

COMPUTER ANALYSIS OF THE LABELED MITOSIS CURVES

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Summary

Motivation: The interpretation of the labeled mitoses curves needs improvement, since for a long time it was based on a simple model with a constant cell flow through the cell-cycle – assumption evidently incorrect now for tissues. The problem of the presence or absence of mother-daughter and daughter-daughter cell cycles correlation do not have direct experimental solution now and needs another kind of proof.

Results: The cell cycle can be described by the differential equation of wave [1] (not to be mixed with wave equation). The comparison of the model with absolute and absent mother-daughter cell cycle speed correlation shows the last one is more convenient for the cell-cycles in tissues.

Introduction

Cell-cycle is a periodic change *G1*, *S*, *G2* and *M* phases in a cell. The method of labeled mitoses based on short time incubation of cells in labeled DNA precursors (incorporating in the *S* phase cells) and registration of labeled metaphase cell proportion as a function of time. [1]. If all cells have the same speed of cell-cycle, than labeled mitoses curve must to be the sequence of trapezium like figures [1]. Real curves are more smooth and the interpretation of those curves require most complex approach (a lot of other experimental facts argue against an unique cell-cycle speed [2]). Here we present a model where the cells traverse cell-cycle with a continuum of speeds distributed normally. Two extreme cases are discussed – absolute and absent mother-daughter cell-cycle correlation.

Model

The cell can be characterized at the moment *t* by the cell-cycle phase θ : $0 \leq \theta \leq 1$. *G1*, *S*, *G2* phases constitute to regions $1 < \theta < \theta_{G1}$, $\theta_{G1} \leq \theta < \theta_S$ and $\theta_S \leq \theta < 1$, where θ_{G1} , θ_S are ending points of *G1* and *S*, correspondingly. At the point $\theta_{G2} = 1$ the mitosis takes place (the length of the mitosis is usually very short in comparison to the total cell-cycle duration). As the result of the mitosis, the cell with $\theta=1$ disappeared and two new cells with $\theta=0$ appeared. The amount of cells, $dN(\theta, t)$, in the interval $[\theta, \theta + d\theta]$ can be expressed through the density of cells, $n(\theta, t)$: $dN(\theta, t) = n(\theta, t)d\theta$. For the density of cells the conservation of the cells flux takes place:

$$\frac{\partial n(\theta, t)}{\partial t} - \beta \frac{\partial n(\theta, t)}{\partial \theta} = 0, \quad (1)$$

where β is the cell cycle speed along the phase θ (without death). If we introduce the cell-cycle speed distribution $n(\theta, t) = n(\theta, \beta, t)$, then the equation (1) becomes:

$$\frac{\partial n(\theta, \beta, t)}{\partial t} - \beta \frac{\partial n(\theta, \beta, t)}{\partial \theta} = 0 \quad (2)$$

The general solution of equation (2) is $n(\theta, \beta, t) = f(\theta - \beta t)\rho(\beta)$, where $f(x)$ and $\rho(x)$ are some arbitrary functions. If few cell cycles take place, then the solution at the point of mitosis is

$$n(1, \beta, t) = \rho_0(\beta) \sum_{i=0}^{\infty} f_0(1 - \beta t + i) \quad (3)$$

The summation in equation (3) is over the cells that were born after i divisions. In order to use equation (3) one can assume the uniform phase distribution, since it is a good approximation in many cases:

$$f_0(x) = \begin{cases} 0 & \text{if } x \leq 0 \\ 1 & \text{if } 0 < x \leq 1 \\ 0 & \text{if } 1 < x \end{cases} \quad (4)$$

The density of labeled cells at the point of mitosis is

$$\Phi_m(t) = \int_{\beta_{\min}}^{\beta_{\max}} \left[\rho_0(\beta) \sum_{i=0}^{\infty} f_{0m}(1 - \beta t + i) \right] d\beta, \quad (5)$$

where:

$$f_{0m}(x) = \begin{cases} 0 & \text{if } x \leq G1 \\ 1 & \text{if } \theta_{G1} < x \leq \theta_S \\ 0 & \text{if } \theta_S < x \end{cases} \quad (6)$$

the density of all the cells at the point of mitosis is

$$\Phi_n(t) = \int_{\beta_{\min}}^{\beta_{\max}} \left[\rho_0(\beta) \sum_{i=0}^{\infty} f_0(1 - \beta t + i) \right] d\beta. \quad (7)$$

The ratio: $z(t) = \frac{\Phi_m(t)}{\Phi_n(t)}$ is the labeled mitoses curve.

In the model described above the daughter cells have the same speed β as their mother cell. It may be not the case in real experiment. It is known that a cell is sensitive to external signals at the «restriction point», θ_r , where the cell can change their speed β . The restriction point is placed within $G1$ phase near $G1-S$ transition. In order to consider this case (speed decorrelation between mother and daughter cells) as well, we developed an alternative model assuming that at the restriction point the speed distribution for daughter cells is changed to the same initial speed distribution, $\rho_0(\beta)$, of all cells at that point. Then the distribution of the cells over cell-cycle speed at the point can be expressed as

$$\rho(\beta, \theta_r, t) = \rho_0(\beta) \sum_{i=0}^{\infty} A_i(t), \quad (8)$$

where $A_i(t)$ is the portion of cells which were born after i divisions. Due to the conservation of the cell flow for those subpopulations, the following recurrent relation takes place:

$$A_{i+1}(t) = \frac{2 \int_{\beta_{\min}}^{\beta_{\max}} \beta A_i(t - \frac{1}{\beta}) \rho_0(\beta) d\beta}{\int_{\beta_{\min}}^{\beta_{\max}} \beta \rho_0(\beta) d\beta}. \quad (9)$$

At the point of mitosis, the density of the cells appeared after i divisions is

$$n_i(1, \beta, t) = A_i(t - \frac{1 - \theta_r}{\beta}) \rho_0(\beta), \quad i > 0. \quad (10)$$

In order to obtain $n_0(1, \beta, t)$, one can use equation (3) for first cell-cycle: $n_0(1, \beta, t) = f_0(1 - \beta t) \rho_0(\beta)$. Then the following expression for $A_1(t)$ is used:

$$A_1(t) = \frac{2 \int_{\beta_{\min}}^{\beta_{\max}} \beta f_0(1 - \beta t) \rho_0(\beta) d\beta}{\int_{\beta_{\min}}^{\beta_{\max}} \beta \rho_0(\beta) d\beta}. \quad (11)$$

Thus, when the initial distribution, $\rho_0(\beta)$, (e.g. normal distribution) and phase distribution, $f_0(\theta)$, (e.g. uniform distribution) are set, one can define theoretical approximation of the labeled mitoses curve, $z(t)$. Essential assumption of both models is the independence of the relative duration of the cell cycle phases (i.e. the positions of θ_{G1} , θ_S and θ_r) on the cell cycle speed, β .

Both models were realized as a computer programs using Labview software assuming normal initial speed distribution and uniform initial phase distribution for the cells. The optimization of the experimental and theoretical labeled mitoses curves were fitted with the variation of θ_{G1} , θ_S and the parameters of cell-cycle speed distribution. The optimization of the labeled mitoses curve for epithelial cells of duodenum of 18 days rat embryos [3] shows that the model with decorrelated mitoses describes the experimental data better than the model with absolute correlation. (χ^2 is equal to 0.0197 and 0.0233, consequently). The similar analysis was performed for a qualitatively

different case: the culture of cells *in vitro*. The labeled mitoses curve for *in vitro* culturing human leucocytes where the inter cell communications is absent [4]. The optimization gives $\chi^2=0.0195$ and 0.0189 for the decorrelated and correlated cases, consequently. Thus our treatment demonstrated more arguments to the absence of maternal-daughter cell-cycle correlation in some tissues.

References

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