Morphologically-structured models of growing budding yeast populations

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Abstract

It has been well recognized that many key aspects of cell cycle regulation are encoded into the size distributions of growing budding yeast populations due to the tight coupling between cell growth and cell division present in this organism. Several attempts have been made to model the cell size distribution of growing yeast populations in order to obtain insight on the underlying control mechanisms, but most were based on the age structure of asymmetrically dividing populations. Here we propose a new framework that couples a morphologically-structured representation of the population with population balance theory to formulate a dynamic model for the size distribution of growing yeast populations. An advantage of the presented framework is that it allows derivation of simpler models that are directly identifiable from experiments. We show how such models can be derived from the general framework and demonstrate their utility in analyzing yeast population data. Finally, by employing a recently proposed numerical scheme, we proceed to integrate numerically the full distributed model to provide predictions of dynamics of the cell size structure of growing yeast populations.

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1. Introduction

Model organisms and in particular the budding yeast Saccharomyces cerevisiae have been instrumental in advancing our understanding of cell cycle regulation. The interplay of complex molecular control mechanisms provides coordination between cell growth and division which is reflected unmistakably in the maintenance of size homeostasis or constancy in cell size. Because of the tight coupling between cell growth and division, study of the cell size distribution of yeast populations at steady state or under perturbed conditions can reveal a wealth of information on the cell cycle regulatory mechanisms. Exploration of cell cycle control mechanisms in yeast based on cell size started with the pioneering studies of Hartwell et al. (1974) using a set
of temperature-sensitive cell cycle mutants while in a recent landmark study Jorgensen et al. (2002) systematically probed the complete set of ~6000 *S. cerevisiae* gene deletion strains to identify about 500 abnormal cell size mutants that helped to elucidate novel pathways involved in the coupling of cell growth and cell division in yeast.

The asymmetric division of the budding yeast and the tight coupling between cell growth and division has challenged the theoretical understanding of the cell size structure of growing yeast populations. Past efforts have centered on modeling the steady-state theoretical age distribution for asymmetric division from which a cell size distribution can be derived assuming dispersion of cell size within each age class (Martegani et al., 1984; Mariani et al., 1986). Here we propose a new rigorous framework for modeling directly the dynamics of size distributions of structured yeast populations. An advantage over simulation-based approaches is that the present framework allows derivation of simpler models through direct analytical manipulations and it also readily extends to modeling of more complex conditions, such as transient growth. Before formulating the model, we will first review some background on cell size control in yeast.

2. Budding yeast cell cycle

Cell growth and division in budding yeast are tightly coupled to ensure size homeostasis during proliferative growth. In *S. cerevisiae* the major point of coordination

![Figures A and B showing the cell cycle of budding yeast](image)
of cell growth and division occurs at “Start” (Fig. 1A), where cells commit to bud emergence and DNA replication only after they reach a critical size (Hartwell et al., 1974; Johnston et al., 1977). The critical size at Start increases in proportion to cell ploidy level and nutrient status (Reed, 1995). Although the molecular mechanisms of cell size control have not been adequately elucidated yet, the timing of Start relative to cell growth rate, both being affected by nutrient conditions, determine the duration of the pre-Start phase and the average cell size of a yeast population. The conventional pathway for the molecular regulation of Start involves activation of the cyclin-dependent protein kinase (CDK) Cdc28 and cyclin 3 (Cdc28/Cln3) complex (Reed, 1995), although a recent study using the complete set of gene deletion strains identified 40 previously-unknown components that also control Start, many of which appear to be involved in ribosome biogenesis (Jorgensen et al., 2002).

Cell growth in the pre-Start (G1) phase is typically uniform and isotropic, however after bud initiation growth becomes localized to the bud and vectorial, adding new cell wall material primarily to the distal end of the bud (Harold, 1990). Newborn cells are typically smaller than their mothers, especially under poor nutrient conditions, and therefore need a longer pre-Start growth phase before they can reach the critical size (Lord and Wheals, 1980) (Fig. 1B). However, the duration of the budded phase appears to be the same for newborn or mother cells (Lord and Wheals, 1983; Vanoni et al., 1983). The above observations form the basis of the morphologically-structured modeling framework that follows.

3. Morphologically-structured modeling framework

Motivated to explain the structural heterogeneity of cell size distributions of growing yeast populations, Vanoni et al. (1983) proposed a modeling framework that integrates most of the known morphological observations occurring in the yeast cell cycle. Unlike prior models of unequal division in yeast that accounted only for two morphologically-distinct sub-populations, daughter and mother cells (Hartwell and Unger, 1977), the new framework accounted for several morphologically-distinct and experimentally observ-
The morphologically-structured modeling framework proposed by Vanoni et al. is depicted schematically in Fig. 3. Specifically, the following assumptions define the framework: (1) the single-cell growth rate or protein synthesis rate is deterministic, i.e. all the cells in the population synthesize protein at the same specific rate; (2) the specific protein synthesis rate of individual cells is equal to the specific growth rate of the overall population; (3) bud initiation and DNA replication occurs when cells reach a critical cell size at Start; (4) the critical size at Start increases with genealogical age of parents; (5) at any given specific growth rate, the duration of the budded period is the same for all daughter and parent cells of all genealogical ages; and (6) all the mass synthesized in the budded phase goes to the newborn daughter cell at division. Assumptions (5) and (1) imply the existence of a critical cell size for division which is proportional to the critical size at Start and assumption (4) implies that this critical size at division increases with the genealogical age of parents. As shown in Fig. 3, smaller parent cells produce smaller daughters that require a longer time to reach critical size $P^0_m$ prior to bud initiation. The size of parent cells in each new generation increases because the critical sizes also increase and these parents produce larger daughter cells which in turn have shorter pre-Start phases and therefore shorter cell cycle durations.

The above structured growth model has been applied successfully to simulate protein distributions from
yeast populations essentially by convoluting the theoretical age distribution of the population derived from the model with a Gaussian kernel to account for the variability in the protein content of cells of a given age (Alberghina et al., 1983; Martegani et al., 1984; Mariani et al., 1986). Next, we present a more comprehensive modeling framework that models directly the size distribution of growing yeast populations by utilizing the rich structure provided by the two-threshold morphologically-structured model for yeast growth and the population balance theory.

4. Multistaged population model of yeast growth

The population balance theory (PBT) provides a natural statistical framework for describing the dynamics of growing populations (Fredrickson et al., 1967; Ramkrishna, 1979, 1985). Unlike continuum models that view microbial populations as homogeneous and “biomass” as an extensive property of the population, PBT acknowledges the segregated or corpuscular nature of microbial populations, i.e. that populations consist of morphologically and physiologically distinct cells of varying subcellular structure and composition. The physiological state of each cell is specified by the physiological state vector, which is a collection of state properties such as total mass, protein content, DNA content or the level of a specific mRNA transcript. The PBT framework supplies equations that describe changes in the joint distribution of the physiological state vector of cells in the population in relation to growth events and environment changes.

Hatzis et al. (1995) later extended the original framework to accommodate the temporal structure that is inherent in growth processes. They considered growth events within each of a sequence of contiguous stages and inter-stage transitions controlled by checkpoints that are functions of the physiological state of the cells. This extended framework forms the basis of our approach for describing the dynamic course of protein distributions during the yeast cell cycle, which effectively amounts to overlaying the above multistaged population balance framework onto the morphologically-structured yeast cell cycle framework of Vanoni et al. (1983).

An equivalent version of the morphologically-structured yeast cell cycle model of Fig. 3 that is compatible with the multistaged population balance framework of Hatzis et al. (1995) is shown in Fig. 4. The model considers $2k_{\text{max}} + 1$ morphologically-distinct classes based on cell budding status and genealogical age (number of bud scars), where $k_{\text{max}}$ is the maximum number of parent generations considered. Here we will address the one-dimensional case where the physiological state within each stage is fully specified by cell mass or total protein (both are used interchangeably). Cell mass increases due to growth within each stage and the probability that cells proceed to the next stage is a function of the corresponding critical size.

We now consider the number density function of cells with mass in $(m, m + dm)$, $f(m, t)$, which is defined such as the total number of cells at time $t$ is:

$$n(t) = \int_{V} f(m, t) \, dm.$$  

(1)

Population balance equations for each morphological class are then formulated in terms of the corresponding number density functions directly from the model of Fig. 4. Two stages are recognized, unbudded ($U$) and budded ($B$), within each genealogical class. Daughter cells ($D$) have genealogical age 0 (no bud scars) and parents of genealogical age $k$ are denoted as $P_k$. At division, daughter cells produce a newborn daughter ($D_U$) and a first generation parent cell ($P^1_U$), whereas a parent cell of genealogical age $k$ produces a newborn daughter cells ($D_U$) and a next generation parent ($P^k_U$). The transitions to budded phase and division are controlled by the indicated critical sizes, which increase with genealogical age.
Fig. 4 and the above-stated assumptions (see Fig. 4 for explanation of notation):

\[
\frac{\partial f_{\text{UB}}(m, t)}{\partial t} + \frac{\partial}{\partial m} \left[ \gamma_k(m) f_{\text{UB}}(m, t) \right]
= -\left[ \gamma_k(m) + D \right] f_{\text{UB}}(m, t)
+ \int_{m}^{\infty} \gamma_k(m') P_{\text{UB}}(m, m') f_{\text{UB}}(m', t) \, dm'
+ \sum_{k=1}^{3} \int_{m}^{\infty} \gamma_k(m') P_{\text{UB}}(m, m') f_{\text{UB}}(m', t) \, dm'
\]

(2)

\[
\frac{\partial f_{\text{UB}}(m, t)}{\partial t} + \frac{\partial}{\partial m} \left[ \gamma_k(m) f_{\text{UB}}(m, t) \right]
= \gamma_k(m) f_{\text{UB}}(m, t) - \left[ \gamma_k(m) + D \right] f_{\text{UB}}(m, t)
\]

(3)

\[
\frac{\partial^2 f_{\text{UB}}(m, t)}{\partial t^2} + \frac{\partial}{\partial m} \left[ \gamma_k(m) f_{\text{UB}}(m, t) \right]
= -\left[ \gamma_k(m) + D \right] f_{\text{UB}}(m, t)
+ \int_{m}^{\infty} \gamma_k(m') P_{\text{UB}}(m, m') f_{\text{UB}}(m', t) \, dm'
+ (1 - \delta_{k,1}) \int_{m}^{\infty} \gamma_k(m') P_{\text{UB}}(m, m') f_{\text{UB}}(m', t) \, dm'
\]

\times (m', t) \, dm' + \delta_{k,1} \int_{m}^{\infty} \gamma_k(m') P_{\text{UB}}(m, m') f_{\text{UB}}(m', t) \, dm'
\]

\times (m', t) \, dm' + \delta_{k,1} \int_{m}^{\infty} \gamma_k(m') P_{\text{UB}}(m, m') f_{\text{UB}}(m', t) \, dm'
\]

(4)

\[
\frac{\partial f_{\text{UB}}(m, t)}{\partial t} + \frac{\partial}{\partial m} \left[ \gamma_k(m) f_{\text{UB}}(m, t) \right]
= \gamma_k(m) f_{\text{UB}}(m, t) - \left[ \gamma_k(m) + D \right] f_{\text{UB}}(m, t)
\]

(5)

where \( \sigma(m) \) is the time-specific transition probability, or transition rate, through Start, i.e. \( \sigma(m) \, dm \) is the probability that an unbudded cell of mass \( m \) will enter Start in the next \( dm \) time units. \( \gamma(m) \) similarly the transition rate through division, \( r(m) \) the single-cell growth rate within a morphological class, i.e. \( dm \cdot r(m) \, dt \) is the rate of increase of cell mass in \( dt \) time units, \( D \) the dilution rate of an open system, \( P(m, m') \), also known as the partitioning function, is the conditional density function of the size of newborn cells given the size of its parent, \( m' \) and \( \delta_{k,1} \) is the Kronecker’s delta.

The left-hand side of the balance equations is the total derivative of \( f(m, t) \) w.r.t. time that accounts for changes in cell mass due to cell growth within each stage, while the right-hand side terms account for contributions to the number density from inter-stage transitions. Considering the RHS of Eqs. (3) and (5) for budded daughters and parents, the first term reflects the increase in the number of budded cells of mass \( m \) from unbudded cells of the same mass traversing Start, and the second term accounts for cells exiting the budded phase, i.e. those that traverse the mitosis checkpoint heading to cell division. The balances for the unbudded classes are more complicated as they include the contributions from dividing cells. As above, the first term in the RHS of Eq. (2) accounts for loss of unbudded daughters due to their traversing Start. The next term accounts for newborn daughter cells of mass \( m \) created from dividing budded daughter cells, and the third term accounts for newborn daughters created from dividing parents of all genealogical ages. Similarly, the second term in the RHS of Eq. (4) is the increase in first generation unbudded parents created from the division of budded daughter cells, the next term represents contributions to unbudded parents from budded parents of the previous generation, and the last term is needed for closure since \( \kappa_{\text{max}} \) is finite, typically less than 25 for yeast (Iazwinski, 1990).

Eqs. (2)–(5) constitute a system of coupled integro-differential equations for the number density functions within each morphologically-distinct stage. Previous studies have reported (Vanoni et al., 1986; Mariani et al., 1986) that during exponential growth the fraction of first-generation parents is equal to the sum of parents of all subsequent generations and that parents with five or more bud scars represent only about 3% of the total population. Therefore considering three parent generations by setting \( \kappa_{\text{max}} = 3 \) and equivalently \( P_{\text{UB}}(m, m') = P_{\text{UB}}(m, m') \) for \( k \geq 4 \), will capture a significant portion of the population structure. In this case, the model consists of eight coupled equations, the solution to which will provide the time-dependent traces of the total cell numbers and of the cell protein distributions within each morphological stage. For the model to be complete, we need to specify the unknown functions of the state vector, i.e. the growth rates, transition rates and partitioning functions for each stage, which constitute the critical link between the number balances and the underlying cell-cycle model. Furthermore, we need to specify the regularity conditions, which define the boundaries of the physiological state space (Fredrickson et al., 1967),
as following:

\[ r_{DU}(0, t) f_{DU}(0, t) = r_{DU}(\infty, t) f_{DU}(\infty, t) = 0 \quad \text{for all } t. \]  

(6)

This expression states that unbudded daughter cells of zero mass cannot grow and that cells of infinite mass cannot exist at any time and analogous regularity conditions apply to the other stages. Finally appropriate initial conditions that specify the state of the population at time \( t = 0 \) need to be provided. However, before attempting to solve the full model we will first consider some special cases of the full model that provide additional insight on the model’s predictions.

5. Non-distributed model of yeast growth

5.1. Non-distributed morphologically-structured model

Simpler models are useful for analyzing experimental data by relating the observed population fractions in the different morphological stages to the durations of stages (Barford and Hall, 1976; Lord and Wheals, 1980; Grover and Woldringh, 1995). Such models can be derived from the general model of Eqs. (2)–(6) through appropriate averaging on the number density functions. Integration of Eqs. (2)–(5) over the entire range of cell mass and using the definition of Eq. (1) and the regularity conditions of Eq. (6) yields the following equations for the total number of cells in each stage:

\[
\frac{dN_{DU}(t)}{dt} = -(\sigma_D + D)N_{DU}(t) + \gamma_D N_{DB}(t) + \sum_{k=1}^{k_{\text{max}}} \gamma_k N_{PB}(t),
\]  

(7)

\[
\frac{dN_{DB}(t)}{dt} = \sigma_D N_{DU}(t) - (\gamma_D + D)N_{DB}(t),
\]  

(8)

\[
\frac{dN_{PB}(t)}{dt} = \sigma_D N_{PU}(t) - (\gamma_P + D)N_{PB}(t) + \delta_K \bar{\gamma}_P N_{PB}(t),
\]  

(9)

This model consists of \( 2(k_{\text{max}} + 1) \) ordinary differential equations that can be integrated easily once the \( 2(k_{\text{max}} + 1) \) unknown parameters that represent the mass-average transition rates for each stage are specified. Predictions of the above model under batch growth \( (D = 0) \) for \( k_{\text{max}} = 3 \) starting with 100 unbudded daughter cells at \( t = 0 \) and using estimates of the transition rate parameters from experimental observations obtained as will be explained below yields the profiles shown in Fig. 5. For the chosen set of parameters, the population reaches exponential balanced growth after 6h, with the daughter cells representing about \( 60\% \) of the population.

![Fig. 5. Profiles of batch population growth from the non-distributed morphologically-structured model. (A) time course of cell numbers within the eight morphological stages, (B) time profiles of daughter and parent cell populations, (C) time course of daughter and parent cell population fractions.](image-url)
5.2. Exponential balanced growth

The number balance model of Eqs. (7)–(10) for batch growth (D=0) can be written in matrix form as:

\[
\frac{d n}{dt} = An.
\]

where \( n = (N_{U1}, N_{DB}, N_{PHU}, \ldots, N_{PHPB}, N_{PB})^T \) is the vector of cell numbers in each morphological stage and \( A \) is the transition rate matrix for the entire model:

\[
A = \begin{bmatrix}
-\delta_D & \gamma_D & 0 & \cdots & 0 & \gamma_{U1} \\
0 & -\sigma_P & -\delta_P & 0 & \cdots & 0 \\
0 & 0 & -\delta_P & -\sigma_P & \cdots & 0 \\
\vdots & \vdots & \vdots & \vdots & \ddots & \vdots \\
0 & 0 & 0 & 0 & \cdots & -\delta_{PQB} & -\sigma_{PQB} \\
0 & 0 & 0 & 0 & \cdots & 0 & -\delta_{PB} & -\sigma_{PB} \\
\end{bmatrix}
\]

Under exponential balanced growth conditions, the number of cells in each stage increases at the same rate, which is equal to the population’s specific growth rate, and therefore the fractions of the population in each class remain constant (see Fig. 5c). Therefore under these conditions we can try pure exponential solutions of Eq. (11) of the form

\[
e^{\mu t} = e^{\mu t}.
\]

Then Eq. (11) yields

\[
A e^{\mu t} = \mu e^{\mu t}.
\]

Eq. (13) is interesting because it states that the population will achieve stable exponential balanced growth if and only if the transition rate matrix \( A \) has a dominant strictly positive eigenvalue, which is equal to the population’s specific growth rate \( \mu \). Furthermore, the vector of steady-state population fractions \( \phi \) is the eigenvector of \( A \) that corresponds to this eigenvalue. Therefore, it is clear that not all possible choices of transition rates will lead to exponential balanced growth according to this model. A sketch of the proof for the conditions of existence of a positive eigenvalue of matrix \( A \) is outlined in Appendix A.

An additional implication is that the population’s specific growth rate \( \mu \) is uniquely determined by the inter-stage transition rates. Eq. (13) provides the means for computing \( \mu \) as the dominant positive eigenvalue of transition matrix \( A \). Alternatively, the specific growth rate can be calculated from the balanced-growth population fractions from the following expression that follows from Eq. (13) and the normalization condition \( e^T \phi = 1 \), where \( e \) is a vector of 1’s:

\[
\mu = e^T A e = \gamma_{U1} \phi_1 + \sum_{k=1}^{K} \phi_{PB} b_k.
\]

Notice that the transition rates at division and the age-specific budding indices determine \( \mu \). The inter-stage transition rates in turn affect the steady-state fractions in the different stages.

5.3. The inverse problem

Frequently one measures the stable population fractions experimentally under exponential balanced growth conditions (D=0) and use these to derive estimates of the transition rates, which cannot be measured directly. The so-called inverse relationships for this model can be derived from Eq. (13) as follows. The product \( A \phi \) can be expressed as a linear combination of the columns of \( A \) and since a given transition rate multiplies each column of \( A \), the product can be expressed equivalently by switching the role of transition rates and population fractions. Specifically,

\[
A \phi = \sum_{j=1}^K \phi_j a_j = \sum_{j=1}^K \phi_j r_j b_j = \sum_{j=1}^K \phi_j r_j b_j = \sum_{j=1}^K \phi_j r_j = \Phi r.
\]

where \( a_j \) is the \( j \)th column of \( A \), \( b_j \) the column vector \( a_j \) divided by the corresponding transition rate \( r_j \), \( \Phi \) is
the matrix of population fractions that has the same structure as \( A \) except that fractions \( \psi_j \) replace rates \( r_j \), \( \psi = (\psi_{DU}, \psi_{DB}, \psi_{DU}, \psi_{DB}, \psi_{PU}, \psi_{PB}, \psi_{DU}, \psi_{DB}, \psi_{PU}, \psi_{PB}, \psi_{DU}, \psi_{DB}, \psi_{PU}, \psi_{PB})^\top \), \( r = (\sigma_1, \gamma_1, \sigma_2, \gamma_2, \sigma_3, \gamma_3, \sigma_4, \gamma_4)^\top \), and \( k = 2(k_{\text{max}} + 1) \). Matrix \( \Phi \) is non-singular provided that \( \psi \) is element-wise positive and from Eqs. (13) and (15) it follows that \( r = \mu \Phi^{-1} \psi \).

Eq. (16) allows the direct estimation of the model’s transition rates from the exponential specific growth rate and the balanced-growth fractions of the population in each class, all of which can be measured experimentally.

**Example 1.** Estimation of transition rates from balanced growth population fractions.

In Section 5.1 the complete model for \( k_{\text{max}} = 3 \) was integrated numerically to produce the traces shown in Fig. 5. We will derive here the equations used to estimate the eight transition rates from the steady-state population fractions in the eight morphological classes of the model.

Direct application of Eq. (16), with \( \Phi \) having the same structure as matrix \( A \) in Eq. (12) but with the rates being replaced by the steady-state fractions, as described above, yields:

\[
T = \frac{1}{\mu} T_p (1 - \psi_{DU})/\psi_{DU} \quad (1 - \psi_{DU} - \psi_{DB})/\psi_{DB} \quad (1 - \psi_{DU} - \psi_{DB} - \psi_{PU})/\psi_{PU} \\
(1 - \psi_{DU} - \psi_{DB})/\psi_{DB} \quad (\psi_{DU} + \psi_{DB} + \psi_{PU}/\psi_{PU}) \quad (\psi_{DU} + \psi_{DB} + \psi_{PU}/\psi_{PU}) \quad (\psi_{DU} + \psi_{DB} + \psi_{PU}/\psi_{PU}) \quad (\psi_{DU} + \psi_{DB} + \psi_{PU}/\psi_{PU}) \quad (\psi_{DU} + \psi_{DB} + \psi_{PU}/\psi_{PU}) \quad (\psi_{DU} + \psi_{DB} + \psi_{PU}/\psi_{PU}) \quad (\psi_{DU} + \psi_{DB} + \psi_{PU}/\psi_{PU}) \quad (\psi_{DU} + \psi_{DB} + \psi_{PU}/\psi_{PU}) \quad (\psi_{DU} + \psi_{DB} + \psi_{PU}/\psi_{PU}) \quad (\psi_{DU} + \psi_{DB} + \psi_{PU}/\psi_{PU}) \quad (\psi_{DU} + \psi_{DB} + \psi_{PU}/\psi_{PU}) \quad (\psi_{DU} + \psi_{DB} + \psi_{PU}/\psi_{PU}) \quad (\psi_{DU} + \psi_{DB} + \psi_{PU}/\psi_{PU}) \quad (\psi_{DU} + \psi_{DB} + \psi_{PU}/\psi_{PU})
\]

where as before, \( r = (\sigma_1, \gamma_1, \sigma_2, \gamma_2, \sigma_3, \gamma_3, \sigma_4, \gamma_4)^\top \).

Experimental measurements from an exponentially growing yeast population with \( \mu = 0.264 \) h\(^{-1}\) provided the following estimates for the steady-state fractions \( \Phi = (0.405, 0.177, 0.08, 0.13, 0.04, 0.07, 0.03, 0.067)^\top \), and substitution in Eq. (17) yields \( r = (0.388, 0.620, 1.115, 0.422, 1.109, 0.370, 2.921, 1.088)^\top \) h\(^{-1}\). These transition rates were used in the simulation shown in Fig. 5. We verified that matrix \( A \) with the above transition rate estimates has a single, distinct positive eigenvalue which is equal to the population’s specific growth rate.

**5.4. Derivation of structured submodels**

Although in recent years considerable progress has been made in instrumentation and methods for measuring state properties and morphological characteristics of yeast populations (Porro and Stienicke, 1995; Porro et al., 1995, 1997), in many cases limited observations might be available for analysis. For example, one might be able to track only two morphologically-distinct classes of cells, daughters and parents. The model of Eqs. (11) and (12) is too detailed for this purpose and therefore it would be desirable to have simpler models with the right structure for the given data.

Consider a \( p \times k \) aggregation matrix \( T_S \) that maps the original \( k \) morphological classes to \( p \) new classes \((p \leq k)\). For example, the aggregation matrix

\[
T_S = \begin{pmatrix}
1 & 0 & 1 & 0 & \ldots & 0 & 0
\end{pmatrix}
\]

maps the \( 2(k_{\text{max}} + 1) \)-dimensional vector \( \mathbf{n} \) to the two-dimensional vector \( \mathbf{n}_S \) of cell numbers in unbudded and budded classes:

\[
T_S \mathbf{n} = \begin{pmatrix}
N_{DU} + N_{DB} + \cdots + N_{P_{\text{max}}}
N_{DU} + N_{DB} + \cdots + N_{P_{\text{max}}}
\end{pmatrix}
\]

By definition of the aggregation matrix, it follows that

\[
T_S \mathbf{r} = \mathbf{r}_S
\]
where \( \mathbf{e} \) is a vector of 1's of the indicated dimension. The balance equations for the simpler, aggregated model can be derived from Eq. (11) using properties of vector \( \mathbf{n} \), and application of Eq. (16) provides the inverse relationships between transition rates and balanced growth population fractions:

\[
\begin{pmatrix}
\sigma_P \\
\gamma_D \\
\sigma_D \\
\gamma_P
\end{pmatrix} =
\begin{pmatrix}
(1 - \psi_{DU}/\psi_{DB}) \\
(1 - \psi_{PU}/\psi_{PB})/\psi_{PB} \\
(1 - \psi_{PU}/\psi_{PB}) \\
(\psi_{PU} + \psi_{PB})/\psi_{PB}
\end{pmatrix}
\]

The corresponding transition matrix for the aggregate model can be derived from Eq. (20):

\[
\mathbf{A}_S =
\begin{pmatrix}
\sigma_D & \gamma_D & 0 & 0 \\
0 & \sigma_D - \gamma_D & 0 & 0 \\
0 & 0 & \sigma_P & -\gamma_P
\end{pmatrix}
\]

and estimation of transition rates of the aggregated submodel. In the above derivation we also used the identities \( \mathbf{n} = \text{diag}(\mathbf{n}_U \mathbf{n}_B) \) and \( \mathbf{n}_U = \text{diag}(\mathbf{n}_{U1} \mathbf{n}_{U2}) \), where \( \text{diag}(\mathbf{n}) \) is the square matrix that has the elements of vector \( \mathbf{n} \) on its diagonal and zeros anywhere else. Finally, we can easily derive expressions similar to Eqs. (14) and (16) for the exponential growth rate and estimation of transition rates of the aggregated model.

**Example 2.** Yeast population model with unbudded and budded daughters and parents.

Lord and Wheals (1980) considered an age model to estimate the duration of the daughter cell cycle time \( (D) \), parent cell cycle time \( (P) \) and length of budded period \( (B) \) from experimental estimates of the fractions in the four different classes. To demonstrate the submodel approach, we will extract from the full model a four-class submodel with the same structure as that considered by Lord and Wheals. The following aggregation matrix:

\[
\mathbf{T}_S =
\begin{pmatrix}
1 & 0 & 0 & \cdots & 0 \\
0 & 1 & 0 & \cdots & 0 \\
0 & 0 & 1 & \cdots & 1 \\
0 & 0 & 0 & \cdots & 1
\end{pmatrix}
\]

maps the \( 2(k_{\text{max}} + 1) \) classes of the original model to the four classes of the aggregated model:

\[
\begin{pmatrix}
N_{UB} \\
N_{UB} \\
N_{UB} + \ldots + N_{UB}^{p_{m+1}} \\
N_{UB} + \ldots + N_{UB}^{p_{m+2}}
\end{pmatrix} =
\begin{pmatrix}
N_{UB} \\
N_{UB} \\
N_{UB} \\
N_{UB}
\end{pmatrix} = \mathbf{n}_S.
\]

The fractions of the population in the four different classes were measured by Lord and Wheals (1980; Table 1) in batch cultures growing at different growth rates and Eq. (21) was used to estimate the transition rates as a function of the growth rate; Lord and Wheals (1980) used expressions that they derived from the ideal cell age distribution to estimate the duration of the different phases. Here, assuming random transitions, i.e. exponential age distributions within each stage, we estimate the average duration of each phase as the inverse of the corresponding transition rates given by Eq. (21).

Fig. 6 shows the resulting trends, which agree very closely with those of Lord and Wheals (1980; Fig. 1). It is very clear that the duration of the unbudded phase of the daughter cells increases linearly with the population’s doubling time and it becomes the longest phase at slower growth rates, suggesting that the daughter cells produced under slow growth conditions are considerably smaller and therefore need a longer time to grow to Start. A similar effect is observed for the duration of the unbudded phase of the parents, albeit considerably less pronounced. Interestingly, the duration of the budded phase for both daughter and parents is independent of the doubling time, although for this data the linear regression line for the parents has a non-trivial slope (\( p \)-value = 0.002). On the other hand, Fig. 6B shows that only the transition rates at Start increase in proportion to the population’s growth rate, revealing the strong coupling that exists between cell growth and cell division.
size control at Start. As expected, the transition rate of the newborn daughter cells controls the population’s growth at slow growth rates, whereas at higher growth rates the transition rate through division becomes the rate limiting step, due to the relative constant duration of the budded phase.

6. Mass-distributed yeast population model

We can now consider the full, mass-distributed model for growth of yeast populations described in Section 4. In order for the model to be complete, we need to specify functional forms for the growth rates, transition rates and partitioning functions, which are all functions of cell mass and are directly linked to the size-control hypotheses of the model.

6.1. Growth rate function

The growth rate function describes the rate of increase in the mass of individual cells within each morphologically-distinct stage of the cell cycle. Growth kinetics can be described either by zero order (linear growth) or first order (exponential growth) kinetics. Hartwell and Unger (1977) assumed that individual yeast cells increase their masses exponentially throughout the entire cell cycle and equal to the overall population (number) specific growth rate, under exponential balanced growth. Tyson et al. (1979) modeled the dependence of cell volume on growth rate based on the same assumption of exponential growth, and later measurements of the cell volume of individual cells provided evidence in support of this (Woldringh et al., 1993). Similarly, Vanoni et al. (1983) also assumed first-order kinetics for the protein content of individual cells, with the rate constant being equal to the specific growth rate (Mariani et al., 1986). Recently, using a double-tagging flow cytometric technique that allows tracking both the cell age and protein contents of individual cells (Porro and Srienc, 1995), Porro et al. (1995) demonstrated convincingly that individual cell protein content increases exponentially during the cell cycle with a rate constant that is the same for both newborn daughters and parents. Furthermore, under balanced growth conditions, this rate constant is the same as the population’s specific growth rate, thus validating the assumptions of Vanoni et al. (1983) and Mariani et al. (1986).

Considering the experimental evidence, we assume first-order kinetics for individual cell mass increase within each stage, i.e. \( r_s(m) = k_s m \), for all stages \( s \), with the rate constants being of the same order of magnitude as the population’s specific growth rate.
6.2. Transition rate functions

The transition rate function for inter-stage transitions specifies the probability that a cell crosses the control point per unit time. For the threshold model presented here, this probability is obviously a function of the critical sizes at Start and division. Wheals (1982) established the size distribution of daughter cells at bud emergence, which had a coefficient of variation (CV) of 25% and approximately normal shape. More recently, Porro et al. (1997) measured the protein distributions of dividing daughter and parent cells by flow cytometry with corresponding CVs of 18 and 27%, respectively. The emergence, which had a coefficient of variation (CV) of 25% and approximately normal shape. More recently, Porro et al. (1997) measured the protein distributions of dividing daughter and parent cells by flow cytometry with corresponding CVs of 18 and 27%, respectively.

The transition rate function \( \lambda(m) \) is linked to the size of the cells at transition by the following relationship (Hatzis et al., 1995):

\[
\lambda(m) = \frac{r(m)h(m)}{\Gamma - \int_0^m H(x)dx},
\]

where \( h(m) \) is the probability density function of the size of cells making the transition, and \( r(m) \) is the single-cell growth rate. Assuming a normal size distribution at transition, the transition rate function becomes:

\[
\lambda^N(m) = \frac{r(m)N(m - P^*)/\sigma_\mu}{1 - \Phi(m - P^*/\sigma_\mu)},
\]

where \( P^* \) is the critical size at transition, \( \sigma_\mu \) is its standard deviation, and \( N(x), \Phi(x) \) are the standard normal probability density and distribution functions. An alternative that offers more flexibility and is computationally more advantageous is to assume a Weibull distribution for the size at transition (Hatzis et al., 1995). In this case, the transition rate function

\[
\lambda^W(m) = \frac{r(m)\alpha}{\beta} m^{\alpha-1},
\]

where \( \alpha, \beta \) are the shape and scale parameters of the distribution. The average size at transition is equal to \( \beta \Gamma(1 + 1/\alpha) \) and the CV of the distribution is \( \sqrt{\Gamma(1 + 2/\alpha)/\Gamma(1 + 1/\alpha^2) - 1} \), where \( \Gamma(x) \) is the gamma function. The shape parameter is adjusted to give the desired CV at transition and then the critical size at transition determines the scale parameter. As shown in Fig. 7, although the shapes of the distributions are almost identical, the transition rate based on the Weibull distribution shows a steeper increase as cells grow beyond the critical size. We used the Weibull functional form in the simulations.

6.3. Partitioning function

The partitioning function describes the probabilistic law of cell mass segregation at division. More specifically, if the random variables \( X, Y, Z \) denote the cell size of budded cells at division, newborn unbudded parent cells, and newborn daughter cells, respectively, the partitioning functions are defined as the conditional probability density functions of \( X \) or \( Y \) given \( Z \). These density functions will either have to be measured directly or modeled based on the assumptions of the postulated cell-cycle model. Assuming that no mass is lost a division, then \( Z = X + Y \), and because all the mass synthesized in the budded phase goes to the bud, \( Y \) is equal to the cell mass at Start. Therefore assuming first-order protein synthesis in the budded phase, \( Z = Ve^{\beta T} \), where \( T_B \) is the duration of the budded phase.

The partitioning function can be expressed in terms of the size distributions of cells at transition and of the distribution of the length of the budded phase that can be measured experimentally or modeled based on simpler assumptions. From Bayes’ theorem the partitioning function of dividing daughters to parents of the next generation can be expressed as

\[
P_{DP}(m, m') = \frac{f_ZZ(m|m') f_Y/Y(m|m') f_X/X(m)}{\int f_ZZ(m'|m) f_Y/Y(m'|m') f_X/X(m') dm'}.
\]
The cell partitioning function is defined as the conditional density function of the cell size of the newborn daughter \( X \) or of the newborn parent \( Y \) given the size of the dividing parent cell \( Z \). Due to the assumptions inherent in the cell-cycle model, the four random variables that describe the yeast cell cycle \( X, Y, Z \) and \( T_B \) are not statistically independent. For example, mass conservation at division implies that \( Z = X + Y \).

Furthermore, assuming first-order kinetics and since all the mass synthesized during the budded phase goes to the bud, \( Z = Ye^{kT_B} \). And since \( Z \) and \( T_B \) are related by the exponential growth law as explained above, a simple transformation expresses the distribution of \( Z \) in terms of that of \( T_B \) as following:

\[
f_{Z|Y}(m'|m) = \frac{1}{km'} f_{T_B} \left( \frac{1}{k} \ln \left( \frac{m'}{m} \right) \right).
\]

Finally, from the above two relationships we get the final expression for the partitioning function of dividing daughter cells to first generation parents:

\[
P_{DP}(m, m') = \frac{1}{km' f_T(m')} f_Y(m) f_{T_B} \left( \frac{1}{k} \ln \left( \frac{m'}{m} \right) \right).
\]

(25)

and the partitioning function of dividing daughter cells to newborn daughters can be expressed as

\[
P_{DD}(m, m') = f_{X|Z}(m|m') = f_Y(m' - m|m') = P_{DP}(m' - m, m').
\]

(26)

Similar expressions can be derived to describe the partitioning of the mass of dividing parents to newborn daughters and parents of the next generation.

The concept of the partitioning function and its specification is rather difficult as it involves distributions of correlated random variables. Thus it is important to understand the relationships between the involved random variables and their distributions. Since the duration of the budded phase is practically independent of the size of daughter (or parent) cells, \( T_B \) and \( Y \) are marginally independent (as are \( T_B \) and \( Z \)). Marginal independence implies that the distribution of \( T_B \) and that of \( Y \), which are required in Eq. (25) to specify the partitioning function, can be measured independently. However, \( T_B \) and \( Y \) are conditionally dependent given \( Z \), since for a given \( Z \) specification of \( Y \) determines \( T_B \) and vice versa. Furthermore, \( Y \) and \( Z \) are neither conditionally nor marginally independent and description of their relationship requires specification of their joint distribution. An important implication related to Eq. (25) is that if the distributions of \( T_B \) and \( Y \) are known, the distribution of \( Z \) cannot be specified independently, since \( T_B \) and \( Z \) are conditionally dependent given \( Y \). Instead, the distribution of \( Z \) is determined from the marginal distributions of \( T_B \) and \( Y \) as following:

\[
f_Z(z) = \int f_{Z|Y}(z|y) f_Y(y) dy
\]

\[
= \frac{1}{k} \int f_{T_B} \left( \frac{1}{k} \ln \left( \frac{z}{y} \right) \right) f_Y(y) dy.
\]

Therefore, complete specification of the partitioning function requires the size distribution at Start and the distribution of the duration of the budded phase, both of which can be determined experimentally. Combination of the above expression and Eq. (25) finally yields:

\[
P_{DP}(m, m') = \frac{f_{T_B}(1/k) \ln(m'/m) f_Y(m)}{\int f_{T_B}(1/k) \ln(m'/y) f_Y(y) dy}.
\]

(27)

Determining the distribution of \( T_B \) would require following a large enough cohort of newborn parent cells
from Start to division. Alternatively, the joint distribution of \( Y \) and \( Z \) could be measured by flow cytometry and this joint density then directly specifies the partitioning function (and the distribution of \( T_B \)). Due to lack of direct experimental data here we have assumed that \( Y \) and \( Z \) are jointly distributed according to a bivariate normal distribution. Then, conditionally on \( Z \), \( Y \) is normally distributed with mean and variance

\[
E[Y|Z = z] = \mu_Y + \rho_{YZ} \left( \frac{\sigma_Y}{\sigma_Z} \right) (z - \mu_Z),
\]

\[
V[Y|Z = z] = \sigma_Y^2 (1 - \rho_{YZ}^2),
\]

and following the arguments outlined above we can show with a little effort that \( \rho_{YZ} = \delta_{Y}/\delta_Z \), where \( \delta \) denotes the CV of the random variable. Then, the partitioning function is given by the following normal density:

\[
P_{DP}(m, m') = N\left( E[Y|m'], \sqrt{V[Y|m']} \right)
\]

The complementary partitioning function for daughters is obtained through Eq. (26). To keep the notation consistent, \( \mu_Y = P_s \) and \( \mu_Z = P_m \).

6.4. Critical sizes as a function of genealogical age

A key feature of the model proposed by Vanoni et al. (1983) is that the critical sizes at Start and division increase with increasing genealogical age of the parent cells. Following Martegani et al. (1984) we assume that the critical cell size of parents increases in each new generation by a fixed amount. More specifically, if \( q \) is the fractional increase in the critical size of first-generation parents relative to daughters, i.e. \( P_1^d - P_1^p = qP_0^d \), and \( a \) is the fractional increase for parents of successive generations, i.e. \( P_k^d - P_k^p = a(P_{k-1}^d - P_{k-1}^p) \), and so on, then

\[
P_k^p = P_0^d \left( 1 + q \sum_{j=0}^{k-1} a^j \right) = P_0^d \left( 1 + q \frac{a^{k+1} - 1}{1 - a} \right),
\]

\( k = 1, 2, \ldots \)

where \( P_0^d \) is the critical size at Start for daughter cells and \( q, a < 1 \). The assumption of a constant budded phase for all cells is equivalent to assuming that the ratio of the two critical sizes is constant, i.e.

\[
h = \frac{P_k^p}{P_k^d} = e^{ku_k}, \quad k = 0, 1, 2, \ldots \]

Fig. 9. Numerical solution of population balance equations. The initial population consisted of 100 unbudded daughter cells (average size = 1.4 ng/cell, CV = 0.2). Additional parameters included: \( P_0^s = 1, \delta_{P_D} = 0.2, 0.15, 0.25, 0.4 \). Integration was performed over a 100-point cell mass grid using a time step of 0.9 s: (a) daughter, parent and total population numbers increase at the same specific exponential growth rate (0.527 h\(^{-1}\)) after an initial lag; (b) fractions of the different subpopulations reach steady-state levels at balanced growth after initial fluctuations; (c) average cell size profiles for the different subpopulations show considerable overlap and reach steady-state values at balanced growth.
Fig. 10. Evolution of the structure of a yeast population from the numerical solution of the population balance model showing the cell size distributions of the component subpopulations. Parameter values were the same as in the previous figure. The size distributions reach steady state after 4 h.
According to this model the average size of daughter cells increases with the genealogical age of the parents, since \( P^m_k - P^s_k = (h - 1)P^s_k \). Therefore in order to ensure that the size of the largest daughter cells will be smaller than the critical size at Start for daughters, i.e., \( P^m_k - P^s_k \leq P^0_s \) for large \( k \), the above constants need to satisfy the following constraint (Mariani et al., 1986):

\[
h \leq \frac{q + 2(1 - \alpha)}{q + 1 - \alpha}.
\]

Mariani et al. (1984) reported from their simulations that values of \( \alpha \) between 0.50 and 0.99 had little effect on the shape of the size distribution and increasing values of \( q \) affect primarily the CV but not the shape of the distribution. The values selected here \((h, q, \alpha) = (1.6, 0.2, 0.5)\) satisfy the stated constraint.

6.5. Numerical solution of population balance model

The population balance equations and boundary conditions Eqs. (2)–(6) together with the functional forms for the growth rates, transition functions and partitioning functions specified above constitute the complete model that can be used to describe the dynamics of cell numbers and cell size distributions within the different, morphologically-distinct yeast subpopulations. The considerable complexity of the model equations necessitates development of specialized techniques for solving the system of equations to obtain the model predictions.

It is possible to derive analytical solutions to the model equations but only for the case of constant substrate concentration, e.g., conditions of early exponential growth, using the successive generations approach of Liou et al. (1997). These solutions are given in Appendix B as they offer insight on the effects of the various factors on the structure of the size distributions of the various subpopulations. For more general models, Hatzis et al. (1995) proposed a Monte-Carlo approach to realize solutions of multi-stage population balance models, which is directly applicable to the model presented here. An advantage of the Monte-Carlo approach is that it automatically extends to more complicated growth situations, such as shift-up experiments and to higher-dimensional state spaces, but a disadvantage is that the computational time required increases with the size of the population.

Numerical integration techniques offer the best compromise between computational demand and model flexibility, although they do not extend easily beyond three-dimensional physiological state spaces. In our case, we applied the numerical integration scheme proposed by Mantzaris et al. (1999), which is a hybrid time-explicit finite difference scheme to solve the model for the constant-substrate case. The hybrid scheme was developed from a combination of the leapfrog and the Lax-Friedrichs schemes to offer better numerical stability and accuracy. The integral terms of the model are evaluated with the Trapezoid rule. Details of the numerical technique and its performance can be found in the original article.

Results from integration of the model for batch growth \((D=0)\) are shown in Fig. 9, where it can be seen that the population reaches balanced growth after 2 h, but the fractions and size of the smaller subpopulations take longer to stabilize. In addition to estimates of the moments of the size distributions, numerical solution of the population balance equations provides the time course of the complete size distribution of the various subpopulations. Typical samples from the evolution of the size distribution are shown in Fig. 10. The initial population distribution is unimodal consisting of unbudded cells and becomes bimodal and even tri-modal before reaching the shape that is typical of exponential protein distributions (see, e.g. Porro et al., 1997). It is intriguing but remains to be validated experimentally whether the size distribution of the population does indeed go through the intricate stages suggested by our model simulations.

7. Conclusions

We have extended the morphologically-structured framework of Vanoni et al. (1983) to model directly the size distributions of growing yeast cell populations. This represents a significant contribution, as the phenomenological assumptions about the cell cycle of the budding yeast are introduced transparently in the model. The framework allows modeling complex
situations involving shift-up or shift-down conditions that can reveal important aspects of the size control mechanisms (Alberghina et al., 1998). Furthermore, although here we considered a mass-structured model, the modeling framework is extendable to more complex descriptions of the yeast cell cycle by expanding the dimensionality of the physiological state representation, e.g. by adding DNA content. An advantage of having a rigorous analytical framework available (as opposed to a simulation algorithm) is that simpler and limiting cases can be derived and evaluated analytically, thus providing additional insight on the effects of the stated assumptions and the values of the parameters involved. We demonstrated this point by deriving expressions that allow estimation of the transition rates through the various morphological classes that provide additional insights on the size regulation mechanism.

The recent availability of stable and accurate methods for the numerical solution of mass-structured population balance models has been a catalyzing development for the use of such models in practice. Continuing developments on this front have produced additional and improved numerical schemes (Mantzaris et al., 2001a,b,c) that will undoubtedly provide additional impetus for complex modeling efforts.

The presented morphologically-structured model can be easily extended to include intracellular or biochemical structure within each morphologically-distinct class. An important extension would be to introduce substrate dependence for the growth rates based, for example, on Monod-like substrate kinetics. Similarly, the mean ratio of the critical sizes \( h \), which is proportional to the length of the budded phase, is known to be lower at very poor nutritional conditions (Alberghina et al., 1991). Such dependency was modeled as a function of substrate levels in Cazzador et al. (1990). In this case the model of Eqs. (2)–(5) will have to be coupled with mass balance equations for the substrates considered (see, e.g. Mantzaris et al., 2001a; Eq. (13)), appropriately formulated to allow for different yield coefficients for each morphological class (see, e.g. Cazzador, 1991) to account for the fact that the cellular metabolism of daughter cells is very different from that of the parent (budded) cells (Strassle et al., 1988). These extended models should be capable of predicting interesting dynamic phenomena such as the sustained oscillations observed in continuous cultures operated under glucose-limited conditions.

As our understanding of the molecular mechanisms of size control in budding yeast improves, we envision to link detailed molecular models of cell cycle control, such the one proposed by Chen et al. (2000, 2004) with our morphological and population balance framework to be able to model directly the assumptions at the molecular level and evaluate their effects on macroscopic, measurable population properties, such as the cell size distribution.

Appendix A. Existence of positive eigenvector of transition matrix

Matrix \( A \) (Eq. (12)) is not symmetric and therefore some of its eigenvalues are complex but since it is real these come in complex conjugate pairs. Furthermore, because \( A \) has \( 2(\max + 1) \), i.e. an even number of eigenvalues, it follows that it has an even number of real eigenvalues and since \( \det(A) < 0 \), the product of the real eigenvalues is negative and therefore \( A \) has at least one or an odd number of strictly positive real eigenvalues.

Ideally, we would like to prove that the dominant eigenvalue of \( A \) is simple and positive and that there exists a corresponding eigenvector with all positive entries representing the population fractions in the different classes under balanced growth conditions. These properties are guaranteed for non-negative, irreducible matrices by the Perron-Frobenius theorem (Meyer, 2000). However \( A \) has negative elements and therefore the theorem cannot be applied directly.

Appendix B. Analytical solution of mass-distributed model for constant substrate

The population balance equations were solved using the method of successive generations of Liou et al. (1997) for constant substrate conditions. The solutions are given below:

A. Unbudded daughter phase

For initial generation \( (n = 0) \):

\[
\frac{f_0(m, t)}{f_0(m, 0)} = f_0(m e^{-\lambda_0 t}) e^{-\rho t} \quad (B.1)
\]
where \( f_0 \) is the initial number density function. For subsequent generations \((n = 1, 2, \ldots)\):

\[
f_{k,n}(m, t) = e^{-\rho(t)} \int_{\mathbb{R}} e^{\nu(t')} \int_{
\bigg[ f_{k,n}'(m')P_{\text{PD}}(m e^{\lambda(t'-t)}, m')
\bigg] \, dr'.
\]

For all generations \((n = 1, 2, \ldots)\) and all genealogical ages \((k = 1, 2, \ldots, n)\):

\[
f_{k,n}(m, t) = e^{-\rho(t)} \int_{0}^{\infty} e^{\nu(t')} \int_{\mathbb{R}} \bigg[ f_{k,n}'(m')P_{\text{PD}}(m e^{\lambda(t'-t)}, m')
\bigg] \, dr'.
\]

where

\[
\rho(t) = \int_{0}^{t} [\sigma_k m e^{\lambda(t'-t)} + \kappa_k] \, dt'.
\]

**References**


