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 ($\text{min}^{-1} \cdot \text{mol}^{-1}$)	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
$\frac{d}{dt}mCLN = f(P_x) - k_{deg} \cdot mCLN$
$\frac{d}{dt}mCLB = f(P_x) - k_{deg} \cdot mCLB$
$\frac{d}{dt}Cln = k_{pCln} \cdot mCLN \cdot B_R \cdot \frac{A}{V} - k_{deg} \cdot Cln$
$\frac{d}{dt}Clb = k_{pClb} \cdot mCLB \cdot B_R \cdot \frac{A}{V} - k_{deg} \cdot Clb$
$\frac{d}{dt}B_R = k_{growth} \cdot \left(\frac{k_R}{k_R+kAm+k_{Ad}}\right) \cdot B_R \cdot \frac{A}{V}$
$\frac{d}{dt}B_{Am} = k_{growth} \cdot \left(\frac{k_{Am}}{k_R+kAm+k_{Ad}}\right) \cdot B_R \cdot \frac{A}{V}$
$\frac{d}{dt}B_{Ad} = k_{growth} \cdot \left(\frac{k_{Ad}}{k_R+kAm+k_{Ad}}\right) \cdot B_R \cdot \frac{A}{V}$

$f(P_x)$	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.

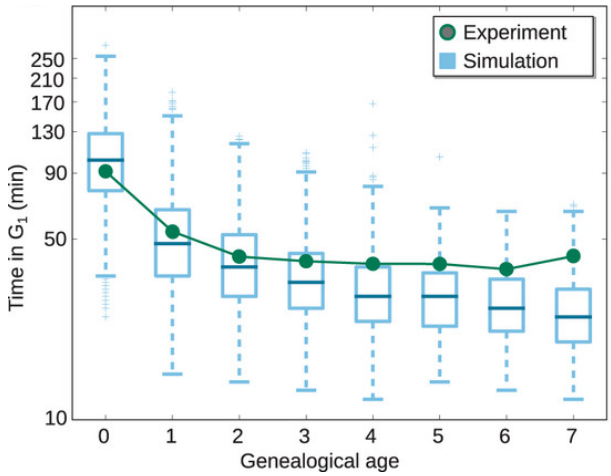

Figure 2.1: Figure taken from (cite spießer 2012 here!!!). Average time spend in G1 depending on genealogical age. Comparison of experimental data and a simulated population

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.

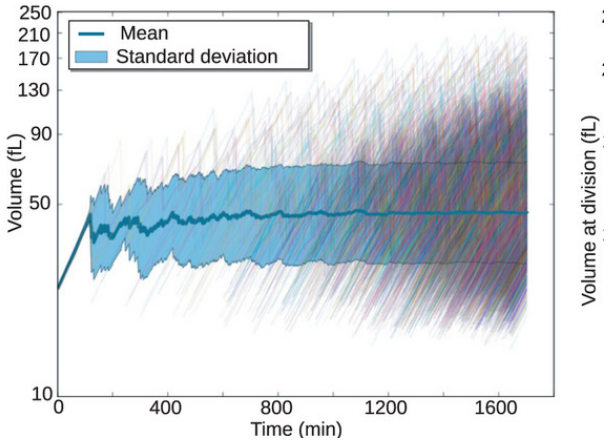

Figure 2.2: Figure taken from (cite spießer 2012 here!!!). A simulated population with cell volume plotted against simulation time. Population mean and standard deviation, single cell trajectories are represented by thin lines.

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.

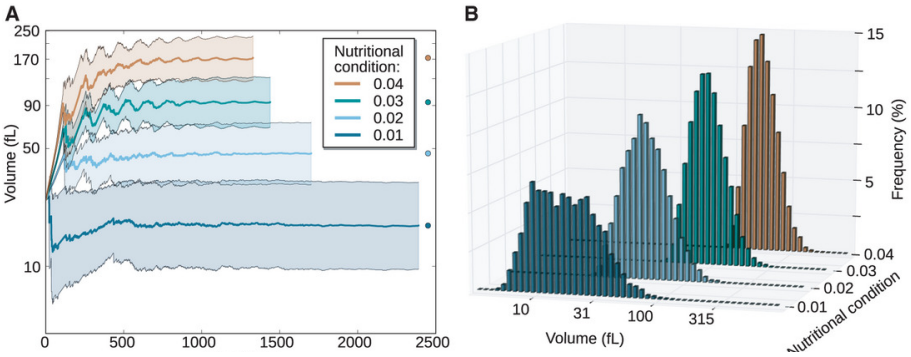

Figure 2.3: Figure taken from (cite spießer 2012 here!!!). (A) Cell volume vs. time(min) of cultures simulated for different parameter values of k_{growth}, mean cell size and standard deviation plotted. (B) Volume distribution at the end of simulations in (A). For high values of k_{growth} average cell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

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.

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)
Π_t	Tugor pressure
c_i	Inner osmolyte concentration

parameter	**decription**	**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	$3.0 \cdot 10^{-16}$	$arb.unit$

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

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

3.3 Main results

Chapter 4

The merged model

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 an autocatalytic process. In the VGM cell growth is a result of thermodynamic properties of the cell and its surrounding, independent of biomass . Both approaches are in conflict with each other, unless complicated (and mostly hypothetical) feedback between biomass and cell volume is introduced. To avoid this, a new volume dependent biomass is needed.

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 become 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 $B = k_D \cdot V$ the osmolyte consumption $\dot{n}_{main}$ reads

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

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 k_D 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

$$\dot{B} = k_D \cdot \dot{V} \tag{4.1}$$

The cost of this amounts to

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

The complete equation for the osmolyte balance is

$$\dot{c} = \frac{\dot{n}_{up}}{V} - \frac{\dot{n}_{main}}{V} - \frac{\dot{n}_{cost}}{V} - \dot{c}_{dilution} \tag{4.2}$$

$$= k_{up} \cdot \frac{A}{V} - k_{main} - (k_{cost} \cdot k_D - c) \cdot \frac{\dot{V}}{V} \tag{4.3}$$

$$\tag{4.4}$$

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*.