

Thesis Title

Humboldt Universität Berlin

Louis Pascal Polczynski

Day Month Year

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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.

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 mammals. While the sequence of events in that cycle is well understood, their regulation leaves open questions. A prominent observation is an apparent critical size that yeast cells have to reach, before they start growing a bud. This phenomena suggests size control, to ensure stable cell cycle progression. Various implementations for this have been proposed, with no definitive answer yet.

1.1 Motivation

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.

- **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). Here called BGM (Biomass Growth Model)

- **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). Here called VGM (Volume Growth Model)

The BGM simulates populations of growing single cells. The rythm of growth and division for these cells is set by a recurring cycle of four phases, the cell cycle. In the first phase they simply grow themselves. During the next three phases buds emerge and then seperate from them. New cells are born. As generation after generation is being simulated this leads to an ever growing number of cells, thus allowing interesting observations on the population level. The model includes a simplified version of the signalling network that controls this cycle. So called cyclins are produced with rates dependent on the available biomass. When these cyclins reach a certain threshold level the cell cycle progresses to the next phase.

The BGM relies on a so called self-replicator, where the cell's biomass produces more biomass. The consequence is exponential growth. While the growth of a yeast population clearly has an exponential phase, there is an ongoing discussion about the nature of single cell yeast growth. Various models have been proposed, the most common being exponential growth and linear growth with several distinct phases.

Goldenbogen et al. proposed another model, the VGM. It assumes the pressure difference between the inside and the outside of the cell to be the driving force for water uptake. A delicate balance between inner osmotic pressure and cell wall pressure shapes the dynamics of the growth process. An extension of the model allows to simulate a mother cell with a growing bud.

It promises to be an interesting next step to integrate this model for single cell growth into the population framework of the BGM. Two current limitations of the VGM that are to be overcome are the preset lengths of the cell phases and the coverage of only one cell cycle.

1.2 The cell cycle of 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. The result is a big mother cell and a smaller daughter cell, as opposed to the more common equal division with two indistinguishable daughter cells. The cell cycle is divided in interphase (I) and mitotic phase (M). The interphase is again subdivided into a first gap phase between division and DNA-replication (G1) where only the mother cell grows. This is followed by a synthesis phase (S) where, after passing the start signal (START) at the end of G1, DNA-replication and bud formation begin. Then comes a second gap phase (G2) where mother and bud continue growing and the duplicated nucleous is transferred to the bud and the Interphase ends. In the last phase, the mitotic phase mother and bud

divide. Because it generally is smaller than older (mother) cells, the new born cell is called a 'daughter' cell during its first cycle.

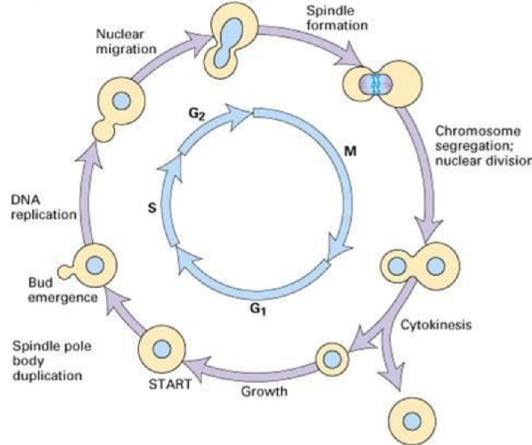


Figure 1.1: Cell cycle in yeast. Figure taken from Jing Cui 2017

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. Two important protein classes in this network are cyclins and cyclin-dependent-kinases (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. 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. Located in the nucleous they bind to strands of DNA and activate or deactivate genes, by changing their morphology and blocking/exposing binding sites for polymerases. The activity of many transcription factors is regulated by phosphorylation and dephosphorylation. When the right cyclin is abundant in sufficient amounts and binding to the CDKs, specific TFs are targeted and the genes for transition to the next cell phase are expressed.

The following simplified description of the network regulating the transition from the G₁ phase to the S phase is based on Barber et al. 2020.

During the early G₁ phase the inhibitor Whi5 is localized in the nucleus of the cell. There it binds to the transcription factor SBF and inactivates it. Active SBF induces the transcription of the genes needed for the phase transition. The cyclin Cln3 binds to the cyclin dependent kinase Cdk1 and phosphorylates Whi5. Phosphrylated Whi5 releases SBF and is exported from the nucleus. The now free SBF induces the transcription of several other cyclins, Cln1 and Cln2 among the first. These cyclins further phosphorylate Whi5, leading to a positive feedback loop and subsequent transition to the S phase.

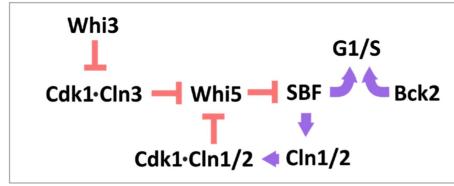


Figure 1.2: Schematic representation of the regulation of the G1 phase in yeast.
Figure taken from Barber et al. 2020

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 daughter 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 *structural 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. Also to account for greater capacities to take up nutrients as the cells' surface increases biosynthetic efficiency and cell surface are assumed to be proportional.

The progress of the cell cycle is regulated by a protein signalling network. 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, as for example the one presented in the introduction. Cyclins themselves do not exhibit catalytic activity, but program free CDKs. The CDKs are assumed to be abundant in sufficient and stable amount. They are not explicitly modeled. The point of phase transition is defined by a threshold amount of cyclin molecules, the transition itself as an instantaneous event.

2.2 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.

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 (hereby 'fixed G2 BGM'). Already this even simpler version produced populations with a quickly converging mean cell size, while the individual cells could continue growing (Figure 2.1a). This convergence was lost, when the scaling of the metabolic capacity with the ratio of the cell surface to the cell volume was removed (Figure 2.1b).

As can be seen in Figure 2.1c the BGM shows a dilution of metabolic biomass.

The growth rate of a population can be varied by adjusting the nutrient composition of the growth medium. It can be observed that fast growing populations have higher average cell sizes and shorter G1 phases. The fixed G2 BGM could already reproduce this. It also predicted a clear decrease in size variability with increasing growth rate (Figure 2.2a). In Spießer et al. 2012 they included experimental results from their laboratory regarding the variability of cell sizes for different growth media (-rates). They confirmed the dependence of the mean cell size on the growth rate, but found that the cell size variability within a culture

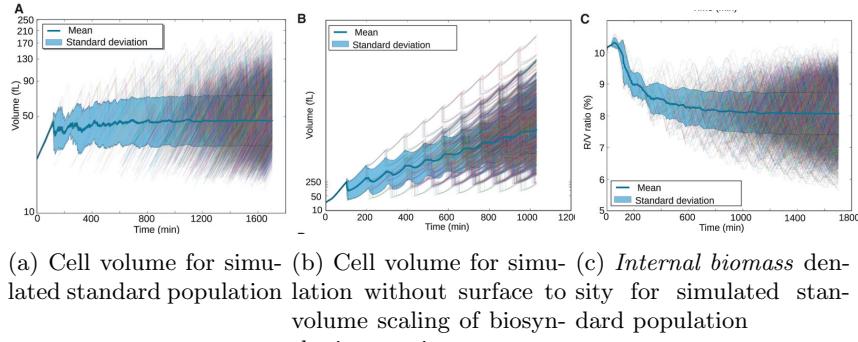
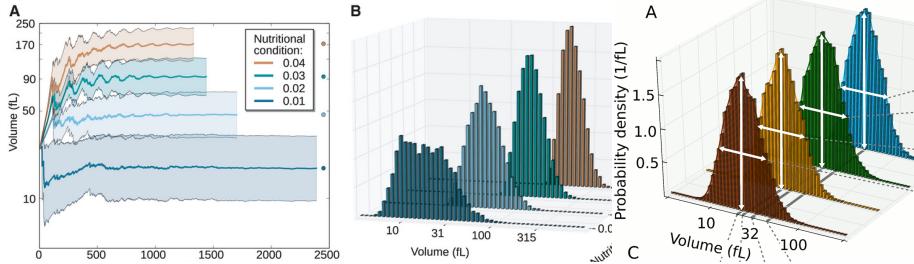


Figure 2.1: Figures taken from Spießer et al. 2012. Mean and standard deviation from population simulation plotted. Thin lines are single cell results.



(a) (A) Cultures simulated for different growth rates, (b) Simulation with variable mean cell size and standard deviation plotted. (B) Variable G2 phase and production distribution at the end of simulations in (A).
 (c) Production of *Clb* localized in the bud.

Figure 2.2: Figures taken from Spießer 2012 and 2015

did not change much with increasing growth rate. The BGM could reproduce this finding, when a variable G2 duration was introduced, governed by a second cyclin *Clb*. This cyclin was assumed to be produced only by the biomass located in the bud (Figure 2.2b).

A classic observation is a distinct drop of the G1 duration between daughter and mother cells (cite torres et al 2018). The BGM reproduces this.

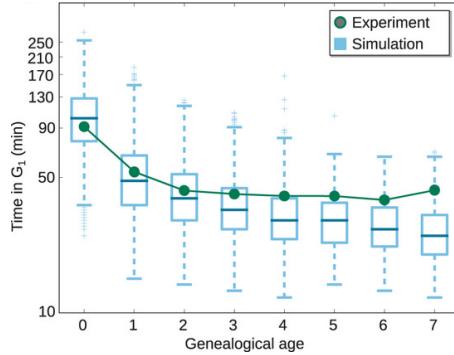


Figure 2.3: Figure taken from (cite spießer 2012 here!!!). Average time spent in G1 depending on genealogical age. Comparison of experimental data and a simulated population

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 turgor 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 turgor 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 amount of osmolytes inside the cell increases with an uptake rate proportional to the cell's surface and decreases with an osmolyte consumption proportional to the cell's volume. The change in concentration is derived by scaling with the cell volume and a dilution term.

The turgor pressure acts on the cell wall and is increased by water influx- the cell wall gets elastically expanded. When a critical turgor pressure is reached, the cell wall expands plastically (permanently) and turgor 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 turgor pressure water flows in and the cell grows. The turgor pressure increases, ultimately leading to a growth stop, but upon reaching the critical value for plastic expansion drops again, allowing some more growth.

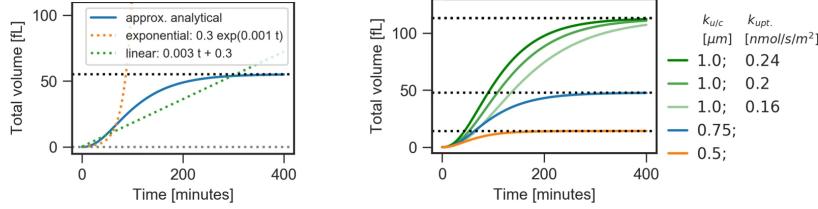
To simulate the growth of a bud during S/G2/M phase, after letting the mother cell grow in G1, a second cell is initialized with small starting volume. Both cells are coupled via exchange terms for water and osmolytes, depending on turgor pressure and osmolyte concentrations. Both mother and bud are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

3.2 Main results

It is often assumed, that single cells grow exponentially. The VGM predicts another, rather sigmoidal growth trajectory (Figure 3.1a). The model was fitted and tested with experimental single cell volume data from light microscopy. Another result of the VGM is the emergence of a limit on the cell volume. This limit is dependent on the ratio of the osmolyte uptake rate and volume dependent consumption (Figure 3.1b). The final cell radius is given by the equation

$$r_{final} = 3 \cdot \frac{k_{uptake}}{k_{consumption}} \quad (3.1)$$

For a fixed ratio the slope of the trajectory depends on the absolute values of k_{uptake} and $k_{consumption}$ (Figure 3.1b). If the mother and the bud are coupled via water and osmolyte exchange the bud can only grow when its cell wall extensibility is several orders of magnitude higher than the mother's.



(a) Cell volume over time. The predicted single cell growth trajectory is neither an exponential nor a linear function, k_{uptake} and $k_{consume}$ increased or decreased but rather sigmoidal. (b) Cell volume over time. The ratio between k_{uptake} and $k_{consume}$ determines the maximum volume. If this ratio is kept constant but function only the growth rate changes.

Figure 3.1: Figures taken from Goldenbogen et al. 2019

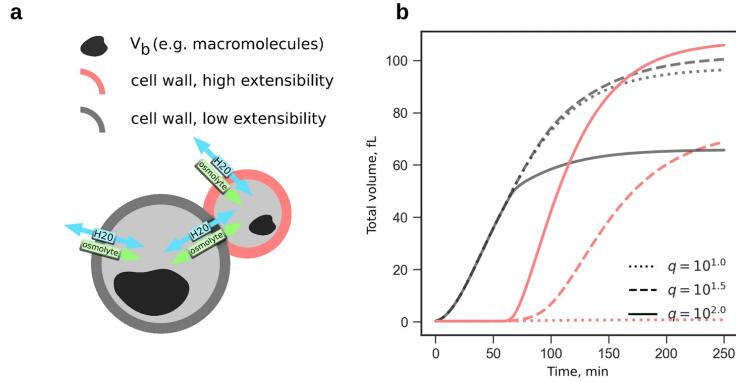


Figure 3.2: Figure take from (cite Goldenbogen here!!). (Left) Scheme of mother/bud coupling. (Right) Growth trajectories of mother and bud for different ratios of their cell wall extensibilities.

Chapter 4

Results

4.1 The merged model

A model is always based on a set of assumptions. Merging two models their respective assumptions have to be examined carefully and if need be revised. In the following sections different aspects of integrating the VGM into the BGM are illuminated.

The merged growth model (MGM) consists of three modules:

- Equations for single cell growth taken from the VGM
- A new biomass function
- Adjusted cyclin equations, similar to the BGM

4.1.1 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 buoyant density of yeast cells varies by around 1% throughout the cell cycle (Baldwin,Kubitschek 1984, Figure 2). Table 1 shows the Buoyant density and relative volume of 7 cell fractions seperated by Percoll equilibrium centrifugation. The top fraction is about 44 % bigger and only 0.9 % less dense than the bottom fraction. In the following the density of yeast is assumed to be independent of the cell volume and constant on a population and single cell level.

In the following this relationship is used to define a volume dependent biomass that is equivalent to the *internal* and *structural* biomass of the BGM.

$$\rho_{cell} = \frac{B}{V} \quad (4.1)$$

B is the weight of the cell's biomass, V the cell volume and ρ_{cell} their constant ratio. As in the VGM the total cell volume V is split in osmotically active and inactive volume V_{os} and V_b .

$$V = V_{os} + V_b \quad (4.2)$$

The volume V_b directly occupied by B depends on density ρ_b of the biomass itself.

$$\rho_b = \frac{B}{V_b} \quad (4.3)$$

Since the cell has to be at least as big as the volume taken up by its' biomass, it is clear that $\rho_b \geq \rho_{cell}$. The two types of biomass of the BGM may be called

- 'structural' biomass B_A
- 'metabolic' biomass B_R

with a total biomass

$$B = B_A + B_R \quad (4.4)$$

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 therefore proportional to the relaxed cell surface A_{ref} :

$$\sigma_A = \frac{B_A}{A_{ref}} \quad (4.5)$$

Assuming all biomass has the same density, the parameter σ_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

$$\sigma_A = \rho_b * d \quad (4.6)$$

Next a function for the *metabolic biomass* is derived. Insert Eq. 4.2 in Eq. 4.1

$$B = \rho_{cell} (V_{os} + V_b) \quad (4.7)$$

with Eq. 4.3

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

Rearranging B on the left side of the equation yields

$$B = \frac{\rho_{cell} \cdot \rho_b}{\rho_b - \rho_{cell}} \cdot V_{os} \quad (4.8)$$

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

$$B_R = \frac{\rho_{cell} \cdot \rho_b}{\rho_b - \rho_{cell}} \cdot V_{os} - B_A \quad (4.9)$$

$$\dot{B}_R = \frac{\rho_{cell} \cdot \rho_b}{\rho_b - \rho_{cell}} \cdot \dot{V}_{os} - \dot{B}_A \quad (4.10)$$

Volume and radius

Contrasting to the VGM the osmotically inactive cell volume is now directly dependent on the biomass content of the cell. Using Eq. 4.7 and 4.8 it can be written as a function of the osmotically active volume.

$$V_b = V_{os} \cdot \frac{\rho_{cell}}{\rho_b - \rho_{cell}} \quad (4.11)$$

Since the total volume is the sum of the osmotically active and inactive volumes it reads as

$$V = \frac{\rho_b}{\rho_b - \rho_{cell}} \cdot V_{os} \quad (4.12)$$

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

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

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

4.1.2 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 therefore 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

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

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 ρ_{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

$$\dot{B} = \rho_{cell} \cdot \dot{V} \quad (4.15)$$

The cost of this amounts to

$$\begin{aligned}\dot{n}_{cost} &= k_{cost} \cdot \dot{B} \\ &= k_{cost} \cdot \rho_{cell} \cdot \dot{V}\end{aligned}$$

The complete equation for the osmolyte balance is

$$\dot{c} = \frac{\dot{n}_{up}}{V} - \frac{\dot{n}_{main}}{V} - \frac{\dot{n}_{cost}}{V} - \dot{c}_{dilution} \quad (4.16)$$

$$= k_{up} \cdot \frac{A}{V} - k_{main} - k_{cost} \cdot \rho_{cell} \cdot \frac{\dot{V}}{V} - c \cdot \frac{\dot{V}}{V} \quad (4.17)$$

$$= k_{up} \cdot \frac{A}{V} - k_{main} - (k_{cost} \cdot \rho_{cell} - c) \cdot \frac{\dot{V}}{V} \quad (4.18)$$

$$(4.19)$$

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.

4.1.3 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 osmotically active molecules, it appears 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.

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, for example 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.

4.1.4 Cyclin expression

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

$$\frac{d}{dt} \left(\frac{P}{V} \right) = \frac{1}{V} \cdot \frac{d}{dt} P - \frac{P}{V^2} \cdot \frac{d}{dt} V \quad (4.20)$$

The change in absolute molecule number can be obtained using the first term . In the BGM a second order kinetic with reactants mRNA and B representing

biosynthetic biomass is assumed. This implies an increase in translated protein per copy number of mRNA with increasing metabolic biomass. Including a first order degradation as well it follows that

$$\frac{1}{V} \cdot \frac{d}{dt} P = k_p \cdot \frac{mRNA}{V} \cdot \frac{B}{V} - k_d \cdot \frac{P}{V} \quad (4.21)$$

Multiplied with the cell volume:

$$\frac{d}{dt} P = k_p \cdot mRNA \cdot \frac{B}{V} - k_d \cdot P \quad (4.22)$$

Previous studies have emphasized the need and evidence for a scaling of copy number of mRNA per gene site with cell volume/biomass (Lin,Amir 2018;Sun,Bowman et al. 2020). In a growing cell constant transcription levels may result in a $\frac{1}{V}$ dilution of the transcriptome and the proteome.

As shown for the BGM in the analysis section this happens indeed. Consequently if the cyclin threshold are implemented as concentrations, cells get stuck in the cell cycle when their cyclin concentrations become to low to reach the thresholds.

For the sake of simplicity and comparability with the original model the MGM was implemented with amount based thresholds. Additionally a version with concentration based thresholds is offered. While the probability of a transcription burst remains constant, the transcription rate during a burst scales with cell size, as was proposed in Sun,Bowman et al. 2020

4.1.5 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. The model didn't have separate compartments for mother and bud. It was therefore 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*). The biomass was assumed to be distributed homogeneous and scaled with the total cell volume. Only when the bud was big enough the cyclin threshold for a phase transition was met and division initialized.

The merged model has separate compartments for mother and bud. Localizing *Clb* reduces to including only the bud's *metabolic biomass*, scaled by its volume in the production of *Clb*.

4.1.6 Coupling of mother and bud

As explained later 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* and the volumina of mother and bud, unless there are explicitly decoupled as for *Clb*.

4.2 Analysis

4.2.1 Mean cell size and size variability on the population level

The MGM produces populations with a quickly converging stable mean cell size, that is dependent on the chosen growth rate of the single cells. A high growth rate results in a high mean cell size. The growth rate can be varied by changing the absolute values of either k_{uptake} and k_{main} or just one of them. For the simulations shown in figure 4.1 a fixed ratio was kept between them as described in Goldenbogen et al. 2019. Notably this does not increase the maximum single cell size, which is determined by the ratio k_{uptake} to k_{main} .

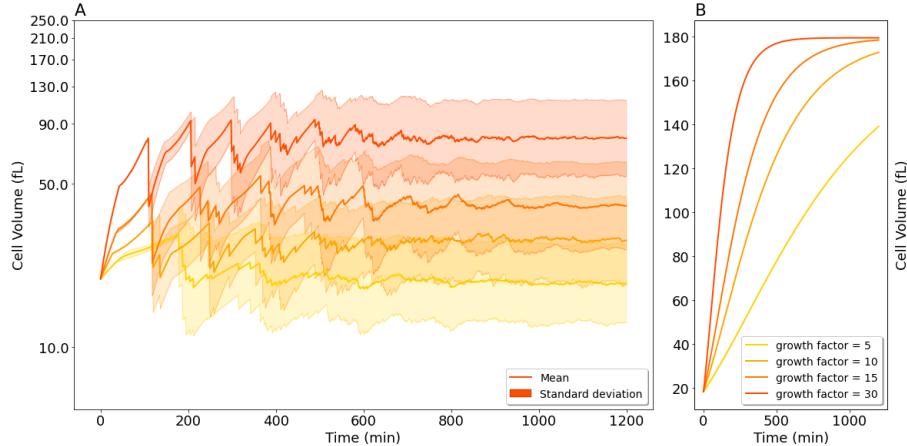


Figure 4.1: (A) Mean cell volume and standard deviation in log scale of simulated population (version with *Clb* production localized in bud) for four different growth factors. (B) Growth trajectory of a single cell at given growth factors.

same figure with changed k_{uptake} to $k_{maintenance}$ ratio?

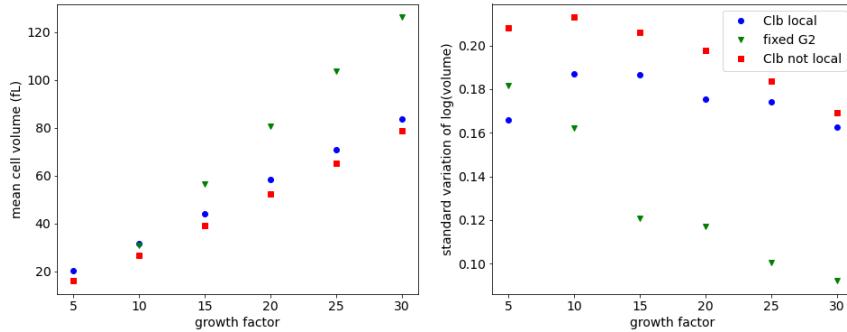


Figure 4.2: Results taken from last timepoint of population simulations. (A) Mean cell volume for different growth factors.(B) Standard deviation of cell volume for different growth factors.

To assess the effect of a localized production of *Clb* three implementations of the MGM where compared. They only differ in their regulation of the G2 phase.

1. fixed G2 duration, no *Clb*
2. *Clb* regulates the G2 duration, *Clb* is produced in mother and bud (not localized)
3. *Clb* regulates the G2 duration, *Clb* is produced only in bud (localized)

The results of the simulations are shown in figure 4.2. An increasing growth factor reduces the size variability of the population, regardless of the G2 phase regulation. The introduction of a *Clb* regulated G2 phase clearly stabilizes the size variability. Localizing the production of *Clb* to the bud seems to reduce the change in size variability again a little bit.

4.2.2 Cell cycle timings

For low growth rates the MGM shows the typical drop and subsequent stabilization of the G1 duration after the first cell cycle of a new born cell. With increasing single cell growth rate this initial step becomes less pronounced. (exp.data/reference??)

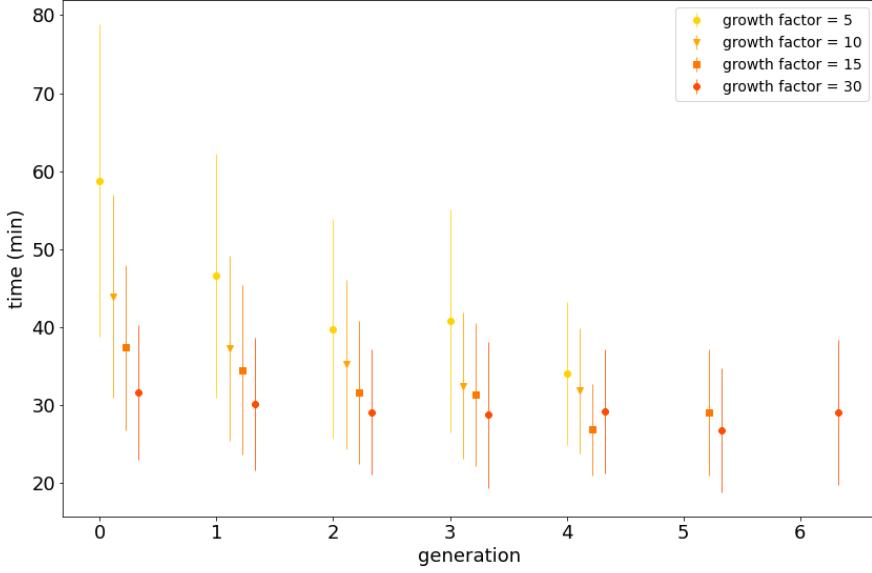


Figure 4.3: Populations simulated with varied growth factor. Mean and standard deviation of time spend in G1 phase at different genealogical age. Generation zero represents daughter cells.

4.2.3 Cyclin expression

The following results were produced with a variation of the BGM. The *Cln* threshold was implemented as critical concentration and not as amount. Even though not explicitly presented here, a concentration based threshold implementation of the MGM produces similar results.

On the population level size homeostasis remains (Figure 4.4a). Spießer et al. therefore concluded the implementation of the cyclin thresholds as an absolute amount was not a size sensing mechanism (Cite Spießer 2012 here!!!). This might not be entirely true. In fact it seems to be more of a reverse size sensing mechanism, where big cells stop budding after a few generations. As the cells grow the concentration of cyclins is ever falling (Figure 4.5) and average G1 durations clearly increase (Figure 4.6). Also simulations with a concentration based threshold are not stable in their behavior when the threshold level is varied. Figure 4.4b is an example. Already small perturbations of the parameters can then lead to either just a handful of cell being born or more than a hundred thousand.

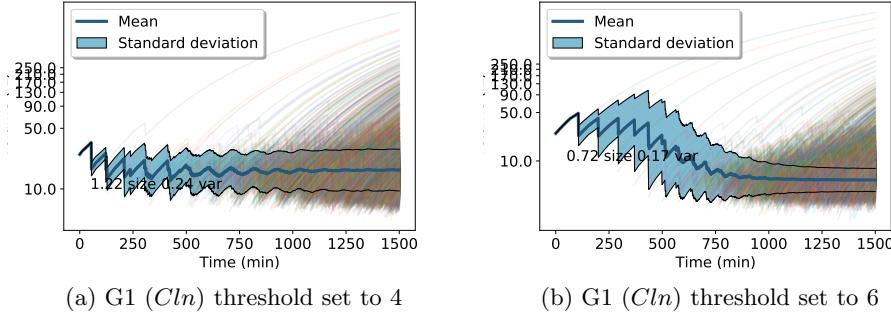


Figure 4.4: Population simulated with concentration based G1 threshold (Cln). Mean volume and standard deviation plotted. Thin lines belong to single cells. Note that older cells stop budding.

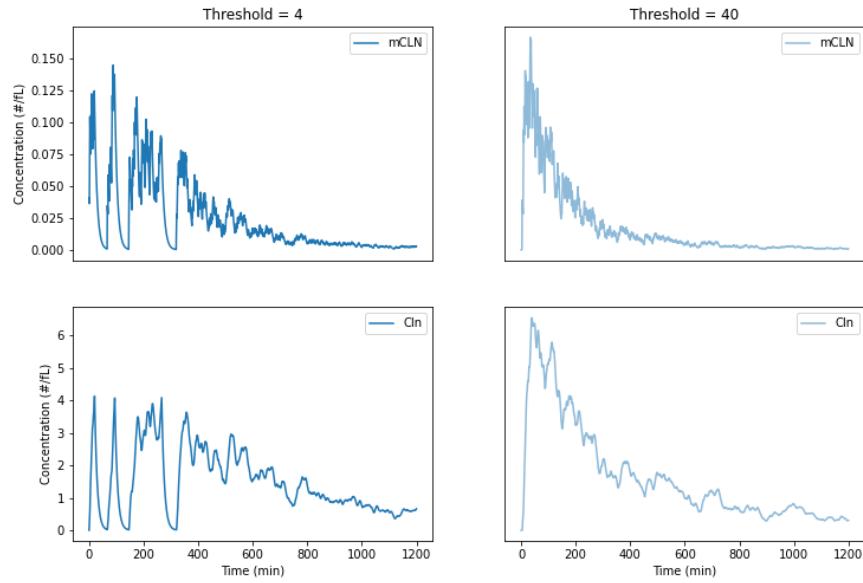


Figure 4.5: Single cell trajectory of mCLN and Cln concentration for a BGM simulation. (Left) Cln threshold set to 4: the cell has several daughter cells before it stops budding. (Right) The Cln threshold is set to 40 and never reached: the cell stays in the first G1 phase.

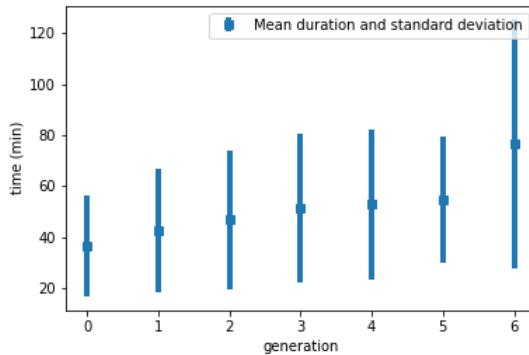


Figure 4.6: Population simulated with concentration based Cln threshold set to 4. Mean G1 duration and standard deviation vs. genealogical age.

Concentration based MGM

In the following section the implementation and some results of a concentration based version of the MGM are reported.

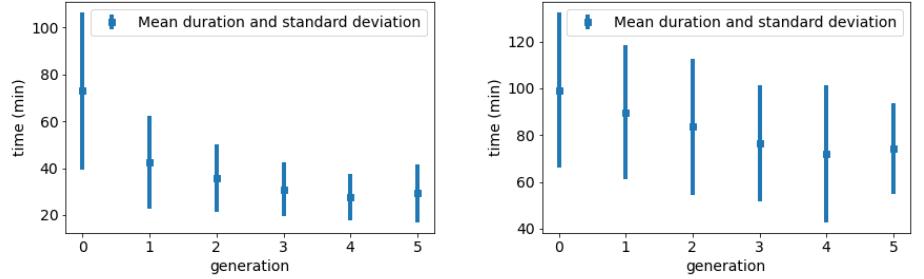
In this version both thresholds, the one for the G1 phase and the one for the G2 phase are set as concentrations. To prevent dilution of the cyclins as described earlier the transcription of mRNA is being scaled with the *metabolic* biomass. In Lin,Amir 2018 it is argued for such biomass scaling under the assumption that the competition of gene sites for RNA-polymerases is the limiting factor for transcription. This assumption is similar to the scaling of the translation rate with the *metabolic* biomass, where the mRNAs compete for ribosomes.

Sun et al. 2020 concluded that an observed scaling of mRNA transcription was achieved by increasing the transcription rate during transcriptional bursts, as opposed to an increasing burst frequency. Therefore in this version of the MGM the transcription of mRNA is implemented as follows:

- When a transcription burst occurs mRNA is produced with a biomass dependent rate
- Whether mRNA is transcribed or not is determined before each time step of the simulation. The probability for a burst is fixed.

Though not shown here the simulated populations show the same size convergence as other implementations. It also reproduces qualitative cell cycle timings, with a clear drop of G1 duration between daughter and mother cells and a subsequent stabilization(Figure 4.8a).

Finally the mRNA and cyclins are no longer diluted, as expected.



(a) Mean and standard deviation of G1 duration for population simulation (b) Mean and standard deviation of G2 duration for population simulation

Figure 4.8: Population simulated with concentration based thresholds and biomass scaling of transcription.

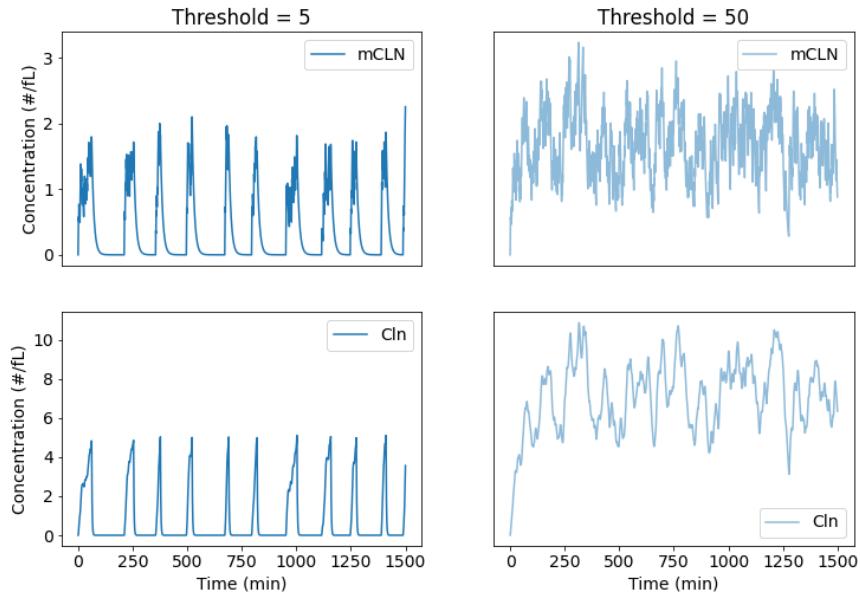


Figure 4.7: Single cell trajectory of mCLN and Cln concentration for a concentration based MGM simulation. (Left) Cln threshold set to 5: The cell budding is normal. (Right) The Cln threshold is set to 50 and never reached: the cell stays in the first G1 phase. In both cases mRNA and cyclin are not diluted.

4.2.4 Coupled multigeneration simulation

When mother and bud are coupled, the cell stops budding after a few generations (Figure 4.9). The reason for this is a decline in osmolyte concentration and turgor pressure in the mother cell. At first the osmolyte concentration of the mother is higher than that of her buds, but falling with every generation. At some points the osmolyte concentration of the buds is higher than the mother's and the turgor pressure starts dropping after the cell division. As water and osmolytes follow the gradient between mother and bud, when a new bud is initiated it first gets drained by the mother. When the mother's turgor pressure and concentration are restored to her normal levels, the bud can start growing. If the pulse of the cell division is set such that the bud can always grow enough, the cell does not stop budding. The MGM implementation with a fixed G2 duration and with *Clb* translation in the whole cell both stop budding. The version with localized *Clb* translation continues budding, as it directly measures the metabolic capacity of the bud.

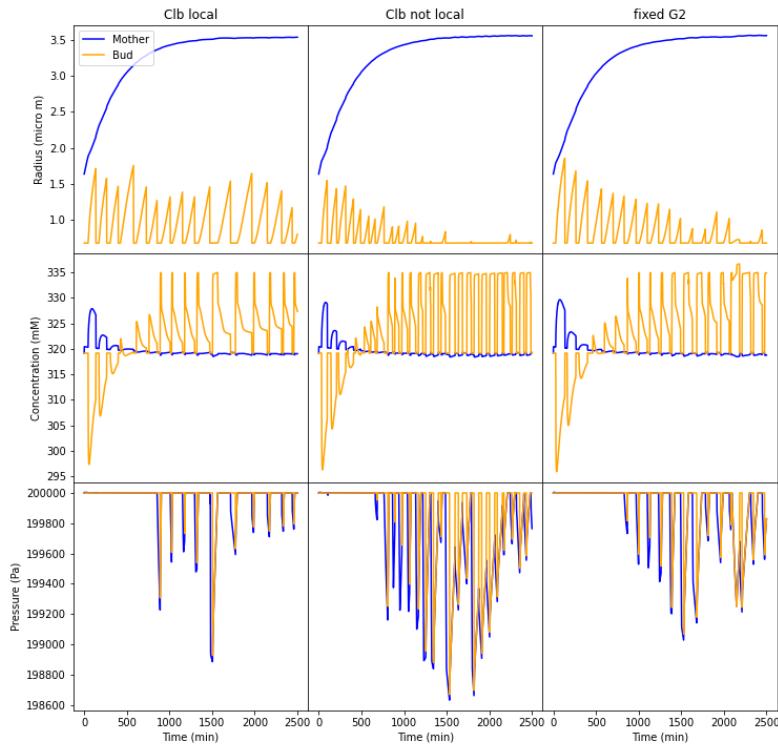


Figure 4.9: Single cell plots of cell radius, osmolyte concentration and turgor pressure of mother (blue) and bud (orange). Simulated with growth factor of 10 and the parameters sed before.

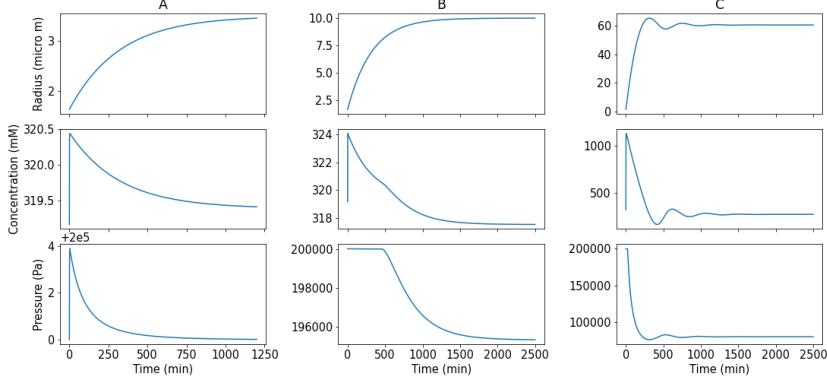


Figure 4.10: Single cell plots of cell radius, osmolyte concentration and turgor pressure. (A) $k_{uptake} = 1$, $k_{maintenance} = 0.3$, $growth_{factor} = 10$. (B) $k_{uptake} = 5.05$, $k_{maintenance} = 0.25$, $growth_{factor} = 100$, $L_p = 1.19 * 0.095 * e - 6$.

Another a lot less puzzling decline of osmolyte concentration and turgor pressure occurs in both, single cell and coupled simulations. The trigger is a critical cell size (Figure 4.10).

Taking a look at the equation for turgor pressure there are two terms scaling with the change in total cell radius r . A term with a positive sign scales with $\frac{1}{r^2}$, another term with a negative sign scales with $\frac{1}{r}$. The term for plastic expansion only acts for turgor pressures above Π_{tc} and is omitted in the following equation.

$$\frac{d}{dt} \Pi_t = \frac{2 \cdot E \cdot d}{1 - \nu} \cdot \frac{\dot{r}}{r^2} - \Pi_t \cdot \frac{\dot{r}}{r} \quad (4.23)$$

For a growing cell ($\dot{r} > 0$) the turgor pressure increases as long as

$$r < \frac{2 \cdot E \cdot d}{1 - \nu} \cdot \frac{1}{\Pi_t} \quad (4.24)$$

Above this critical radius the turgor pressure Π_t of the growing cell can only decrease. For a single cell this increases the growth rate of the radius, as is clear from the equation for water uptake:

$$\frac{d}{dt} r_{os} = -L_p \cdot (\Pi_t + R \cdot T \cdot (c_e - c_i)) \quad (4.25)$$

The equation for osmolyte concentration has a similar feature.

$$\frac{d}{dt} c_i = k_{uptake} \cdot \frac{A}{V} - k_{maintenance} - \frac{c_i}{V} \cdot \frac{\dot{V}}{V} \quad (4.26)$$

Rewritten for a spherical cell with radius r it reads as

$$\frac{d}{dt} c_i = 3 \cdot \frac{k_{uptake}}{r} - k_{maintenance} - 3 \cdot \frac{c_i \dot{r}}{r} \quad (4.27)$$

The critical radius for which the osmolyte concentration declines is similar to the already known final cell radius given by Eq. 3.1

$$r < 3 \cdot \frac{k_{uptake} - c_i \cdot \dot{r}}{k_{maintenance}} \quad (4.28)$$

The cell grows in a regimen where the osmolyte concentration decreases slowly, with a rate close to zero. When the critical radius for the decline of turgor pressure is reached (Ineq. 4.24) \dot{r}_{os} and therefore \dot{r} increases (Eq. 4.25). This reduces the critical radius for the decline of the osmolyte concentration (Ineq. 4.28). Again according to Eq. 4.25 the radius grows slower. Given the right set of parameters this can even result in oscillatory behavior of the system around the maximum cell radius defined in Eq. 3.1 (Figure 4.10).

Chapter 5

Discussion

Even though yeast populations can clearly exhibit exponential growth behavior, various models for single cell yeast growth have been proposed. In this thesis a single cell model for yeast growth was integrated in an already existing model for cell growth and cell cycle regulation. While the original model assumed a self-replicator approach to model single cell growth, the newly integrated model links cell volume, osmolyte concentration and turgor pressure to explain single cell growth.

The biomass of the single cells was defined as volume dependent, assuming a fixed total cell density. It was implicitly assumed that only proteins, ribosomal RNA and cell wall components contribute to the cell mass. This is a very simplistic view, as for example carbohydrates can make up a big part of the cell's biomass, depending on the growth rate (T.Nissen et al. 1997, Table 4; H.Lange,J.Heijnen 2001, Table 5). It was also assumed that all types of biomass have the same density. This leaves open space for further explorations on the effect of more detailed modeling of the cell composition on the results presented before. An increasing protein and RNA content for higher growth rates, could for example shorten cyclin regulated cell phases with increasing growth rate even more.

The MGM reproduces key aspects of the BGM. Simulated populations converge to a growth rate dependent stable mean cell size. Depending on the chosen growth rate the mean G1 duration of the population shows the characteristic drop between daughter and first generation mother cells. The introduction of a cyclin regulated G2 duration reduces the dependence of the size variability of a population on the growth rate.

The implementation of the cyclin thresholds as absolute molecule number hides the fact that on the single cell level mRNA, protein and biomass concentrations are falling, as the cell grows. An implementation of the MGM with scaling mRNA transcription reproduces the characteristic behavior of the original model, but 'fixes' the dilution issue.

Appendix A

Appendix

A.1 BGM

In the tables below all species, parameters and equations used in the original BGM are listed. 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 ($\text{min}^{-1} \cdot \text{mol}^{-1}$)	0.589	0
k_{pCln}	production rate Cln ($\text{min}^{-1} \cdot \text{mol}^{-1}$)	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 (min^{-1})	0.1	0.1
P_x	probability of mRNA transcription (min^{-1})	0.4	0.4
$threshold$	amount of Cln (G1)/ Clb (G2) needed to trigger phase transition (mol)	150	150

ODE	Equation
$\frac{d}{dt} mCLN = f(P_x) - k_{deg} \cdot mCLN$	$A_{m/d} = B_{Am/Ad}$
$\frac{d}{dt} mCLB = f(P_x) - k_{deg} \cdot mCLB$	$V_{m/d} = A_{m/d}^{\frac{3}{2}}$
$\frac{d}{dt} Cln = k_{pCln} \cdot mCLN \cdot B_R \cdot \frac{A}{V} - k_{deg} \cdot Cln$	$A = A_m + A_d$
$\frac{d}{dt} Clb = k_{pClb} \cdot mCLB \cdot B_R \cdot \frac{A}{V} - k_{deg} \cdot Clb$	$V = V_m + V_d$
$\frac{d}{dt} B_R = k_{growth} \cdot \left(\frac{k_R}{k_R + k_{Am} + k_{Ad}} \right) \cdot B_R \cdot \frac{A}{V}$	
$\frac{d}{dt} B_{Am} = k_{growth} \cdot \left(\frac{k_{Am}}{k_R + k_{Am} + k_{Ad}} \right) \cdot B_R \cdot \frac{A}{V}$	
$\frac{d}{dt} B_{Ad} = k_{growth} \cdot \left(\frac{k_{Ad}}{k_R + k_{Am} + k_{Ad}} \right) \cdot B_R \cdot \frac{A}{V}$	
$f(P_x)$	Function executed in regular time intervals (implement as once per simulated minute), adds one molecule of mRNA with probability P_x .

A.2 VGM

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)
Π_t	Tugor pressure
c_i	Inner osmolyte concentration

parameter	description	value	unit
V_{os}^0	Initial volume of solid components	10	μm^3
V_b^0	Initial volume of solid components	3	μm^3
c_i^0	Initial inner osmolyte concentration	322.2	$ mM$
Π_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	$1.19 \cdot 10^{-6}$	$\frac{\mu m}{s \cdot Pa}$
Π_{tc}	Critical tugor pressure	$2.0 \cdot 10^5$	Pa
d	Cell wall thickness	0.115	μm
Φ	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	$3.0 \cdot 10^{-16}$	<i>arb.unit</i>

ODE
$\frac{d}{dt} V_{os} = -L_p \cdot A \cdot (\Pi_t + \Pi_e - \Pi_i)$
$\frac{d}{dt} V_b = 0.2 \cdot V_{ref}$
$\frac{d}{dt} V = \dot{V}_{os} + \dot{V}_b$
$\frac{d}{dt} V_{ref} = \frac{\Phi \cdot r}{2 \cdot d} \cdot f(\Pi_t) \cdot V_{ref}$
$\frac{d}{dt} \Pi_t = \frac{E \cdot 2d}{r} \cdot \frac{\dot{V}}{V_{ref}} - E \cdot \Phi \cdot f(\Pi_t) - \Pi_t \cdot \frac{\dot{V}}{V}$
$\frac{d}{dt} c_i = k_{uptake} \cdot \frac{A}{V} - k_{cons} - c_i \cdot \frac{\dot{V}}{V}$
$f(\Pi_t) = \max(\Pi_{tc} - \Pi_t, 0)$
$A = (4\pi)^{\frac{1}{3}} \cdot (3V)^{\frac{2}{3}}$
$r = \left(\frac{3}{4\pi}V\right)^{\frac{1}{3}}$

A.3 MGM

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

Species	description
V_{os}	Osmolitically active volume, increases with water influx
V_b	Volume of solid components, proportional to reference volume
r	Total cell radius
R_{ref}	Reference Radius, radius of relaxed cell without elastic expansion (grows by plastic expansion)
Π_t	Tugor pressure
c_i	Inner osmolyte concentration
B_R	internal biomass
B_A	structural biomass
B	total biomass
$mCLN$	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

parameter	specification	G1	S/G2/M
k_{growth}	growth rate (arb.unit)	0.029	0.029
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k_{pCln}	production rate Cln ($\text{min}^{-1} \cdot \text{mol}^{-1}$)	0	1.606
k_{deg}	degradation rate of cyclins and mRNA (min^{-1})	0.1	0.1
P_x	probability of mRNA transcription (min^{-1})	0.4	0.4
$threshold$	amount of Cln (G1)/ Clb (G2) needed to trigger phase transition (mol)	150	150

parameter	description	value	unit
V_{os}^0	Initial volume of solid components	10	μm^3
V_b^0	Initial volume of solid components	3	μm^3
c_i^0	Initial inner osmolyte concentration	322.2	mM
Π_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	$\frac{\text{J}}{\text{mol} \cdot \text{K}}$
T	Temperature	293.0	K
L_p	Membrane water permeability	$1.19 \cdot 10^{-6}$	$\frac{\mu\text{m}}{\text{s} \cdot \text{Pa}}$
Π_{tc}	Critical tugor pressure	$2.0 \cdot 10^5$	Pa
d	Cell wall thickness	0.115	μm
Φ	Cell wall extensibility	$1.0 \cdot 10^{-7}$	$\frac{1}{\text{s} \cdot \text{Pa}}$
E	Young's modulus	$2.58 \cdot 10^6$	Pa
k_{uptake}	Osmolyte uptake rate constant	$2.5 \cdot 10^{-16}$	$\frac{\text{mM}}{\text{s} \cdot \mu\text{m}^2}$
$k_{maintenance}$	constant osmolyte consumption	$3.0 \cdot 10^{-16}$	arb.unit
k_{cost}	number of osmolytes per biomass unit	set to zero	mM/g
ρ_{cell}	cell density	0.5	g/fL
ρ_b	biomass density	1.1	g/fL

ODE
$\frac{d}{dt} V_{os} = -L_p \cdot A \cdot (\Pi_t + \Pi_e - \Pi_i)$
$\frac{d}{dt} V_b = \frac{k_D}{k_b - k_D} \cdot V_{os}$
$\frac{d}{dt} r = \left(\frac{3}{12\pi} \cdot \frac{k_b}{k_b - k_D} t \right)^{\frac{1}{3}} \cdot \frac{dV_{os}}{V_{os}^{\frac{2}{3}}}$
$\frac{d}{dt} R_{ref} = \frac{\Phi \cdot R_{ref} \cdot r}{2 \cdot d} \cdot f_1(\Pi_t)$
$\frac{d}{dt} \Pi_t = \frac{2 \cdot E \cdot d}{1 - \nu} \cdot \left(\frac{dr}{r^2} - \frac{\dot{R}_{ref}}{R_{ref}} \cdot \frac{1}{r} \right) - \frac{dr}{r} \cdot \Pi_t$
$\frac{d}{dt} c_i = 3 \cdot \frac{k_{uptake}}{r} - k_{maintenance} - 3 \cdot \frac{dr}{r} \cdot (c_i + k_D \cdot k_{cost})$
$\frac{d}{dt} B_A = k_A \cdot 8 \cdot \pi \cdot R_{ref} \cdot \dot{R}_{ref}$
$\frac{d}{dt} B_R = \frac{k_D \cdot k_b}{k_b - k_D} \cdot \dot{V}_{os} - \dot{B}_A$
$\frac{d}{dt} B = \dot{B}_A + \dot{B}_R$
$\frac{d}{dt} mCLN = f_2(P_x) - k_{deg} \cdot mCLN$
$\frac{d}{dt} mCLB = f_2(P_x) - k_{deg} \cdot mCLB$
$\frac{d}{dt} Cln = k_{pCln} \cdot mCLN \cdot \frac{B_R}{V} - k_{deg} \cdot Cln$
$\frac{d}{dt} Clb = k_{pClb} \cdot mCLB \cdot \frac{B_R}{V} - k_{deg} \cdot Clb$
$f_1(\Pi_t) = \max(\Pi_{tc} - \Pi_t, 0)$
$A = 4 \cdot \pi \cdot r^2$
$f_2(P_x)$ Function executed in regular time intervals (implement as once per simulated minute), adds one molecule of mRNA with probability P_x .