Population Balance Equations for Cell and Microbial Cultures Revisited

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The first chemically structured model of growth of a microbial or cell population proposed in 1967 by Fredrickson et al. took account of the particulate nature of the population. This model assumed implicitly that cells passed through the cell cycle continuously without transitions between recognizable cell cycle phases. This article discusses how to generalize or revise the first model so that passages of cells through a series of recognizable cell cycle phases can be accounted for. The 1967 article assumed that cell fission can occur anywhere in state space, although with high probability only in a limited domain of that space. Extension of this concept to cell cycle transitions leads to a model that violates a principle of determinism. Revised models that subdivide state space into nonoverlapping subdomains associated with each phase of the cell cycle are developed to obviate this unacceptable situation. The new models introduce the need for information about cell behavior that does not currently exist and thereby provide motivation for an experimental program aimed at supplying the data necessary for their identification and validation.

Introduction

It is well known that mass and other single properties of the cells of a growing and proliferating cell population are distributed rather than uniform, and that the distributions of such properties evolve with time. Early attempts to construct a mathematical model of the evolution of the distribution of cell age, the time elapsed since a cell was formed by fission of its mother cell, were made by Kendall (1948), Von Foerster (1959), and Fredrickson and Tsuchiya (1963). Later, Eakman et al. (1966) attempted to model the evolution of the distribution of cell mass. It was soon recognized that the models developed were applicable only to the highly restricted situation called balanced growth, because cell age or cell mass is a good predictor of cell behavior only in this situation. That is to say, when growth is not balanced, two cells of the same age or the same mass can have quite different behavior.

Age and mass are what we might call *external* properties of a cell because one does not have to probe inside the cell to measure them. Fredrickson et al. (1967) realized that a key to constructing models that would be applicable in situations of non-balanced growth was to introduce *multiple internal* properties of cells. They chose these properties to be the masses of the biochemical components of which a cell is composed so that the vector whose elements are the component masses of a cell determines its state, or physiological state, as they put it. They called this vector the *physiological state vector* of the cell. Such a model, which takes account of changes of the chemical composition of a cell but not of changes in the spatial locations of the cell's components, is called *chemically structured*. These authors defined a density function for the distribution of states at any given time, and they derived an equation of change for this density function in a well-stirred vessel where the density function is independent of spatial position in the culture vessel. The equation of change is simply a balance equation that accounts for the various processes that change the number of cells in a cohort; it is now usually called a *population balance equation*.

Cells go through a series of stages or phases during their life cycle, but the population balance equation of Fredrickson et al. (1967) took no account of this. Thus, the question arises of how one should generalize and/or revise the foregoing equation so that one can have a model for how the distributions of states of cells in various phases of the life cycle evolve in time. This question is addressed in this article.

Review of the Population Balance Equation of Fredrickson et al. (1967)

In the article of concern, growth was assumed to be a strictly deterministic process. Growth changes the masses of the components of a cell; so, the authors regarded it as mo-

tion of cells in state space. Fission was assumed to be a random process which could occur when the cell was anywhere in physiological state space, but which would have high probability only when the cell was in a rather restricted domain of state space. A further assumption was that, at fission, the component masses of a mother cell were partitioned between the daughter cells in a way that satisfies some probabilistic law, as well as the principle of conservation of component masses.

When written for a batch culture, the population balance equation of Fredrickson et al. (1967) was (notation changed)

$$\frac{\partial N_m(\boldsymbol{m},t)}{\partial t} + \nabla_m \cdot \left[\dot{\boldsymbol{m}}(\boldsymbol{m},s) N_M(\boldsymbol{m},t)\right] = -\kappa(\boldsymbol{m},s) N_m(\boldsymbol{m},t) + 2\int_{\Omega_m} p(\boldsymbol{m},\boldsymbol{m}')\kappa(\boldsymbol{m}',s) N_m(\boldsymbol{m}',t) dV_m' \quad (1)$$

In this equation, $\boldsymbol{m}$ is the physiological state vector, $N_m(\boldsymbol{m},t)dV_m$ is the number of cells in the culture that at time t have their state $\boldsymbol{m}$ contained in the element of "volume" dV_m of physiological state space, $\nabla_m \cdot$ is the divergence operation in multidimensional state space, $\dot{\boldsymbol{m}}(\boldsymbol{m}, \boldsymbol{s})$ is the *growth rate vector* of a cell of state $\boldsymbol{m}$ when it is present in a medium where the concentrations of the medium substances are the elements of the vector $\boldsymbol{s}$, $\kappa(\boldsymbol{m}, \boldsymbol{s})dt$ is the fraction of cells having state $\boldsymbol{m}$ at time t that divide in the time interval t to $t + dt$, and $p(\boldsymbol{m}, \boldsymbol{m}')dV_m$ is the fraction of daughter cells having state $\boldsymbol{m}$ contained in the element dV_m of physiological state space produced when a mother cell of state $\boldsymbol{m}'$ divides. Evidently, $N_m(\boldsymbol{m},t)$ is the density of the distribution of states ($\equiv$ component masses) at time t. The subscript m on the density may seem redundant, but it is needed because later on another quantity, age, will be introduced into the description of state. The function $\kappa(\boldsymbol{m}, \boldsymbol{s})$ is called the *fission rate function* and the function $p(\boldsymbol{m},\boldsymbol{m}')$ is called the *partitioning function*. Conservation of component masses requires that the partitioning function satisfy

$$p(\boldsymbol{m},\boldsymbol{m}') = p(\boldsymbol{m}' - \boldsymbol{m},\boldsymbol{m}') \quad (2)$$

that is, the production of daughter cells of states $\boldsymbol{m}$ and $\boldsymbol{m}' - \boldsymbol{m}$ are equally likely when a mother cell of state $\boldsymbol{m}'$ divides. In addition to the equation of change or population balance equation for the density function $N_m(\boldsymbol{m},t)$ an equation of change for the state vector of the cellular environment $\boldsymbol{s}$ is needed. This is given by Fredrickson et al. (1967) and will not be considered here because, in order to focus attention on internal processes in cells, it will be assumed that the vector $\boldsymbol{s}$ is constant.

Fredrickson et al. (1967) assumed that meaningful state vectors are always contained in a closed, simply connected, finite domain Ω_m of state space. Let $\partial\Omega_m$ be the boundary of this domain. The authors assumed that what they called a "regularity condition" applied on $\partial\Omega_m$ and they wrote this as

$$\dot{\boldsymbol{m}}(\boldsymbol{m},s) N_m(\boldsymbol{m},t) = \boldsymbol{0} \quad \forall \boldsymbol{m} \in \partial\Omega_m \quad (3)$$

When this equation is true, it is plain that growth will never take a cell within Ω_m out of that domain. Fredrickson and Mantzaris (2002) reconsidered this regularity condition, showed that it is not completely correct, and replaced it with what they called *containment conditions* on the growth rate vector and boundary conditions on the density function.

In particular, assume that the boundary $\partial\Omega_m$ of Ω_m is composed of two mutually exclusive parts $\partial\Omega_m^0$ and $\partial\Omega_m^{\min}$. The first of these is such that the growth of a cell can take a cell to, but not across, this part of the boundary of Ω_m. This statement imposes no constraint on the density function but it does require that the growth rate vector be such that

$$\boldsymbol{n} \cdot \dot{\boldsymbol{m}}(\boldsymbol{m},s) = 0 \quad \forall \boldsymbol{m} \in \partial\Omega_m^0 \quad (4)$$

where $\boldsymbol{n}$ is a unit outer normal vector to $\partial\Omega_m$. This equation was called a *containment condition* by Fredrickson and Mantzaris (2002). The portion $\partial\Omega_m^{\min}$ of the boundary $\partial\Omega_m$ is the portion of the boundary of state space defined by the minimum requirements on cell composition for viability. In a nonstarvation medium cells satisfying the minimum requirements grow *away from* $\partial\Omega_m^{\min}$ toward the interior of Ω_m so the containment condition (Eq. 4) does not apply there. Instead, Fredrickson and Mantzaris (2002) showed that (Eq. 4) must be replaced by a *boundary condition* on the density function everywhere on $\partial\Omega_m^{\min}$ and the boundary condition is

$$N_m(\boldsymbol{m},t) = 0 \quad \forall \boldsymbol{m} \in \partial\Omega_m^{\min} \quad (5)$$

When cells are starving, growth is negative in the sense that the states of cells in the interior of Ω_m would be moving toward $\partial\Omega_m^{\min}$ rather than away from it, and cell death would occur when a cell crosses $\partial\Omega_m^{\min}$. Presumably, there would be only one cell cycle phase in such a situation, the boundary $\partial\Omega_m$ of Ω_m would be composed of two parts Ω_m^0 and $\partial\Omega_m^{\min}$, and the boundary condition (Eq. 5) would be replaced by $N_m(\boldsymbol{m},t) = 0$ for all $\boldsymbol{m}$ on Ω_m^0. No boundary or containment condition could be prescribed on $\partial\Omega_m^{\min}$. It will be assumed in what follows that cells are not starving.

In Fredrickson et al. (1967), the regularity condition (Eq. 3) should, therefore, be replaced by the containment and boundary conditions 4 and 5, as indicated.

Definition of a Cell Cycle Transition and Phase

In biology a cell cycle transition is said to occur when there is an abrupt change in what a cell is doing. Classic examples are the $G_1 - S$ transition of a mammalian cell, at which DNA synthesis begins, and the $S - G_2$ transition, at which DNA synthesis stops. In the interval of time between these two transitions, the cell that undergoes them is said to be in the S (for synthesis) phase of the cell cycle.

In this article, cell cycle transitions and cell cycle phases must be defined in a mathematical way so that the concepts can be reflected in the equations that are to be written. With the chemically structured model of cell growth that is adopted here, there are two ways that this can be done. The DNA synthesis example cited above suggests one of them. Initiation or cessation of a chemical reaction must result in a discontinuous change in the growth rate vector of a cell, and thus, one way to define a cell cycle transition so that it is reflected in the equations is to say that such a transition occurs when a discontinuity in the growth rate vector occurs. This was the definition of a cell cycle transition given in Fredrickson and Mantzaris (2002). Growth is not the only thing that a cell does, however; it is also getting ready for the

next cell cycle transition or for fission. In the initial attempt to take account of the passage of cells through a cell cycle it will be assumed that such transitions are random events, and that the probability that a transition will occur in a short interval of time depends on the cell's state at the beginning of that interval. Another way to define a cell cycle transition is then to say that such a transition occurs when a discontinuity in the function that gives the transition probability occurs. A discontinuity in either the growth rate vector or the function that gives the probability of transition, or, in general, in both of these things, will, therefore, define a cell cycle transition in this article. A cell is said to be in a certain phase of the cell cycle during the interval of time between successive cell cycle transitions.

Initial Attempt to Write Population Balance Equations for Various Cell Cycle Phases

Obviously, any generalization of the model of Fredrickson et al. (1967), so that it accounts for cell cycle phases, must involve a population balance equation for cells in each cell cycle phase. In the spirit of the older model, it is assumed that transitions from one cell cycle phase to the next occur at random states anywhere in physiological state space, and, to handle this, one introduces into the equations a set of *transition rate functions* defined such that $\boldsymbol{\kappa}^{p,p+1}(\boldsymbol{m})dt$ is the fraction of cells in cell cycle phase p having state vector $\boldsymbol{m}$ at time t that undergo transition to cell cycle phase $p+1$ in time interval t to $t+dt$. In general, one would assume that the transition rate functions depend on $\boldsymbol{s}$, as well as on $\boldsymbol{m}$, but, since we are assuming $\boldsymbol{s}$ is constant, the dependence of the transition rate functions on this vector will not be indicated. Assuming that cells do not skip cell cycle phases and do not go backwards in the cell cycle (except at fission), the population balance equation for the first cell cycle phase, that of daughter cells, is then

$$\frac{\partial N_m^1(\boldsymbol{m},t)}{\partial t}+\nabla_m\cdot\left[\dot{\boldsymbol{m}}^1(\boldsymbol{m})N_m^1(\boldsymbol{m},t)\right]=-\kappa^{1,2}(\boldsymbol{m})N_m^1(\boldsymbol{m},t)+2\int_{\Omega_m}p(\boldsymbol{m},\boldsymbol{m}')\kappa^{P,1}(\boldsymbol{m}')N_m^P(\boldsymbol{m}',t)dV_m' \quad (6)$$

whereas for intermediate cell cycle phase p, $p=2,3,\ldots,P-1$, the population balance equation is

$$\frac{\partial N_m^p(\boldsymbol{m},t)}{\partial t}+\nabla_m\cdot\left[\dot{\boldsymbol{m}}^p(\boldsymbol{m})N_m^p(\boldsymbol{m},t)\right]=\kappa^{p-1,p}(\boldsymbol{m})N_m^{p-1}(\boldsymbol{m},t)-\kappa^{p,p+1}(\boldsymbol{m})N_m^p(\boldsymbol{m},t) \quad (7)$$

and for the last cell cycle phase, the fission phase, it is

$$\frac{\partial N_m^P(\boldsymbol{m},t)}{\partial t}+\nabla_m\cdot\left[\dot{\boldsymbol{m}}^P(\boldsymbol{m})N_m^P(\boldsymbol{m},t)\right]=\kappa^{P-1,P}(\boldsymbol{m})N_m^{P-1}(\boldsymbol{m},t)-\kappa^{P,1}(\boldsymbol{m})N_m^P(\boldsymbol{m},t) \quad (8)$$

Here, $N_m^p(\boldsymbol{m},t)$ and $\dot{\boldsymbol{m}}^p(\boldsymbol{m},s)$ are the density function and growth rate vector, respectively, for cells of cell cycle phase p, P is the total number of cell cycle phases so that the P^{th} phase is the phase of cell division, and $p(\boldsymbol{m},\boldsymbol{m}')$ is a partitioning function already defined. According to the definition of a cell cycle transition given above, it must be true that there is a discontinuity in either the growth rate vector or the transition rate function, or, perhaps in both, at a cell cycle transition. This implies that at least one of the inequalities $\dot{\boldsymbol{m}}^p(\boldsymbol{m})\neq\dot{\boldsymbol{m}}^q(\boldsymbol{m})$ or $\kappa^{p,p+1}(\boldsymbol{m})\neq\kappa^{q,q+1}(\boldsymbol{m})$ must be true if $p\neq q$.

Solutions are sought for each of the Eqs. 6–8 in the domain Ω_m and the same boundary and containment conditions apply to each phase. The boundary condition on the density function of the p^{th} phase is

$$N_m^p(\boldsymbol{m},t)=0 \quad \forall\boldsymbol{m}\in\partial\Omega_m^{\min} \quad \text{and} \quad \forall p \quad (9)$$

and the containment condition on the growth rate vector of that phase is

$$\boldsymbol{n}\cdot\dot{\boldsymbol{m}}^p=0 \quad \forall\boldsymbol{m}\in\partial\Omega_m^0 \quad \text{and} \quad \forall\text{p} \quad (10)$$

Of course, initial conditions for each of the density functions are needed, too, and that is also true of the model of Fredrickson et al. (1967).

The foregoing equations were not derived or presented in Fredrickson and Mantzaris (2002), but they were mentioned as a model taking account of cell cycle phases that is an alternative to the model actually presented in their article. However, in subsequent thinking about Eqs. 6–10, the present author came to the conclusion that they are not an acceptable alternative to the equations presented in Fredrickson and Mantzaris (2002). The rest of this article will explain why Eqs. 6–10 are unsatisfactory, and several alternate sets of equations that, in the author's opinion, are satisfactory, will be presented.

Critique of the Initial Attempt at Generalization

Equations 6–8 assume that cell cycle transitions can occur anywhere in physiological state space so solutions are sought for these equations in the domain Ω_m of state space, and the same boundary and containment conditions (Eqs. 9 and 10) apply to each phase. The assumption stated allows situations like that shown in Figure 1 to occur. This figure, which is for the simplest mathematically nontrivial case of a two-dimensional (2-D) physiological state space, shows trajectories of state change for two cells during some interval of time. Both cells are in the same cell cycle phase, call it p, at the beginning of the interval. This phase happens to be one for which component 2 of the biomass is not being synthesized. Cell 1 remains in cell cycle phase p during the entire interval of time considered, but cell 2 undergoes a transition to phase $p+1$ at condition A; the new cell cycle phase is one in which component 2 of the biomass is synthesized. As a result of the transition, there is a discontinuity in the slope of the trajectory of cell 2 at condition A, and the trajectory rises and *crosses the trajectory* of cell 1 at condition B. Thus, at condition B, the two cells are in different cell cycle phases and they have different growth rate vectors, neither is undergoing a cell cycle transition, but *they have the same physiological state*. Although the figure shows this for a simple 2-D state space, the same condition can occur in a completely general model which takes account of all biochemical components of the cells. Occurrence of this condition is unacceptable, as it violates a principle of determinism that is always assumed to be applicable to models of dynamical systems. The principle

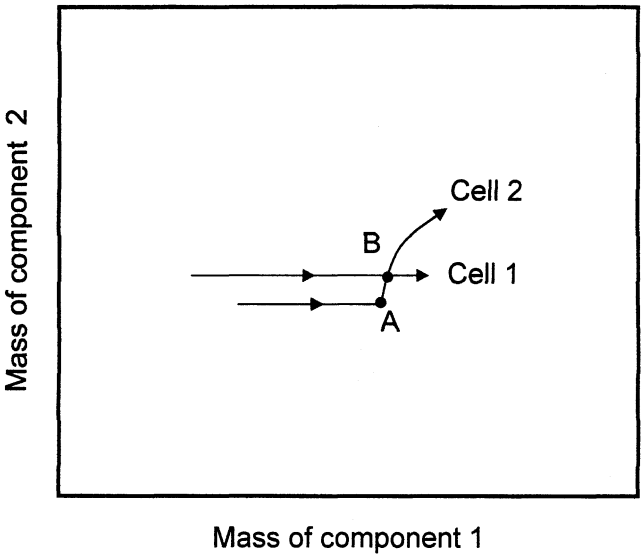

Figure 1. Consequence of the assumption that a cell cycle transition can occur anywhere in state space: crossing of the trajectories of state change of two cells.

is that (complete) specification of the state of a dynamical system determines *uniquely* what the system is doing. According to this principle, cells having the same physiological state and not currently undergoing a cell cycle transition cannot have different growth rate vectors and cannot be in different cell cycle phases. The qualifying phrase "not currently undergoing a cell cycle transition" is added to exclude the situation at a transition, where an infinitesimal change of the state vector is accompanied by a finite change in the growth rate vector; the principle of determinism does not forbid this.

In practice, we cannot include all biochemical components of a cell in a chemically structured model, and considerable lumping of components must be done. Cybernetic models (see, for example, Ramkrishna, 2002) handle this by introducing nonphysical variables—cybernetic variables—in addition to the variables in the physiological state vector $\boldsymbol{m}$ to handle the regulatory features of cellular metabolism. Cybernetic variables, being nonphysical, do not satisfy the principle of conservation of mass that the variables in $\boldsymbol{m}$ must satisfy. In a chemically structured, cybernetic model of cell growth, the state of a cell is specified completely by the *two* vectors $\boldsymbol{m}$ and $\boldsymbol{x}$, where $\boldsymbol{x}$ is the vector of cybernetic variables; so, the growth rate vector $\dot{\boldsymbol{m}}$ is determined uniquely by the three vectors, $\boldsymbol{m}$, $\boldsymbol{x}$, and the environmental state vector $\boldsymbol{s}$. It might seem from this that a condition where trajectories of cell state in a physiological state space cross does not violate the principle of determinism stated above because the cells at the crossing have different $\boldsymbol{x}$ vectors, even though their $\boldsymbol{m}$ vectors are the same. However, in cybernetic models, the cybernetic variables are determined *uniquely* by the two vectors $\boldsymbol{m}$ and $\boldsymbol{s}$, so that the $\boldsymbol{x}$ vectors, as well as the $\boldsymbol{m}$ vectors of cells at a condition where trajectories cross, are the same; so, the cells cannot have different growth rate vectors.

In summary, the principle of determinism stated above does not allow cells having the same physiological state, and not undergoing a cell cycle transition, to do different things, like have different growth rates. Since the assumption that cell cycle transitions can occur anywhere in physiological state space leads to situations where cells having the same physiological state and not undergoing a cell cycle transition *do*, in fact, different things, the assumption is invalid and the Eqs. 6–8 based on it, must be rejected.

Construction of a New Model to Account for the Occurrence of Cell Cycle Phases

In a previous section a cell cycle transition was defined to be a situation at which a discontinuous change in what a cell does occurs, and a cell was said to be in a certain cycle phase during the time between successive cell cycle transitions. The second part of this statement is not an unambiguous definition of what a cell cycle *phase* is, and, as was shown above, it allows cells in different cell phases to occupy the same point in physiological state space, a condition which is forbidden by the principle of determinism stated in the previous section. To avoid a violation of the principle of determinism, we must make a more precise definition of what we mean by a cell cycle phase, and, in particular, we must require that *cells in different cell cycle phases have physiological states in different, nonoverlapping subdomains of physiological state space*. If we do this, we shall have given an unambiguous definition of what we mean by a cell cycle phase: a cell is in a certain cell cycle phase when its physiological state is in a certain subdomain of physiological state space. We shall now explore the consequences of this definition.

The boundaries between nonoverlapping subdomains of state space are hypersurfaces in state space, and the first thing we have to do to construct a model that does not violate the principle of deteminism is to answer the question of how these hypersurfaces are determined.

As mentioned above, cells do different things in different phases of the cell cycle and so the hypersurfaces must be places where cells make a discontinuous change in at least one thing that they are doing. "What they are doing" can refer to the cell growth rate, so the hypersurfaces can be places where discontinuities in the growth rate vector occur. In fact, if we consider transitions between phases p and $p+1$, where $p=1,2,\ldots,P-2$, and use a chemically structured model, then, the only thing in the model that can change discontinuously at such transitions is the growth rate vector. With such a model, the hypersurfaces in state space at which transitions between phases 1 and 2, 2 and 3, …, $P-2$ and $P-1$ occur are *places where discontinuities in the growth rate vector occur.*

The transition between phases $P-1$ and P can also be marked by a discontinuity in the growth rate vector. However, if this transition is considered to be a *commitment to fission*, this commitment is then indeed a discontinuous change in what the cell is doing; so, the transition between phases $P-1$ and P need not involve a discontinuity in the growth rate vector.

There is a corollary to these statements. Conservation of component masses allows the growth rate vector to change discontinuously, but it forbids discontinuous changes in the composition state vector of a *single cell*. Therefore, a cell cannot undergo a transition to another cell cycle phase when it is in the *interior* of one of the subdomains of state space men-

tioned above. Thus, transitions from one cell cycle phase to another occur when growth processes bring the state of a cell to and across one of the boundaries between the aforementioned different subdomains of state space. In this view, *transitions between cell cycle phases are deterministic rather than random events in the sense that they occur when deterministic conditions on cell state are satisfied*. To be sure, the *times* between successive cell cycle transitions can be random, but that does not invalidate the notion that transitions are deterministic rather than random.

One might argue that transitions between cell cycle phases are fuzzy rather than sharp in the sense that there is a probability rather than a certainty of transition, and that the probability only becomes different from zero near hypersurfaces in state space. If this is to be a real effect, the aforementioned probability must become non-zero when the state is still a finite distance from a hypersurface. However, this will not do as it is in fact a cell cycle transition in the *interior* of a subdomain of state space. Thus, cell cycle transitions are sharp and not fuzzy.

There are two exceptions to the conclusion that a transition cannot occur when the state of the cell is in the interior of one of the subdomains into which state space is divided. If the transition is a *fission*, so that it results in the formation of *two* cells, then the principle of conservation of masses of components can be satisfied even though discontinuous changes in cell state occur. The model of Fredrickson et al. (1967) is an example of this exceptional case, because it assumes there is only one cell cycle phase; however, more general examples are possible and will be described below in what will be called the set of models A.

The other exception is when the transition is a *mutation*. In the chemically structured model being considered here the genome of a cell is modeled as a single component of the biomass. Occurrence of a mutation does not change the mass of the genome, so it can happen when the cell is in the interior of a subdomain of state space. Mutation does change the identity of the cell; it becomes a cell of a different population. In order to handle this situation, we would have to write sets of population balance equations for the various populations of cells which are present. Source and sink terms accounting for the occurrence of mutations would occur in these equations. Each of the sink terms would involve a mutation rate function and each source term would involve a mutation rate function, and a probability that a mutation occurring in a certain cell cycle phase of a cell of one population will produce a cell of the same state in a certain cell cycle phase of a different population. Inclusion of the source and sink terms for mutation would require expansion of the notation to keep track of different populations. Since mutation, like cell death by starvation, is peripheral to the main objective of this article, it will be assumed that mutations do not occur and it will thus not be necessary to make the notation more complicated than it is.

Many of the foregoing ideas have been stated already by Fredrickson and Mantzaris (2002), but the argumentation that justifies them that have just been presented was not provided in that article. Instead, those authors dwelt on the difficulty of coming up with some sort of mechanistic model that would predict how the transition rate functions $\boldsymbol{\kappa}^{p-1,p}(\boldsymbol{m})$ that appear in Eqs. 6–8 depend on the state vector $\boldsymbol{m}$. The model that they described, and the additional models that are now to be described, do not employ such transition rate functions because they do not allow cell cycle transitions to occur anywhere in physiological state space, but restrict their occurrence to hypersurfaces in that space.

Four models for cell fission

Fredrickson and Mantzaris (2002) advanced a trio of hypotheses about the last or fission phase of the cell cycle. The first of these was that transition to this phase is *commitment to fission*, but not fission itself. The second was that the occurrence of fission depends on the *time* that has elapsed since the cell entered the fission phase, but is independent of its state when it entered that phase. The third is that the time to fission is not deterministic, but rather satisfies some law of probability. Since growth processes continue when a cell is in the fission phase, fissions will indeed occur at random states, but the distribution of states of dividing cells is determined by (a) the aforementioned probabilistic law, (b) the growth rate vector of the cell, and (c) the distribution of states of cells entering the fission phase.

The discussion given above makes it clear that other sets of hypotheses about the fission phase of the cell cycle are possible, and, in the balance of this article, three of them in addition to the set of Fredrickson and Mantzaris (2002) will be considered. The set of hypotheses of Fredrickson and Mantzaris (2002) will be called model A_1.

The hypotheses of model A_3 are the same as those of model A_1, except it is assumed that the occurrence of fission depends not only on the time that has elapsed since the cell entered the fission phase, but also on the *state* that it had when it entered that phase.

The hypotheses of model A_3 are the same as those of model A_1 except it is assumed that the occurrence of fission depends only on the *state* of the cell and is independent of the time elapsed since the cell entered the fission phase, as well as of the *state that it had when it entered that phase*.

Models A_1-A_3 share the feature that fission occurs when the state of a cell is in the interior of subdomain Ω_m^P and they are, therefore, considered to be subcases of a general case A.

The single hypothesis of the fourth model, which shall be referred to as B, is that fission, rather than simply commitment to fission, occurs when a deterministic condition of cell state is achieved. According to this model, fission occurs when the state of a cell reaches some part of the boundary of subdomain Ω_m^P. Model B is, therefore, a degenerate case of model A_3.

Population Balance Equations, Boundary Conditions, and Containment Conditions for the Four Models of Fission

Assume that there are P phases in the cell cycle. Phase 1 is the phase of daughter cells, phases 2, 3, …, $P-1$ are intermediate phases, and Phase P is the phase of fission.

Equations and conditions for the phase of daughter cells, cell cycle phase 1

Cell cycle transitions remove cells from this phase at the boundary $\partial\Omega_m^{1,2}$ between subdomains Ω_m^1 and Ω_m^2, but fis-

sions of cells of phase P add cells to the interior of Ω_m^1. Thus, the population balance equation for cells of this phase is

$$\frac{\partial N_m^1(\boldsymbol{m},t)}{\partial t} + \nabla_m \cdot \left[\dot{\boldsymbol{m}}^1(\boldsymbol{m}) N_m^1(\boldsymbol{m},t)\right] = (\text{Source term})_\alpha$$
$$\forall \boldsymbol{m} \in \Omega_m^1, \alpha = A_1, A_2, A_3, B \quad (11)$$

The source term, which is non-negative, is different for the four models, as indicated, and will be described below. The boundary $\partial\Omega_m^1$ of Ω_m^1 is the sum $\partial\Omega_m^1 = \partial\Omega_m^{\min} + \partial\Omega_m^{0,1} + \partial\Omega_m^{1,2}$, where $\partial\Omega_m^{o,p}$ is the portion of $\partial\Omega_m^p$ that is not crossed by cells and $\partial\Omega_m^{\min}$ is the boundary of composition space defined by the minimum requirements on cell composition for viability. As explained by Fredrickson and Mantzaris (2002), there is a boundary condition on N_m^1 at this latter boundary and it is

$$N_m^1(\boldsymbol{m},t) = 0 \quad \forall \boldsymbol{m} \in \partial\Omega_m^{\min} \quad (12)$$

No condition can be placed on N_m^1 at the portion $\partial\Omega_m^{1,2}$ of the boundary between subdomains Ω_m^1 and Ω_m^2. The containment condition on $\dot{\boldsymbol{m}}^1$ on $\partial\Omega_m^{o,1}$ is

$$\boldsymbol{n} \cdot \dot{\boldsymbol{m}}^1 = 0 \quad \forall \boldsymbol{m} \in \partial\Omega_m^{0,1} \quad (13)$$

Equations and conditions for intermediate cell cycle phase p, where p = 2, 3, …, P − 1

For this phase, the same equations and conditions hold for all four models A_1-A_3, and B they are the ones given for an intermediate cell cycle phase in Fredrickson and Mantzaris (2002). Cell cycle transitions add cells to this phase at the boundary $\partial\Omega_m^{p-1,p}$ between subdomains Ω_m^{p-1} and Ω_m^p, and they remove cells from it at the boundary $\partial\Omega_m^{p,p+1}$ between subdomains Ω_m^p and Ω_m^{p+1}. Cells with states in the interior of Ω_m^p do not undergo cell cycle transitions, nor are they formed by fissions of cells of phase P. Thus, the population balance equation for cells of this phase is

$$\frac{\partial N_m^p(\boldsymbol{m},t)}{\partial t} + \nabla_m \cdot \left[\dot{\boldsymbol{m}}^p(\boldsymbol{m}) N_m^p(\boldsymbol{m},t)\right] = 0 \quad \forall \boldsymbol{m} \in \Omega_m^p \quad (14)$$

The boundary condition on N_m^p at the portion $\partial\Omega_m^{p-1,p}$ of the boundary between subdomains Ω_m^{p-1} and Ω_m^p is that given by Fredrickson and Mantzaris (2002) and is

$$-\boldsymbol{n} \cdot \left[\dot{\boldsymbol{m}}^p N_m^p - \dot{\boldsymbol{m}}^{p-1} N_m^{p-1}\right] = 0 \quad \forall \boldsymbol{m} \in \partial\Omega_m^{p-1,p} \quad (15)$$

This equation says that the loss of one cell from phase $p-1$ by transition to phase p is accompanied by gain of one cell by phase p. It also satisfies the principle of conservation of component masses. No condition can be placed on N_m^p at the portion $\partial\Omega_m^{p,p+1}$ of the boundary between subdomains Ω_m^p and Ω_m^{p+1}. Let the boundary $\partial\Omega_m^p$ of Ω_m^p be the sum $\partial\Omega_m^P = \partial\Omega_m^{p-1,p} + \partial\Omega_m^{o,p} + \partial\Omega_m^{p,p+1}$, where $\partial\Omega_m^{o,p}$ is the portion of $\partial\Omega_m^p$ that is not crossed by the growth of cells. A containment condition is needed on $\dot{\boldsymbol{m}}^p$ on $\partial\Omega_m^{o,p}$ and it is

$$\boldsymbol{n} \cdot \dot{\boldsymbol{m}}^p = 0 \quad \forall \boldsymbol{m} \in \partial\Omega_m^{o,p} \quad (16)$$

Equations and conditions for cell cycle phase P, Model A for fission

Cell cycle transitions add cells to this phase at the boundary $\partial\Omega_m^{P-1,P}$ between subdomains Ω_m^{P-1} and Ω_m^P fissions remove cells from this phase. Model A assumes that fissions occur when the states of cells are in the interior of Ω_m^P, and Model A_1 assumes that the probability of fission of a cell in time interval t to $t+dt$ is independent of the state of the cell, but instead depends on how long the cell has been in cell cycle phase P, that is, has been committed to fission at time t. If we refer to this time as *age* and give it the symbol a, then the fraction of P-phase cells that have age a at time t that divide in the time interval t to $t+dt$ is defined to be $\gamma(\alpha)dt$, where $\gamma(\alpha)$ is called a fission rate function. Evidently, we must keep track of age when we use this model and, thus, it is an additional state variable for P-phase cells. We must, therefore, define a new density function $N_{ma}^P(\boldsymbol{m},a,t)$ such that $N_{ma}^P(\boldsymbol{m},a,t)dV_m da$ is the number of cells that at time t have composition state $\boldsymbol{m}$ contained in the infinitesimal volume dV_m of composition space and age in the fission phase between a and $a+da$. The population balance equation for P-phase cells when Model A_1 is used is (Fredrickson and Mantzaris, 2002).

$$\frac{\partial N_{ma}^P(\boldsymbol{m},a,t)}{\partial t} + \nabla_m \cdot \left[\dot{\boldsymbol{m}}^P(\boldsymbol{m}) N_{ma}^P(\boldsymbol{m},a,t)\right] + \frac{\partial N_{ma}^P(\boldsymbol{m},a,t)}{\partial a} = -\gamma(a) N_{ma}^P(\boldsymbol{m},a,t) \quad (17a)$$

Two boundary conditions are needed for this equation and Fredrickson and Mantzaris (2002) have shown that they are

$$N_{ma}^P(\mathbf{m},0,t) = 0 \quad \forall \boldsymbol{m} \in \Omega_m^P \quad \text{but} \quad \boldsymbol{m} \notin \partial\Omega_m^{P-1,P}, \quad (18)$$

$$-\boldsymbol{n} \cdot \left[\dot{\boldsymbol{m}}^P(\boldsymbol{m}) N_{ma}^P(\boldsymbol{m},a,t) - \dot{\boldsymbol{m}}^{P-1}(\boldsymbol{m})\delta(a) N_m^{P-1}(\boldsymbol{m},t)\right] = 0, \quad \forall \boldsymbol{m} \in \partial\Omega_m^{P-1,P} \quad (19)$$

where $\delta(a)$ denotes Dirac's delta function. As noted above, the growth rate vector need not be discontinuous on $\partial\Omega_m^{P-1P}$ when the transition from phase $P-1$ to phase P is regarded as a commitment to fission. If the growth rate vector is not discontinuous, then the boundary condition (Eq. 19) becomes

$$N_{ma}^P(\boldsymbol{m},a,t) = \delta(a) N_m^{P-1}(\boldsymbol{m},t), \quad \forall \boldsymbol{m} \in \partial\Omega_m^{P-1,P} \quad (19a)$$

A containment condition on $\dot{\boldsymbol{m}}^P$ on the portion $\partial\Omega_m^{o,P} = \partial\Omega_m^P - \partial\Omega_m^{P-1,P}$ of the boundary of ΩP_m is needed and it is

$$\boldsymbol{n} \cdot \dot{\boldsymbol{m}}^P = 0 \quad \forall \boldsymbol{m} \in \partial\Omega_m^{o,P} \quad (20)$$

In Model A_2 the fission rate function is a function of $\boldsymbol{m}_o$, the state that the cell had when it entered the fission phase, as well as of its age a. This state can be calculated for a P-phase cell that has state $\boldsymbol{m}$ and age a by backward integration of the equation $d\boldsymbol{m}/dt = d\boldsymbol{m}/da = \dot{\boldsymbol{m}}(\boldsymbol{m})$. The state at age 0 is evidently a function of $\boldsymbol{m}$ and a, so that if we call this (vector-valued) function $\boldsymbol{g}(\boldsymbol{m},a)$, we would then write the transition rate function as $\gamma(a,\boldsymbol{m}_o) = \gamma(a,\boldsymbol{g}(\boldsymbol{m},a))$ rather than as

$\gamma(a)$. Thus, the population balance equation for P-phase cells is

$$\frac{\partial N_{ma}^P(\boldsymbol{m},a,t)}{\partial t}+\nabla_m\cdot\left[\dot{\boldsymbol{m}}^P(\boldsymbol{m})N_{ma}^P(\boldsymbol{m},a,t)\right]+\frac{\partial N_{ma}^P(\boldsymbol{m},a,t)}{\partial a}=-\gamma(a,\boldsymbol{g}(\boldsymbol{m},a))N_{ma}^P(\boldsymbol{m},a,t) \quad (17b)$$

and the boundary and containment conditions are Eqs. 18–20.

Like Models A_1 and A_2, Model A_3 assumes that P-phase cells divide when their states are in the interior of Ω_m^P, but it assumes that the probability of fission depends on the state of the cell rather than on its age. The fraction of P-phase cells of state $\boldsymbol{m}$ at time t that divide in time interval t to $t+dt$ is defined to be $\kappa^P(\boldsymbol{m})dt$. Hence, for Model A_3, it is unnecessary to keep track of age in the fission phase and the population balance equation for cells of this cell cycle phase is

$$\frac{\partial N_m^P(\boldsymbol{m},t)}{\partial t}+\nabla_m\cdot\left[\dot{m}^P(\boldsymbol{m},s)N_m^P(\boldsymbol{m},t)\right]=-\kappa^P(\boldsymbol{m})N_m^P(\boldsymbol{m},t) \quad (21)$$

The boundary condition on N_m^P at the portion $\partial\Omega_m^{P-1,P}$ of the boundary of Ω_m^P is

$$-\boldsymbol{n}\cdot\left[\dot{\boldsymbol{m}}^P N_m^P-\dot{\boldsymbol{m}}^{P-1}N_m^{P-1}\right]=0 \quad \forall\boldsymbol{m}\in\partial\Omega_m^{P-1,P} \quad (22)$$

and the containment condition on $\dot{\boldsymbol{m}}^P$ on the portion $\partial\Omega_m^{o,P}=\partial\Omega_m^P-\partial\Omega_m^{P-1,P}$ of the boundary of Ω_m^P is

$$\boldsymbol{n}\cdot\dot{\boldsymbol{m}}^P=0 \quad \forall\boldsymbol{m}\in\partial\Omega_m^{o,P} \quad (23)$$

Equations and conditions for cell cycle phase P, Model B for fission

Model B assumes that fission does not occur so long as the state of the cell is in the interior Ω_m^P, but that it does occur when growth brings the cells to a portion of the boundary of Ω_m^P that I shall call $\partial\Omega_m^{\text{fiss}}$. Hence, when Model B is adopted, the population balance equation for the fission phase of the cell cycle is of the same form as that for intermediate cell cycle phases and is

$$\frac{\partial N_m^P(\boldsymbol{m},t)}{\partial t}+\nabla_m\cdot\left[\dot{\boldsymbol{m}}^P(\boldsymbol{m},s)N_m^P(\boldsymbol{m},t)\right]=0 \quad \forall\boldsymbol{m}\in\Omega_m^P \quad (24)$$

The boundary condition on N_m^P is

$$-\boldsymbol{n}\cdot\left[\dot{\boldsymbol{m}}^P N_m^P-\dot{\boldsymbol{m}}^{P-1}N_m^{P-1}\right]=0 \quad \forall m\in\partial\Omega_m^{P-1,P} \quad (25)$$

No condition can be placed on N_m^P at the portion $\partial\Omega_m^{\text{fiss}}$ of the boundary of subdomain Ω_m^P. However, a containment condition for $\dot{\boldsymbol{m}}^P$ on $\partial\Omega_m^{o,P}=\partial\Omega_m^P-\partial\Omega_m^{P-1,P}-\partial\Omega_m^{\text{fiss}}$ is needed and it is

$$\boldsymbol{n}\cdot\dot{\boldsymbol{m}}^P=0 \quad \forall\boldsymbol{m}\in\partial\Omega_m^{o,P} \quad (26)$$

Source terms for population balance equations for the first cell cycle state

The source terms for Models A_1, A_2, A_3 and B, are

$$(\text{Source term})_{A_1}=2\int_{\Omega_m^P}p(\boldsymbol{m},\boldsymbol{m}')dV_m'\int_0^\infty\gamma(a)N_{ma}^P(\boldsymbol{m}',a,t)da \quad (27)$$

$$(\text{Source term})_{A_2}=2\int_{\Omega_m^P}p(\boldsymbol{m},\boldsymbol{m}')dV_m'\int_0^\infty\gamma(a,\boldsymbol{g}(\boldsymbol{m}',a))N_{ma}^P(\boldsymbol{m}',a,t)da \quad (28)$$

$$(\text{Source term})_{A3}=2\int_{\Omega_m^P}p(\boldsymbol{m},\boldsymbol{m})\kappa(\boldsymbol{m}')N_m^P(\boldsymbol{m}',t)dV_m' \quad (29)$$

$$(\text{Source term})_B=2\int_{\partial\Omega_m^{fiss}}p(\boldsymbol{m},\boldsymbol{m}')\left[\boldsymbol{n}\cdot\dot{\boldsymbol{m}}^P(\boldsymbol{m}')\right]N_m^P(\boldsymbol{m}',t)dA_m' \quad (30)$$

In these equations, dA_m and dV_m represent elements of "area" on a hypersurface in state space and an element of "volume" in this space, respectively.

Reduction of the Equations in the Simplest Cases

It is of interest to see what the population balance equations for the models presented reduce to when there is only one cell cycle phase. In order for there to be only one phase, it must be true that: (1) the growth rate vector is nowhere discontinuous in Ω_m; and (2) daughter cells are committed to fission when they form. Model A_1 for this situation reduces to the single population balance equation

$$\frac{\partial N_{ma}(\boldsymbol{m},a,t)}{\partial t}+\nabla_m\cdot\left[\dot{\boldsymbol{m}}(\boldsymbol{m})N_{ma}(\boldsymbol{m},a,t)\right]+\frac{\partial N_{ma}(\boldsymbol{m},a,t)}{\partial a}=-\gamma(a)N_{ma}(\boldsymbol{m},a,t) \quad (31)$$

which must satisfy the two boundary conditions

$$N_{ma}(\boldsymbol{m},a,t)=0 \quad \forall\boldsymbol{m}\in\Omega_m^{\min} \quad (32)$$

$$N_{ma}(\boldsymbol{m},0,t)=2\int_{\Omega_m}p(\boldsymbol{m},\boldsymbol{m}')dV_m'\int_0^\infty\gamma(a)N_{ma}(\boldsymbol{m}',a,t)da \quad (33)$$

In addition, the containment condition

$$\boldsymbol{n}\cdot\dot{\boldsymbol{m}}(\boldsymbol{m})=0 \quad \forall\boldsymbol{m}\in\partial\Omega_m^o \quad (34)$$

must be satisfied by the growth rate vector. Since there is only one cell cycle phase, the age a is in this case the age of the cell: the time elapsed since it was formed by fission.

If we define

$$N_a(a,t)\equiv\int_{\Omega_m}N_{ma}(\boldsymbol{m},a,t)dV_m \quad (35)$$

then it is easy to see that the integration of each term of Eq. 31 over Ω_m and use of the boundary condition 32 and the containment condition 34 yield

$$\frac{\partial N_a(a,t)}{\partial t} + \frac{\partial N_a(a,t)}{\partial a} = -\gamma(a) N_a(a,t) \tag{36}$$

Similarly, integration of the boundary condition (Eq. 33) over Ω_m yields

$$N_a(0,t) = 2\int_0^\infty \gamma(a) N_a(a,t)\, da \tag{37}$$

Equations 36 and 37 are the equations of the age model of von Foerster (1959) and Fredrickson and Tsuchiya (1963). It should be remarked in connection with these equations that, unless growth happens to be balanced, the assumption is unrealistic that the fission rate is dependent only on age when there is only one cell cycle phase.

If we define

$$N_m(\boldsymbol{m},t) \equiv \int_0^\infty N_{ma}(\boldsymbol{m},a,t)\, da \tag{38}$$

and then integrate Eq. 31 over all ages, we will get Eq. 1, the population balance equation of Fredrickson et al. (1967), with the difference that the fission rate function, which has to be defined by

$$\kappa(\boldsymbol{m},t) \equiv \frac{\int_0^\infty \gamma(a) N_{ma}(\boldsymbol{m},a,t)\, da}{\int_0^\infty N_{ma}(\boldsymbol{m},a,t)\, da} \tag{39}$$

will in general be a function of time, as well as of state. In this sense, Model A_1 does not reduce to the model of Fredrickson et al. (1967).

The equations for Model A_2 when there is only cell cycle phase are the same as Eqs. 31–39 except that $\gamma(a)$ has to be replaced by $(\gamma[a,\boldsymbol{g}(\boldsymbol{m}, a)]$.

When there is only a single cell cycle phase, Model A_3 reduces to the single population balance equation

$$\frac{\partial N_m(\boldsymbol{m},t)}{\partial t} + \nabla_m \cdot \left[\dot{\boldsymbol{m}}(\boldsymbol{m}) N_m(\boldsymbol{m},t)\right] = -\kappa(\boldsymbol{m}) N_m(\boldsymbol{m},t) + 2\int_{\Omega_m} p(\boldsymbol{m},\boldsymbol{m}')\kappa(\boldsymbol{m}') N_m(\boldsymbol{m}',t)\, dV'_m \tag{40}$$

This equation must satisfy the containment condition

$$\boldsymbol{m} \cdot \dot{\boldsymbol{m}}(\boldsymbol{m}) = 0 \quad \forall \boldsymbol{m} \in \partial\Omega_m^o \tag{41}$$

and the boundary condition

$$N_m(\boldsymbol{m},t) = 0 \quad \forall \boldsymbol{m} \in \partial\Omega_m^{\min} \tag{42}$$

Again, when there is only one cell cycle phase, Model B reduces to the single population balance equation

$$\frac{\partial N_m(\boldsymbol{m},t)}{\partial t} + \nabla_m \cdot \left[\dot{\boldsymbol{m}}(\boldsymbol{m}) N_m(\boldsymbol{m},t)\right] = 2\int_{\partial\Omega_m^{fuss}} p(\boldsymbol{m},\boldsymbol{m}')\left[\boldsymbol{n}\cdot\dot{\boldsymbol{m}}(\boldsymbol{m})\right] N_m(\boldsymbol{m}',t)\, dA'_m \tag{43}$$

Once more, the containment and boundary conditions 41 and 42, respectively, must be satisfied, but now it must be realized that the boundary $\partial\Omega_m$ of Ω_m has three parts: $\partial\Omega_m^o$, $\partial\Omega_m^{\min}$, and $\partial\Omega_m^{\text{fiss}}$.

The foregoing equations were given by Fredrickson et al. (1967). Equation 40 was their principal result, and Eq. 43 was given as the balance equation for the "degenerate case" of the population balance equation (see Eq. 5.10 of the 1967 article). It appears now that these results are limited to the quite restrictive situation in which there are no discontinuities in the growth rate vector anywhere in state space.

Factors Affecting the Distributions of Properties of Dividing Cells

The distribution of properties of *dividing cells* is experimentally measurable in principle, and it is of great interest because it reflects the internal dynamics of growth and fission. Let $h(\boldsymbol{m},t)$ be the density of the distribution of states of dividing cells at time t. That is, $h(\boldsymbol{m},t)dV_m$ is the fraction of all the cells that divide in the time interval t to $t+dt$ that have states in the element of "volume" dV_m of state space. Fredrickson and Mantzaris (2002) showed that, when Model A_1 is adopted, the density is given by

$$h(\boldsymbol{m},t) = \frac{\int_0^\infty \gamma(a) N_m^P(\boldsymbol{m},a,t)\, da}{\int_{\Omega_m^P} dV'_m \int_0^\infty \gamma(a) N_m^P(\boldsymbol{m}',a,t)\, da} \tag{44}$$

Evidently, calculation of $h(\boldsymbol{m},t)$ requires knowledge of $N_m^P(\boldsymbol{m},t)$, as well as of the fission rate function $\gamma(a)$. Knowledge of the former quantity has to be obtained by simultaneous solution of the *complete set* of balance Eqs. 11, 14, and 17, not just the balance equation for $N_m^P(\boldsymbol{m},t)$. Of course, the balance equations have to be solved subject to the attendant boundary conditions Eqs. 12, 15, and 18 and 19, as well as appropriate initial conditions. The solution of the equations, and so the density $h(\boldsymbol{m},t)$, will depend on the growth rates $\dot{\boldsymbol{m}}^p(\boldsymbol{m})$ of the various cell cycle phases, the fission rate function $\gamma(a)$, and the partitioning function $p(\boldsymbol{m},\boldsymbol{m}')$. The fission rate function and the partitioning function reflect the assumptions that the age of a mother cell at fission and the partitioning of the masses of a mother cell's components between its daughter cells are not deterministic, but are subject to probabilistic laws. On the other hand, the growth rate has been assumed to be a completely deterministic process. Thus, in model A_1, fission and partitioning of components, but not growth, are the random processes that contribute to making the states of dividing cells distributed rather than uniform.

It is certainly within the realm of possibility, and, indeed, it seems probable, that the growth of cells also has elements of randomness, and Ramkrishna (2000, Sec. 2.10) and Fredrickson and Mantzaris (2002) have shown how that might be incorporated into the balance equations. Such incorporation would change the population balance equations from first-order partial differential-integral equations to second-order partial differential-integral equations, and it seems that this would change the predicted density function $h(\boldsymbol{m},t)$ in a qualitative, not just a quantitative, way. If this conjecture is correct, one might be able to use the predicted changes to devise an experiment for determining whether or not randomness of growth rate is a significant effect for a given population of cells.

Discussion

Since it is difficult to imagine that there is a set of conditions which result in cell fission, rather than in commitment to fission, it would seem to be labor lost to use Model B in a program designed to identify and validate a population balance model for microbial and cell growth. This model was given here for the sake of completeness and also because a special case of it had been given in Fredrickson et al. (1967). The three subcases of Model A are not subject to the same recommendation, since they assume a condition for commitment to fission, rather than a condition for fission itself. Of these three models, A_1, the model of Fredrickson and Mantzaris (2002), is the simplest, and it would be this author's choice for a model to be used in the kind of program described above. However, the hypothesis that the fission rate function $\gamma(a)$ is a function only of age ($\equiv$ time elapsed since the cell became committed to fission) might be restrictive, and it might be necessary to turn to the more complicated models A_2 and A_3. Model A_2 is more flexible and also more intuitively appealing than model A_3.

In this author's opinion, the population balance models A (and also B) presented herein and, in part, earlier by Fredrickson and Mantzaris (2002), call for a radical change in the way one thinks about the theory of microbial and cell population growth. In the model presented by Fredrickson et al. (1967) there were no internal boundary conditions, the fission rate function was state-dependent, and the topology of state space played no role. In the present models, there are internal boundary conditions, the fission rate function is state-independent but age dependent (in the simplest case), and the need for transition rate functions for transitions between cell cycle phases is replaced by the need to know the topology of state space.

With regard to the topology of state space, Fredrickson and Mantzaris (2002) assumed that it was *linear* in the sense that no branching occurs. That is, these authors assumed that, under growth conditions, a cell in cell cycle phase p can only undergo transition to one cell cycle phase $p+1$ and there is no possibility for it to change to phase $p+1$ *or* to a *different* phase $p'+1$. The population balance equations given above could be generalized to account for branching, but that will not be done here.

Again, Fredrickson and Mantzaris (2002) assumed that the topology of state space is independent of environmental conditions, but the senior author of that article now thinks that this assumption cannot be true. As the environment is changed, it seems likely that the sizes, and, perhaps, also the locations, of the subdomains in state space defining the various cell cycle phases will change. Depending on the nature of the environmental changes, one can think that some cell cycle phases might disappear and others might appear. Motion of the boundaries of the subdomains defining the states of cells in different cell cycle phases will not affect the population balance equations for the phases but it will affect the boundary and containment conditions. For example, the boundary condition 15 will have to be replaced by

$$-\boldsymbol{n}\cdot\left[(\dot{\boldsymbol{m}}^{p}-\boldsymbol{v}^{p-1,p})N_m^{p}-(\dot{\boldsymbol{m}}^{p-1,p})N_m^{p-1}\right]=0 \quad \forall \boldsymbol{m}\in\partial\Omega_m^{p-1,p} \tag{45}$$

and the containment condition 16 by

$$\left[\boldsymbol{n}\cdot(\dot{\boldsymbol{m}}^{p}-\boldsymbol{v}^{o,p})\right]=0 \quad \forall \boldsymbol{m}\in\partial\Omega_m^{o,p} \tag{46}$$

In these equations, $\boldsymbol{v}^{p-1,p}$ is the local velocity in state space of the boundary between subdomains $p-1$ and p and $\boldsymbol{v}^{o,p}$ is the local velocity of the portion of the boundary of subdomain p across which cells do not pass. Since cells are neither produced nor consumed at a boundary through which they do pass and the densities N_m^{p} and N_m^{p-1} and are positive, the velocity $\boldsymbol{v}^{p-1,p}$ must be such that $[\boldsymbol{n}\cdot(\dot{\boldsymbol{m}}^{p}-\boldsymbol{v}^{p-1,p})]$ and $[\boldsymbol{n}\cdot(\dot{\boldsymbol{m}}^{p-1}-\boldsymbol{v}^{p-1,p})]$ have the same sign.

Population balance equations are often stated to be intractable, meaning, no doubt, that the equations are difficult or impossible to solve, even numerically, or that it is difficult or impossible to solve what Ramkrishna (2000, Chapter 6) calls the inverse problem, namely, the problem of finding out experimentally what expressions should be used for growth rates, fission rates, and component partitioning functions. Probably, the thought that such equations are intractable is a prime reason, perhaps the prime reason, why so little use has been made of them. However, considerable progress in the development of efficient numerical techniques for solving the equations has been made recently (Mantzaris et al., 2001a,b,c), and it seems that the notion needs to be reevaluated that population balance equations are intractable.

Conclusions

(1) Equations 6–8, which seem to be a straightforward generalization of the population balance equation of Fredrickson et al. (1967) to account for the occurrence of cell cycle phases, lead to a violation of a principle of determinism and, thus, must be rejected.

(2) Considerations of the aforementioned principle of determinism require that cells in different cell cycle phases have states in different, nonoverlapping subdomains of state space.

(3) The same considerations require that boundaries between the subdomains be sharp, not fuzzy.

(4) Boundaries between the subdomains are hypersurfaces in state space where cells change what they do. With the kind of model considered (chemically structured but not compartmentalized), this means that the boundaries must be hypersurfaces where discontinuities in cell growth rate or/and commitment to fission occur.

(5) Conservation of masses of cell components forbids occurrence of cell cycle transitions when the states of cells are in the interior of one of the aforementioned subdomains of state space. Therefore, transitions occur when growth brings the states of cells to and across the boundaries between these subdomains.

(6) Conservation of masses of cell components does not forbid fission or mutation of a cell when its state is in the interior of one of the aforementioned subdomains.

(7) Four different models based on the foregoing conclusions have been developed and presented. Internal boundary conditions are essential parts of the models.

(8) Such models focus attention on the need to obtain knowledge of the *topology* of state space and of the effects of changing environmental circumstances on that topology.

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