \left( \frac{3}{4 \cdot \pi} \cdot \frac{\rho_b}{\rho_b - \rho_{cell}} \cdot V_{os} \right)^{\frac{1}{3}} \tag{4.13}$$

$$\dot{r} = \frac{1}{3} \left( \frac{3}{4 \cdot \pi} \cdot \frac{\rho_b}{\rho_b - \rho_{cell}} \right)^{\frac{1}{3}} \frac{\dot{V}_{os}}{V_{os}^{\frac{2}{3}}} \tag{4.14}$$

### 4.1.1 Upper Bound On The Increase Of Basic Biomass

During bud growth the *basic biomass* grows.

$$\dot{B}_N > 0$$

By definition the amount of biomass in a cell can not be negative. To prevent this to occur during bud growth an upper limit for $\dot{B}_N$ has to be formulated, such that $\dot{B}_R$ does not become negative. Using the definition of $\dot{B}_R$ (Eq. 4.10) it holds that

$$\dot{B}_N \leq \frac{\rho_{cell} \cdot \rho_b}{\rho_b - \rho_{cell}} \cdot \dot{V}_{os} - \dot{B}_A \tag{4.15}$$

This inequality can be used to define an adaptive production of basic biomass. Be $k_N$ in $[0, 1]$, setting the ratio of metabolic and basic biomass production and

$$\Delta_{\dot{B}} := \frac{\rho_{cell} \cdot \rho_b}{\rho_b - \rho_{cell}} \cdot \dot{V}_{os} - \dot{B}_A$$

Then an adaptive $\dot{B}_N$ can be defined as

$$\dot{B}_N = k_N \cdot \Delta_{\dot{B}} \tag{4.16}$$

$\dot{B}_R$ is therefor effectively

$$\dot{B}_R = (1 - k_N) \cdot \Delta_{\dot{B}} \tag{4.17}$$

## 4.2 Osmolyte consumption

An important aspect of the VGM is the balance of osmolytes. Their uptake is proportional to the surface and the consumption is proportional to the volume. The ratio $\frac{k_{up}}{k_{cons}}$ defines the final cell size. The consumption of osmolytes being proportional to cell volume cannot really account for the costs of cell growth. When the cell stops growing, the corresponding costs should go to zero. Instead they are constant. It is therefor better understood as a maintenance cost: proteins degrade and have to be reproduced, signalling and cellular transport need

energy etc. A short calculation shows, that maintenance costs due to reproduction of degraded biomass can indeed be approximated as proportional to cell volume. Assuming a first order degradation kinetic $\dot{B}_{deg} = k_{deg} \cdot B$ and constant biomass to volume ratio $B = \rho_{cell} \cdot V$ the osmolyte consumption $\dot{n}_{main}$ reads

$$\dot{n}_{main} = k_{cost} \cdot \dot{B}_{deg}$$
$$= k_{cost} \cdot k_{deg} \cdot \rho_{cell} \cdot V$$

$k_{deg}$ is the degradation rate constant, $k_{cost}$ the number of osmolyte molecules consumed per unit of produced biomass. The constants $k_{cost}$, $k_{deg}$ and $\rho_{cell}$ can be summed up in one as $k_{main}$. This is equivalent to the $k_{cons}$ in the VGM.

To quantify the cost of biomass production a new term is needed. As stated before biomass and volume are assumed to be proportional. The change in biomass as a function of the volume then is

$$\dot{B} = \rho_{cell} \cdot \dot{V} \tag{4.18}$$

The cost of this amounts to

$$\dot{n}_{cost} = k_{cost} \cdot \dot{B}$$
$$= k_{cost} \cdot \rho_{cell} \cdot \dot{V}$$

The complete equation for the osmolyte balance is

$$\dot{c} = \frac{\dot{n}_{up}}{V} - \frac{\dot{n}_{main}}{V} - \frac{\dot{n}_{cost}}{V} - \dot{c}_{dilution} \tag{4.19}$$

$$= k_{up} \cdot \frac{A}{V} - k_{main} - k_{cost} \cdot \rho_{cell} \cdot \frac{\dot{V}}{V} - c \cdot \frac{\dot{V}}{V} \tag{4.20}$$

$$= k_{up} \cdot \frac{A}{V} - k_{main} - (k_{cost} \cdot \rho_{cell} - c) \cdot \frac{\dot{V}}{V} \tag{4.21}$$

$$\tag{4.22}$$

The new cost term is very similar to the dilution term. It does not change the fundamental behaviour of the system, as it only slows down the cell's growth. The final radius of the cell is untouched by it and remains $r_{final} = 3\frac{k_{up}}{k_{main}}$. Nonetheless it clarifies osmolyte consumption and opens up the possibility of more elaborate implementations of growth costs.

## 4.3 Cyclin network

### 4.3.1 Nutrient supply

The uptake of nutrients is proportional to the cell's surface. In the VGM this is expressed by the term for osmolyte uptake in the osmolyte equation. Given the fact that nutrients make up most of the osmolytically active molecules, it is reasonable to equate both. The BGM accounts for this proportionality by

scaling the production terms of cyclin and biomass with the cell surface. The argument being that biosynthetic efficiency increases with nutrient availability. While this is very much true, nutrient availability is not primarily measured in cell surface but nutrient concentration. There is no experimental evidence for a proportional relationship between cell surface and nutrient concentration. In the merged model the concentration of osmolytes/nutrients is a direct output. Consequently the surface scaling of the biosynthetic capacity has to be removed. It could be replaced by the osmolyte concentration, either as plain proportionality or in the form of a Michaelis-Menten kinetic. Since it is almost constant at any time of the simulation it is left out.

### 4.3.2   Bud localization of Clb production

Regulating the duration of the G2 phase via the bud localization of critical G2 cyclins was an important proposition of the BGM. It was implemented using only a fraction of the *internal biomass*, proportional to the bud's share of the total cell volume, in the synthesis of this cyclin (*Clb*). Only when the bud was big enough the cyclin threshold for a phase transition was met and division initialized.
The merged model no longer offers a shared biomass. Therefor localizing *Clb* reduces to including only the bud's *metabolic biomass* in its production.

## 4.4   Coupling of mother and bud

As explained earlier the coupling of mother and bud via exchange terms for water and osmolytes turns out to be rather problematic. For a start it was omitted. The dampened growth of the mother due to resource drain towards the bud was imitated by stopping the growth of the mother during S/G2/M phase, as was done already in the BGM. Nonetheless the model code offers a working option to enable water and osmolyte transfer. The production of cyclins is still coupled by simply adding up the *metabolic biomass* of mother and bud, unless there are explicitly decoupled as for *Clb*.

# Chapter 5

# Cyclin expression

The general equation for the change of concentration of a protein $\left(\frac{P}{V}\right)$ in a growing cell cann be written as:

$$\frac{d}{dt}\left(\frac{P}{V}\right) = \frac{1}{V} \cdot \frac{d}{dt}P - \frac{P}{V^2} \cdot \frac{d}{dt}V \tag{5.1}$$

The change in absolute molecule number can be obtained using the first term. Assuming a second order kinetic with reactants $mRNA$ and $B$ representing biosynthetic biomass and a first order degradation it follows

$$\frac{1}{V} \cdot \frac{d}{dt}P = k_p \cdot \frac{mRNA}{V} \cdot \frac{B}{V} - k_d \cdot \frac{P}{V} \tag{5.2}$$

multiplied with the cell volume:

$$\frac{d}{dt}P = k_p \cdot mRNA \cdot \frac{B}{V} - k_d \cdot P \tag{5.3}$$

In order to maintain concentration homeostasis of proteins and mRNA in growing cells

- protein translation per mRNA has to increase

- mRNA transcription per gene copy has to increase

Without directly mentioning it, the first assumption is already made in the BGM, by assuming the second order kinetic with the biomass as a reactant of the mRNA. The same number of mRNA molecules yields a higher protein production, when the internal biomass increases.
The second assumption is not included and mRNA numbers are volume independent. In a concentration based implementation this leads to dilution of mRNA. Now depending on the ratio $\frac{B}{V}$ this may lead to dilution of the proteins.