EFFECTS OF CELL POPULATION HETEROGENEITY ON THE DYNAMICS OF CELL POPULATIONS

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Abstract: A novel methodological and computational framework for the quantitative assessment of the effects of cell population heterogeneity on the dynamics of cell populations is presented. We focus on populations of cells carrying an artificial genetic network, consisting of a system of two promoter-repressor pairs. Detailed numerical simulations indicate that taking into account cell population heterogeneity leads to agreement with experimental data, while neglecting it results in significant qualitative and quantitative differences both transiently and at balanced growth.

Keywords: cell population heterogeneity, genetic networks, cell population balance models, cell-to-cell variability,.

1. INTRODUCTION

Biological systems are undoubtedly complex. Their complexity originates from a variety of sources. First, the DNA of organisms is comprised of a large number of genes, which, depending on the intracellular state, maybe on or off or have intermediate expression levels. This, in turn gives rise to a huge number of possible gene states. In addition, cells contain a large variety of chemical components, including ribonucleic acids, lipids, amino-acids, proteins, and metabolites of many different chemical compositions. These cellular components participate in many different processes, including signal transduction, DNA transcription, DNA replication, translation of mRNA into proteins, transport between different cellular compartments or between the cell and the extracellular space, as well as transformation of chemical compounds into metabolic products. Furthermore, products of one set of processes typically affect (inhibit or enhance) the rates of another set of processes, leading to highly nonlinear interactions. Finally, intracellular processes occur at multiple, vastly different time scales. For example, cell proliferation may occur at the time scale of minutes or hours or days depending on the strain or cell type, the media, and the environmental conditions. However, regulation molecules typically exert their influence in the time scale of seconds.

All of the aforementioned sources of complexity are related to events at the *single-cell level*. However, the majority of the powerful experimental techniques that are available today (e.g. DNA arrays, 2-D gels, LC-MS etc), collect measurements from entire *cell populations*, instead of individual cells. In addition, the objective of most biotechnological applications is to maximize the productivity of products formed by a population of cells instead of maximizing the production in an individual cell. Since, in systems engineering, the definition of a system depends on the purposes and specific objectives of the study, these considerations lead us to define a biological system as a system consisting of a cell population rather than one comprised of an individual cell and its components.

Such a biotechnologically meaningful definition necessitates the consideration of an extra level of complexity originating from the fact that cell populations are *heterogeneous* systems in the sense that cellular properties are unevenly distributed amongst the cells of a population. Thus, at any given point in time, cells of an isogenic cell population contain different amounts of DNA, mRNA, proteins, metabolites etc. In short, cell population heterogeneity can be defined as phenotypic variability amongst the cells of an isogenic cell population. This biological fact has been known for a long time. As early as 1945, Delbrück showed significant variations in phage burst sizes (Delbrück, 1945). Moreover, cell population heterogeneity has been observed in cell division times (Powell, 1956), the lysogenic states of phage-infected cells (Ptashne, 1987), the tumbling and smooth-swimming states of flagellated bacteria (Spudich and Koshland, 1976), flagellar phases (Stocker, 1949), induction or repression states of bacterial differentiation (Russo-Marie, et al., 1993), sporulating cultures of *B. subtilis* containing fusions between sporulation genes and *lac*Z (Chung and Stephanopoulos, 1995), while population heterogeneity in β-galactosidase activities of cell populations expressing the lac operon genes has been demonstrated in various systems (Novick and Weiner, 1957). Recently, through an elegant set of experiments, the inherently heterogeneous nature of various isogenic *E. coli* strains was also established using two different fluorescent proteins as reporters (Elowitz, et al., 2002).

The phenotype of each cell depends on the type and number of genes that are expressed at any given point

in time, as well as on the subsequent, numerous intracellular reactions comprising what is simply known as metabolism. The outcome of these cellular processes is greatly influenced by the extracellular environmental conditions, while, intracellularly, these processes are tightly controlled by *regulatory* molecules. Due to the instrumental role that regulatory molecules play in the determination of single-cell phenotype, phenotypic variability among the cells of a population is tightly related to regulation of gene expression and, hence, the architecture and dynamics of single-cell regulatory networks. The astonishing recent progress in genomic research has offered the exciting capability of synthesizing artificial genetic networks with pre-specified, tunable functions. This, in turn, presents the revolutionary opportunity to engineer desirable cellular properties by building the organism's gene-regulatory architecture from appropriate, interconnected genetic blocks with well-defined regulatory functions. However, before embarking into the exciting challenge of building the organism's regulatory architecture (Guet, et al., 2002), it is of fundamental importance to understand how each specific gene-regulatory module affects the phenotype of the entire cell population.

Based on these considerations, the natural, fundamental question for the Systems Biologist becomes: "How does one accurately describe the dynamics of cell populations?" The traditional approach consists of formulating chemically structured continuum models. These are ordinary differential equation (ODE) models describing the dynamics of *population average* intracellular concentrations and their coupling with the dynamics of the nutrients and extracellular environmental conditions, in general. Models of this type contain a significant amount of biological detail on processes that occur at the single-cell level. Moreover, they are rather easy to formulate and simulate. In addition, there exist well-developed, general, theoretical and computational tools, which utilize ODE models for process design, control and optimization. Finally, there exists a variety of advanced parameter estimation techniques allowing the routine determination of parameters that appear in such models from appropriate experimental data. However, chemically-structured continuum models are characterized by fundamental limitations. First, by construction, they do not describe how moments of the cell property distribution higher than the first evolve with time, hence providing only a limited view of the biological system of interest. One might argue that in many instances, knowledge of just the population average dynamics is sufficient. However, in order to obtain closure in deriving a set of equations describing just the first moments of the cell property distribution, continuum models inherently assume that a cell population is a homogeneous system. More specifically, it is assumed that a cell population behaves like a lumped biophase, where all cells behave exactly like the average population cell. Such an assumption obviously neglects the effects of cell population heterogeneity and might lead to inaccurate predictions of the dynamics of the average.

Such a restrictive assumption is not present in the cell population balance (CPB) modeling approach (Ramkrishna, 2000). These models describe the dynamics of the entire cell property distribution, thus naturally accounting for the heterogeneous nature of cellular processes, nutrient uptake and product formation. Furthermore, they can accommodate all the information contained in continuum models on the reaction kinetics occurring at the single-cell level. In addition, they explicitly incorporate in their formulation the division process, as well as the mechanism by which dividing cells partition their material into two daughter cells. Thus, one of the fundamental sources of cell population heterogeneity is explicitly accounted for in the mathematical formulation. However, CPB models consist of first-order, hyperbolic, partial integro-differential equations describing the time evolution of the cell property distribution, which are typically, nonlinearly coupled with integro-ordinary differential equations describing the dynamics of the extracellular environment. Such formulations are characterized by significant mathematical complexity and their numerical simulation represents a challenging task. This mathematical complexity in turn results in additional difficulties in developing control and optimization strategies for biological systems described by CPB models.

In this work we present a novel methodological framework that isolates the quantitative effects of cell population heterogeneity for a given set of biomolecular reactions occurring at the single-cell level by combining the chemically-structured continuum with the cell population balance modeling approaches. In section 2 we present the derivation of this framework, and we sketch the general computational algorithm that was developed in order to quantitatively assess the effects of cell population heterogeneity on the dynamics of cell populations. Finally, in section 3 we apply the presented mathematical/computational framework to a well-known artificial gene switching network, known as the genetic toggle (Gardner et al., 2000).

2. METHODOLOGICAL FRAMEWORK

Motivated by the natural way that CPB models account for population heterogeneity, we developed a general modeling and computational framework aiming at: a) quantitatively assessing the effects of population heterogeneity on the dynamics of cell populations for a given single-cell gene-regulatory network and b) accurately predicting the entire cell property distribution dynamics.

To achieve our aim, we observe that the fundamental difference between chemically-structured continuum models and CPB models is that the former models assume that all cells in the population behave identically to the average cell, while the latter are more general and do not make this restrictive

assumption. We also note that, by construction, continuum models can only predict the dynamics of average cellular properties, and none of the other cell distribution characteristics. Therefore, for a given reaction network, in order to isolate the effects of extrinsic population heterogeneity, it suffices to develop a framework for comparing the predictions for the average cellular property dynamics made by a CPB model with those made by its corresponding continuum model. The question that naturally arises is the following: *Given a CPB model, what is its corresponding continuum formulation*?

The starting point of our approach for addressing this question is the generalized CPB model subject to regularity boundary conditions (Ramkrishna, 2000):

$$\frac{\partial N(\mathbf{x},t)}{\partial t}+\nabla_{\mathbf{x}}\cdot\left[\mathbf{r}(\mathbf{x},\mathbf{S})N(\mathbf{x},t)\right]+\Gamma(\mathbf{x},\mathbf{S})N(\mathbf{x},t) = 2\int_G \Gamma(\mathbf{y},\mathbf{S})p(\mathbf{x},\mathbf{y},\mathbf{S})N(\mathbf{y},t)\,d\mathbf{y}-DN(\mathbf{x},t) \quad (1)$$

$$\int_G \nabla_{\mathbf{x}}\cdot\left[\mathbf{r}(\mathbf{x},\mathbf{S})N(\mathbf{x},t)\right]d\mathbf{x}=0 \quad (2)$$

where **x** is an r-dimensional vector describing the cellular state; G is the space of admissible states; S is a p-dimensional vector with the extracellular substrate concentrations; D is the dilution rate in continuous bioreactors; N(**x**,t)d**x** is the number of cells which at time t, have a state between **x** and **x**+d**x**; **r**(**x**,**S**), Γ(**x**,**S**), and p(**x**,**y**,**S**) describe single-cell, state dependent processes. They represent, respectively, the r-dimensional vector with the net reaction rates, the scalar division rate and the partition probability density function. The latter describes the mechanism by which a mother cell of state **y** partitions its cellular material into two daughter cells of state **x** and **y**-**x**. We note that the integro-partial differential equation (1) is, in general, nonlinearly coupled with a set of integro-ordinary differential equations describing the dynamics of the p substrates **S** (not shown). Moreover, in the case of a batch reactor with excess substrates and in the absence of cell death, the *state distribution function* N(**x**,t) does not reach a steady state as the cells continue to grow in the exponential phase. Manipulation of eqs. (1) and (2), as well as properties of p(**x**,**y**,**S**), leads to the equation describing the dynamics of the *number density function* n(**x**,t), which is defined as the ratio of N(**x**,t) over the cell density $N_t(t)=\int_G N(\mathbf{x},t)\,d\mathbf{x}$:

$$\frac{\partial n(\mathbf{x},t)}{\partial t}+\nabla_{\mathbf{x}}\cdot\left[r(\mathbf{x},\mathbf{S})n(\mathbf{x},t)\right]+\Gamma(\mathbf{x},\mathbf{S})n(\mathbf{x},t) = 2\int_G \Gamma(\mathbf{y},\mathbf{S})p(\mathbf{x},\mathbf{y},\mathbf{S})n(\mathbf{y},t)\,d\mathbf{y} - n(\mathbf{x},t)\int_G \Gamma(\mathbf{x},\mathbf{S})n(\mathbf{x},t)\,d\mathbf{x} \quad (3)$$

Due to the presence of the extra nonlinear term accounting for dilution due to cell proliferation, n(**x**,t) reaches a steady state, known as the *state of balanced growth*. At this state, N(**x**,t) reaches a time-invariant shape, while cells continue to proliferate. Taking the first moment of eq. (3) and applying conservation of mass for each cellular component at cell division, leads to a set of equations describing the dynamics of the *average* intracellular concentrations ($\bar{x}_i$, i =1,...,r):

$$\frac{d\bar{x}_i}{dt}=\int_G r_i(\mathbf{x},\mathbf{S})n(\mathbf{x},t)\,d\mathbf{x}-\bar{x}_i\int_G \Gamma(\mathbf{x},\mathbf{S})n(\mathbf{x},t)\,d\mathbf{x} \quad (4)$$

Notice that the dynamics of the average intracellular concentrations are coupled with those of the number density function (eq. (3)). To derive the corresponding continuum model, it suffices to apply to eq. (4), the fundamental assumption of continuum models, namely, that all cells in the population behave exactly like the average cell. This can be mathematically captured by representing n(**x**,t) as a product of delta functions centered around the average intracellular states:

$$n(\mathbf{x},t)=\prod_{i=1}^{r}\delta\left(x_i-\bar{x}_i(t)\right) \quad (5)$$

Substitution of (5) into (4) yields the continuum model, which corresponds to the CPB model:

$$\frac{d\bar{x}_i}{dt}=r_i\left(\bar{\mathbf{x}},\mathbf{S}\right)-\Gamma\left(\bar{\mathbf{x}},\mathbf{S}\right)\bar{x}_i \quad (i=1,...,r) \quad (6)$$

The difference in the predictions of equations (4) and (6) constitutes the basis for quantitatively assessing the effects of population heterogeneity for a given biomolecular network. Notice that both sets contain the same parameters. The only difference is that eqs. (4) account for the heterogeneous nature of cell growth, while eqs. (6) do not. A careful inspection of eqs (4) and (6) reveals that the effects of population heterogeneity can safely be neglected when the single-cell division and reaction rates are either state-independent or depend linearly on the cellular state. In all other cases, differences between the two approaches are expected. Finally, notice that the proposed framework is bidirectional: Given a CPB one can derive the corresponding continuum model as described. Similarly, given a continuum model describing the dynamics of a certain network, the corresponding CPB model can be derived, by relaxing the homogeneity assumption captured in eq. (5). One also needs to model the partitioning mechanism. However, p(**x**,**y**,**S**) affects the dynamics of the average only indirectly, since it does not explicitly appear in the equations describing the population average dynamics (eqs. (4)).

2.1 *Numerical Algorithm*

The computational challenge in applying this theoretical framework lies in the necessity to solve the cell population balance model. To this end, significant progress has been made in recent years (Mantzaris, et al., 1999; Mantzaris et al., 2001a,b,c).

The most efficient algorithms suggested in the literature share one common characteristic: they discretize the cell population balance equation in a fixed domain. However, the upper bounds of the physiological state space are typically known only initially. Moreover, large differences in the time scales involved in the time evolution of the cell property distribution may lead even the most efficient, fixed-domain algorithms to significant inaccuracies. Motivated by the above considerations, we developed a moving boundary algorithm for the solution of population balance models. The number density function in the moving physiological state domain was expanded using a Galerkin spectral method with sinusoidal basis functions, since this set of basis functions requires a low number of modes in order to accurately capture the dynamics of the distribution. For the time integration of the system of ODEs resulting after expansion of the distribution function using a finite number of basis functions, the one-step, time explicit Runge-Kutta 4^{th} order algorithm was found to be the most efficient (Mantzaris, et al., 2001b). This algorithm results in a more accurate and efficient simulation of the population balance model, since at each time step, only the part of the physiological state space where cells exist is discretized. It simulated very efficiently population balance equations describing vastly different (cellular and non-cellular) processes. The details of the derivation and the performance tests are presented elsewhere (Mantzaris, 2003).

3. THE GENETIC TOGGLE SYSTEM

Motivated by the potential of artificial genetic networks to tailor the phenotype of various strains according to specific biotechnological needs, we focus our attention on a specific artificial genetic network, known as the pTAK genetic toggle. It was constructed by Gardner and coworkers (Gardner et al., 2000) and consists of two promoter-repressor pairs. In particular, the *lac*I repressor gene is under the control of the *cIts* (phage λ repressor) - repressed P_Ls1con promoter, while the λ repressor gene is under the control of the *lac*I-repressed, IPTG-inducible Ptrc-2 promoter. The gfpmut3 gene expressing fluorescent GFP serves as the reporter and is placed after the *cIts* gene. The population dynamics of cells carrying the genetic toggle were well-characterized by Gardner and co-workers using flow cytometry (Gardner, et al., 2000).

To predict the induction dynamics, the authors also developed a two-state, chemically-structured continuum model, which, as stated earlier assumes *a homogeneous population behavior*. The model describes the expression dynamics of the two genes according to the dimensionless equations:

$$\frac{dx_1}{dt} = \frac{a_1}{1+x_2^{\beta}} - x_1 \qquad (7)$$

$$\frac{dx_2}{dt} = \frac{a_2}{1+\left(\frac{x_1}{f\left(\left[IPTG\right]\right)}\right)^{\gamma}} - x_2 \qquad (8)$$

where a_1, a_2 are the effective rates of synthesis of the two genes; β, γ are Hill coefficients describing cooperative inhibition of the expression of each gene by the gene product of the other; f([IPTG]) is a function describing the induction process of the Ptrc-2 promoter (controlling the expression of the λ repressor) by the extracellular inducer isopropyl-β-D-thiogalactopyranoside (IPTG):

$$f\left(\left[IPTG\right]\right) = \left(1+\frac{\left[IPTG\right]}{K}\right)^{\eta} \qquad (9)$$

The parameter values that were used in all simulations are given in Table 1 and are identical to those used in the original work of Gardner and co-workers (Gardner, et al., 2000).

Equations (7) and (8) constitute the continuum model, which was used as the basis for the development of the corresponding CPB model through the application of the theoretical framework presented earlier. We assumed that the division rate depends on the λ repressor expression according to an expression of the form:

$$\Gamma\left(x_1,x_2\right) = \frac{x_2^m}{\int_0^{x_{1,max}}\int_0^{x_{2,max}} x_2^m n\left(x_1,x_2,t\right)dx_2} \qquad (10)$$

where m is a parameter. Notice that the average division rate obtained from eq. (10) is equal to 1, in agreement with the average division rate given by the continuum model. Finally, we assumed that the partitioning function is equal with respect to the lac repressor and a symmetric beta distribution with respect to the λ repressor:

$$p\left(x_1,x_2,z_1,z_2\right) = \delta\left(x_1-\frac{z_1}{2}\right)\cdot\left(\frac{x_2}{z_2}\right)^q\cdot\left(1-\frac{x_2}{z_2}\right)^q \qquad (11)$$

where δ is the Dirac function; x_1,x_2 and z_1,z_2 are the expression levels of the two genes in the daughter and mother cells respectively, and q is a parameter.

Table 1: Parameter values used in simulations

Parameter	Value
a_1	156.25
a_2	15.6
β	2.5
γ	1.0
K	2.9618×10^{-5}M
η	2.0015
m	3
q	10

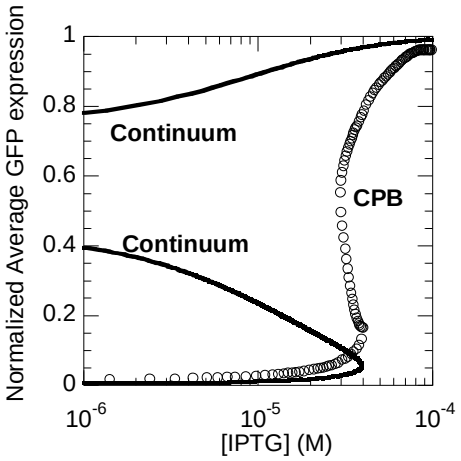

Figure 1: Average, normalized expression levels at balanced growth. Solid line: continuum model; Open circles: Cell population balance model

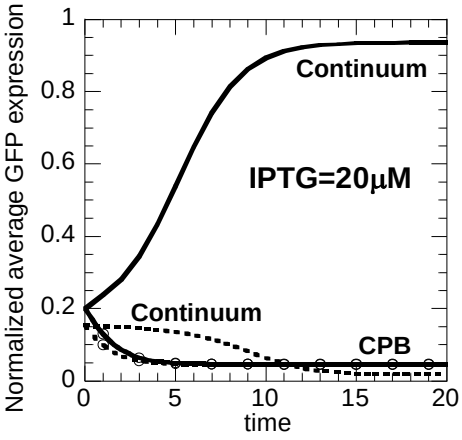

Figure 2: Transient simulations for the average, normalized expression levels for high and low initial average expression level at [IPTG]=20μM. Solid line: continuum model-high initial average; Dashed line: continuum model-low initial average; Solid line-open circles: CPB-high initial average; Dashed line-open circles: CPB-low initial average.

3.1. Results

The theoretical framework discussed in section 2 was applied in order to understand the interplay between the single-cell dynamics of the genetic toggle system and those of the entire cell population. The natural bifurcation parameter was the extracellular IPTG concentration. The comparison between the predictions of the two modeling approaches for the normalized, average balanced growth GFP expression levels (quantifying the expression levels of the λ repressor) is shown in Figure 1. Notice that both modeling approaches predict similar balanced growth average expression levels at high induction levels. Notice also the agreement between the modeling approaches in the prediction of the bifurcation point around 40 μM IPTG. However, the continuum model predicts a huge range of IPTG concentrations where the system exhibits steady-state multiplicity, with the upper and low states being stable, while the intermediate is unstable. On the contrary, taking into account population heterogeneity leads to a much narrower region of multiple steady states. The predictions of the CPB model are in excellent agreement with the experimental measurements of Gardner and co-workers (figure 5a, Gardner, et al., 2000). In particular, they showed that the population displays a much sharper transition from the low to the high state with a narrow region of steady-state multiplicity around the transition point of 40 μM IPTG.

Analogous similarities and differences are observed transiently. In particular, figure 2 shows two sets of simulations for low-intermediate induction levels ([IPTG]=20μM). In the first set, the initial average expression level is lower than in the second set. Notice that vastly different phenotypes are predicted by neglecting population heterogeneity. Low initial average expression level leads to an uninduced state, while for the higher initial condition the system rests at a highly induced state. On the contrary, after some initial dynamics and for both initial conditions, the inclusion of population heterogeneity leads the system to an uninduced state, which is higher than the one predicted by the continuum model.

To better understand the source of the discrepancy between the predictions of the two models, we show the dynamics of the entire number density function for the same IPTG concentration (figure 3a). The system initially exhibits some transient bimodal distributions before reaching a unimodal distribution with a low mean value. These bimodal transient distributions have also been experimentally observed (see Figure 6 in (Gardner et al., 2000)). Despite the low average value, there exist some cells with at relatively high expression state, accounting for the fact that the average expression level at [IPTG]=20μM predicted by the CPB model is higher than the one predicted by the continuum model, (see Figure 2).

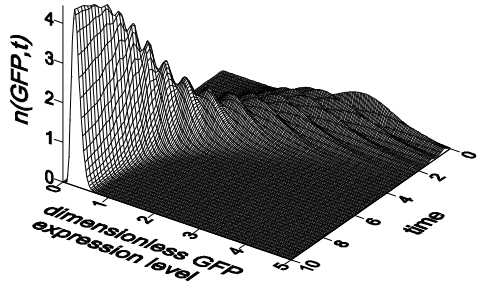

Figure 3: The dynamics of the GFP (or λ repressor) number density function for [IPTG]=20μM as predicted by the CPB model.

The effects of population heterogeneity are less pronounced at high induction levels as figure 4 shows. In this case, most cells exist above the threshold separating the low and high state and consequently the phenotype of the cell population is not significantly affected by the uneven distribution of expression levels. This result can also be explained mathematically: at high induction levels, the expression rate of the λ repressor is almost state-independent and, as explained earlier, linear or constant rate expressions result in less significant

quantitative effects of cell population heterogeneity. The small differences in the predictions of the two modeling approaches are mainly attributed to the nonlinearity of the division rate.

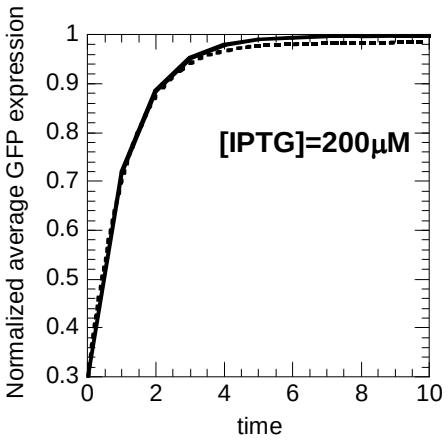

Figure 4: Transient simulations for the average, normalized expression levels at [IPTG]=200μM. Solid line: continuum; Dashed line: CPB model.

4. CONCLUSIONS

We have developed a theoretical/computational framework for isolating the quantitative effects of cell population heterogeneity for a given set of intracellular processes. The framework was applied to study the population dynamics of cells carrying an artificial genetic network, consisting of two promoter-repressor pairs. Our studies clearly showed that neglectimg cell population heterogeneity, may lead to significant qualitative and quantitative errors in the predictions of average population behavior, both transiently and at steady state. Thus, even in cases where only the prediction of the average is of interest, cell population heterogeneity needs to be accounted for. The effects of cell population heterogeneity were shown to be more pronounced at low-intermediate induction levels and smaller at high induction levels. This behavior is in qualitative agreement with experimental data (Elowitz, et al., 2002). The cell population balance modeling approach which accounts for cell-to-cell variability predicted a narrower region of multiple steady states, in agreement with experimental data (Gardner et al., 2000). This qualitative behavior was found to be insensitive to the exact division and partitioning mechanisms (results not shown). Thus, accounting for the heterogeneous nature of cellular processes leads to predictions with less rich dynamics. This, in turn, is an indication that cell-to-cell variability may be responsible for the robustness of cell populations as well as their adaptation ability to various environmental perturbations.

5. ACKNOWLEDGMENTS

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