# MATHEMATICAL AND COMPUTER MODELLING REGULATORY MECHANISMS FOR PROGRAMMED CELL DEATH (APOPTOSIS)

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Abstract. The system for removing needless cells (programmed cell death - apoptosis) is important in coordination of structural-functional organisation of large number of cells in an organism. Discovery of cells suicide phenomenon has allowed revising old notions about regulation mechanisms of cellular homeostasis and importance of this mechanisms disturbance during development of large number of diseases including cancer, auto-immune diseases and infection processes. Intuitive understanding activity of the regulatory system connected by means of complex interaction mechanisms between positive and negative feedback loops is very difficult. Here it is necessary formal mathematical methods and computer tools for modelling corresponding regulatory mechanisms. This work deals with some results of the quantitative analysis of simplest regulatory mechanisms for apoptosis process based on the methods for mathematical modeling and computer simulation.

**1 Introduction** Apoptosis is the programmed cell death, an energetically dependent, and genetically controlled process, which is started by special signals, and saves an organism from unwanted, transformed and enfeebled cell's [1, 2]. This type of cells death is not accompanied by inflammatory processes unlike the necrosis [1]. The discovery of such a cell suicide phenomenon has allowed revising old notions about regulation mechanisms of cellular homeostasis and has emphasized the importance of this mechanism's disturbance in the development of a large number of diseases including cancer, auto-immune diseases and infection processes [1, 2, 3, 4].

Investigations in the decade 1995-2005 show that regulatory mechanisms of programmed cell death are very complex and are practically not changed during yhe evolution process. These facts give researchers a basis to discuss the fundamental biological role of apoptosis [1]. It was established that the cell death process by apoptosis consists of four separate stages: initial, effector, degradation and absorption. The start and realisation of the initial phase are very complex mechanisms actuating pro-apoptotic and anti-apoptotic processes. In the effector stage the regulation mechanism of pro-apoptotic and anti-apoptotic systems determines the choice of further cells fortune. If the pro-apoptotic system is predominant, then the cell"is sentenced" to death. Degradation stage is presented by typical morphological and biochemical changes and this stage is uncontrolled and inconvertible [5]. During end stage active phagocytes absorb apoptotic corpuscles [6]. Regulation disturbance in each phase can led to development of pathological processes [5, 6].

An intuitive understanding of the activity of the regulatory system associated by means of complex interaction mechanisms between positive and negative feedback loops is very difficult. Formal mathematical methods and computer tools for modelling and imitating corresponding regulatory mechanisms are necessary. Only natural cell death (necrosis) is usually considered in the mathematical modelling of cellular processes [7, 8]. In [9, 10] a

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mathematical model of cellular population kinetics restoring after irradiation is considered. This model takes both possible variants of cell death (necrosis and apoptosis) into account. Based on quantitative analysis of cellular population during embryonic development and mathematical modelling, K.Hardy et al [11] have revealed the essential role of cells apoptosis for organ formation.

The initial phase of programmed cell death and questions on quantitative analysis of apoptosis realization by caspase activation are considered in [12] using methods for mathematical modelling based on ordinary differential equations. Here using law of masses action at creation of equations like Michaelis-Menten kinetics approaches, analysis of stabilities nature of caspases cascade during apoptosis is carried out. In [13] questions on quantitative analysis of ways for apoptosis realization are considered. Assuming that protein consumption is minimal at choosing apoptosis biochemical pathways, authors research possible ways for application of optimization methods for control of apoptosis processes using mathematical modelling. Tomlinson I. and Bodmer W.F. quantitatively analyzed consequences after apoptosis mechanism failure. Using the discrete mathematical model of cells population, authors have shown a possibility for the tumors appearance due to breaking apoptosis and differentiation mechanisms [14]. Decision making on beginning apoptosis in effector phase is one of the main processes of programmed cell death. This work deals with some questions on mathematical modelling and control of the regulatory mechanisms of interconnected activity between a pro-apoptotic and anti-apoptotic systems in effector phase.

**2 Apoptosis regulatorika equations in an effector phase** In apoptosis effector phase, when the "black ticket" is received by cell, there appears question on starting suicide mechanisms or continuing functional activity. Here enormous "cellular bureaucratic apparatus" consisting of the pro-apoptotic and anti-apoptotic systems [15, 16, 17] carefully weighs up all the pros and cons. Based on interconnected activity of the pro-apoptotic and anti-apoptotic systems and results of their antagonistic "fight" the question on self-dependent liquidation is solved. Considering genetic conditionality of programmed cell death we can assume that pro-apoptotic and anti-apoptotic systems are controlled by certain gene-regulators. We suppose that

- starting apoptosis is realised if activity of the pro-apoptotic gene system is dominant;
- antagonistic "fight" between pro-apoptotic and anti-apoptotic systems is carried out on the basis of activity inhibition of corresponding genes;
- pro-apoptotic system is originally active and its functioning can actuate anti-apoptotic system.

Then, based on functional-differential equations of living systems regulatorika [18, 19] we can write the following system for regulatorika equations for interconnected activity between pro-apoptotic and anti-apoptotic system of programmed cell death:

$$\frac{dx_i(t)}{dt} = \left(a_i + l_i \prod_{k=1}^{r} x_k(t-h)\right) exp\left(-\sum_{k=1}^{r} \alpha_{1ik} x_k(t-h) - \sum_{l=1}^{m} \beta_{1il} y_l(t-h)\right) - \theta_i x_i(t);$$

$$i = 1, 2, ..., r$$

$$\frac{dy_j(t)}{dt} = b_j \left(\prod_{k=1}^{r} x_k(t-h)\right)\left(1 + c_j \prod_{k=1}^{m} y_k(t-h)\right) \times \tag{1}$$

$$\times exp\left(-\sum_{k=1}^{r}\alpha_{2jk}x_k(t-h)-\sum_{l=1}^{m}\beta_{2jl}y_l(t-h)\right)-\rho_j y_j(t), \qquad j=1,2,...,m$$

where $x_i(t), y_j(t)$ are the values, expressing activity products number for corresponding gene-regulators of the pro-apoptotic and anti-apoptotic systems; $\theta_i, \rho_j$ are the parameters, characterising average live time for corresponding gene-regulators activity products; $h$ is the time, required for feedback realisation in cell's regulatory system; $a_i, b_j$ are the parameters characterising providing of considered system with resources; $\{\alpha\}, \{\beta\}$ are repression parameters; $i=1,2,...,r$; $j=1,2,...,m$; $r,m$ are natural numbers $(r\geq 0, m\geq 0)$ , expressing the number of gene regulators for the pro-apoptotic and anti-apoptotic systems; $r$ and $m$ may identified by the degrees of self-conjugate of considered systems; all parameters are non-negative constants.

**3 Qualitative study** For analysing behaviour characteristics for solutions of functional-differential equations (1) we can use small values for $r$ and $m$. For example, simplest system (1) with zero self-conjugate degrees has the following form:

$$\begin{cases}\dfrac{dx(t)}{dt}=a\exp\left(-\alpha_1 x(t-h)-\beta_1 y(t-h)\right)-\theta x(t);\\[2ex] \dfrac{dy(t)}{dt}=bx(t-h)\exp\left(-\alpha_2 x(t-h)-\beta_2 y(t-h)\right)-\rho y(t),\end{cases} \tag{2}$$

where $x(t), y(t)$ are the functions, expressing genes-regulators number activity products for pro-apoptotic and anti-apoptotic systems in cell at time $t$ accordingly; $a, b$ are the parameters characterizing providing pro-apoptotic and anti-apoptotic systems with resources, accordingly; $\theta$ and $\rho$ are the parameters, characterizing average time for genes-regulators activity products live for pro-apoptotic and anti-apoptotic systems accordingly; $h$ is the characteristic time of feedbacks in cell. Values of all parameters and initial conditions are non-negative.

We can simplify the equations (1) assuming self-repression absence in pro-apoptotic and anti-apoptotic systems. Then denoting $\epsilon_1 = 1/\theta h$, $\epsilon_2 = 1/\rho h$ we have minimal model system for functional-differential equations (1) based on (2) in the following form:

$$\begin{cases}\epsilon_1\dfrac{dx(t)}{dt}=ae^{-y(t-1)}-x(t);\\[2ex] \epsilon_2\dfrac{dy(t)}{dt}=bx(t-1)e^{-x(t-1)}-y(t),\end{cases} \tag{3}$$

where $\epsilon_1, \epsilon_2, a, b$ are positive parameters. $\epsilon_1, \epsilon_2$ are called regulatorika parameters for pro-apoptotic and anti-apoptotic systems accordingly; $a, b$ are the parameters of pro-apoptotic and anti-apoptotic cell's potential accordingly.

In order to analyze the characteristic solutions of (3) we use the methods for the qualitative study of delay-differential equations [20, 21, 22] and ordinary differential equations [23, 24, 25]. We apply special programs [26] when accompanying the analytical studies by computer calculations.

Let $x_0, y_0$ be the equilibrium point for system (3). Then

$$ae^{-y_0} - x_0 = 0; \qquad bx_0e^{-x_0} - y_0 = 0, \tag{4}$$

where $a > 0, b > 0$. The trivial equilibrium point is absent. Using (4) we get

$$bx_0e^{-x_0} - \ln\left(\frac{a}{x_0}\right) = 0. \tag{5}$$

This relation (5) can be presented in the following form

$$\frac{e^{x_0}}{x_0}\ln\left(\frac{a}{x_0}\right) = b.$$

Let us consider

$$F(x_0) = \frac{e^{x_0}}{x_0}\ln\left(\frac{a}{x_0}\right).$$

Analysing $F(x_0)$ and its derivative functions behaviour (Figure 1) we can conclude that one equilibrium point (3) exists always. The existence of two and three equilibrium points (Figure 2) depends on the following condition

$$b_1 \le F(x_0) \le b_2,$$

where $b_1, b_2$ $(0 < b_1 \le b_2)$ are the roots of equation

$$(x-1)\ln\left(\frac{a}{x}\right) = 1.$$

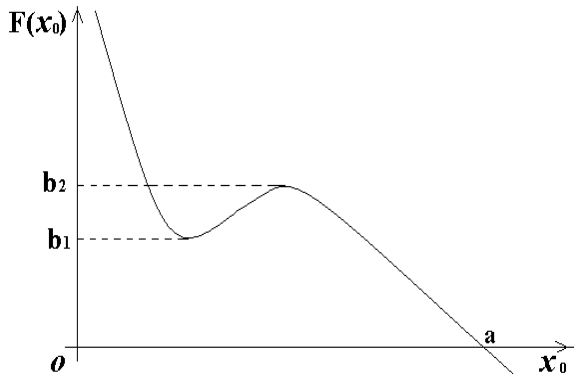


Figure 1: The nature of $F(x_0)$ behaviour.

Let us consider the case when there is only one equilibrium point (Figure 3). We see (Figure 3) that in neighborhood of the considered equilibria the isoclinals form four areas of homogeneous behavior:

- Area A: the solution grows by both variables;
- Area B: the solution grows by variables "y";
- Area C: the solution decreases by both variables;

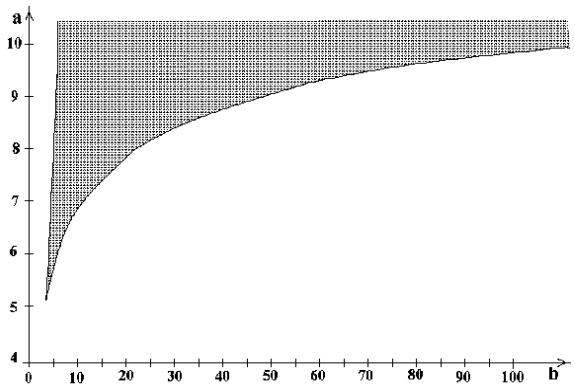


Figure 2: The existence area for positive equilibrium points (3).

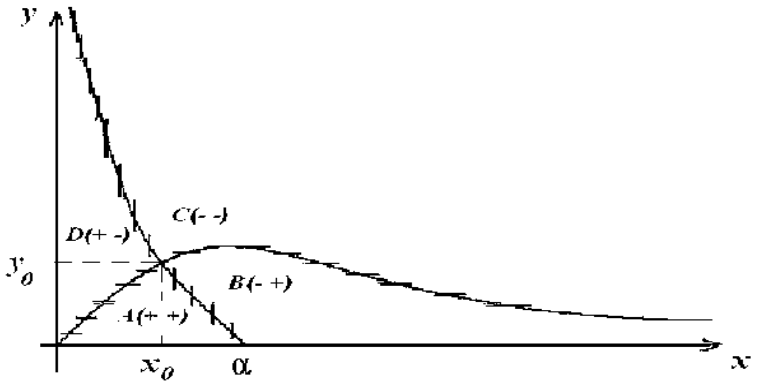


Figure 3: The isoclinic lines and gradients for the system (3).

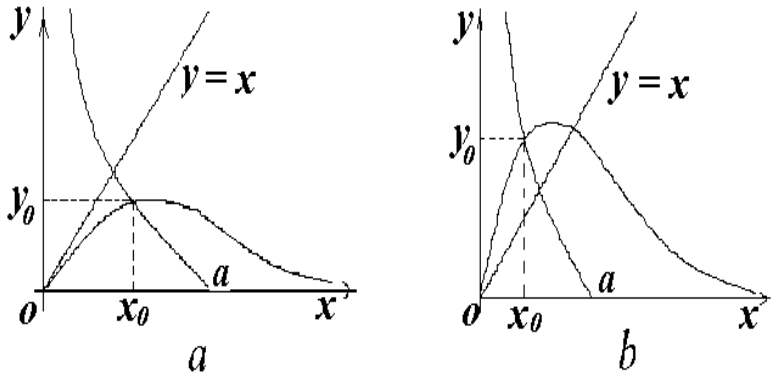


Figure 4: Predominance of pro-apoptotic ($a, y_0 < x_0$) and anti-apoptotic ($b, y_0 > x_0$) systems.

- Area D: the solution decreases by variable "y" and grows by "x".

Qualitative studying the behaviour of system (3) solutions shows (Figure 3) that the considered positive equilibria is the attractor, attracting solutions from the first quadrant of phase space. Based on the permitting suggestions we can take that, if $y_0 < x_0$ then cell come in apoptosis process, otherwise the anti-apoptotic system does not allow cell to come in apoptosis (Figure 4). For potential apoptosis presence at a positive values of the equilibria coordinates we must have predominance of pro-apoptotic systems ($y_0 < x_0$). Under this condition from second equation of (4) we have

$$be^{-x_0} < 1.$$

Hence we need the threshold value $x_0$ for predominance of the cell pro-apoptotic system ($x_0 > \ln b$). Using (4) at $y_0 = x_0$ we get

$$a = b \ln b.$$

Consequently, if $y_0 < x_0$ we have

$$a > b \ln b. \tag{6}$$

The condition (5) allows to choose the area for apoptosis start (Figure 5) in the parametric space (3) and to define cell predetermination to pro-apoptotic (anti-apoptotic) processes if the equilibrium point is stable.

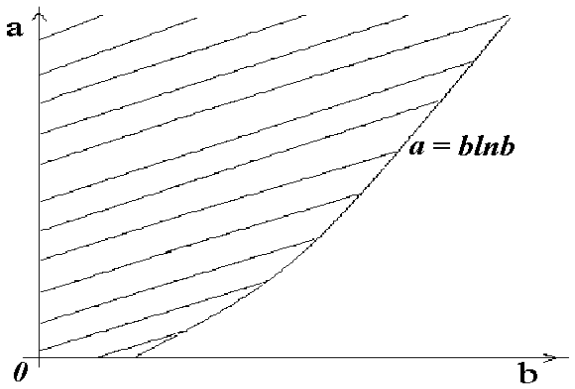


Figure 5: The area of apoptosis start in the parametric space of the system (3).

Let us consider a possibility of stability failure for positive equilibria using Lyapunov method. We replace $x(t)$ by $x_0 + x_1(t)$, $y(t)$ by $y_0 + y_1(t)$, where $x_1(t)$ and $y_1(t)$ are small. Linearizing (3) neighborhood of the equilibrium $(x_0, y_0)$ we have

$$\epsilon_1 \frac{dx_1(t)}{dt} = -x_0 y_1(t-1) - x_1(t);$$

$$\epsilon_2 \frac{dy_1(t)}{dt} = (be^{-x_0} - y_0)x_1(t-1) - y_1(t).$$

We get the characteristic equation (taking into account that $y_0 = bx_0e^{-x_0}$) :

$$\epsilon_1\epsilon_2\lambda^2 + (\epsilon_1 + \epsilon_2)\lambda + 1 + y_0(1 - x_0)e^{-2\lambda} = 0. \tag{7}$$

The analysis shows that (7) can have positive roots, for example, if

$$y_0 > \frac{1}{x_0 - 1}. \tag{8}$$

It follows that the equilibrium points are unstable if $x_0 < 1$ (Figure 6). According to (4) we have

$$y_0 = ln\left(\frac{a}{x_0}\right), \qquad y_0 = bx_0 e^{-x_0}$$

and hence, we get instability conditions (8) in the following form

$$a > x_0 e^{1/(x_0-1)}, \qquad b > \frac{e^{x_0}}{x_0}\frac{1}{(x_0-1)}. \tag{9}$$

The computer calculation allows to evaluate instability area for positive equilibrium points (3) (Figure 7).

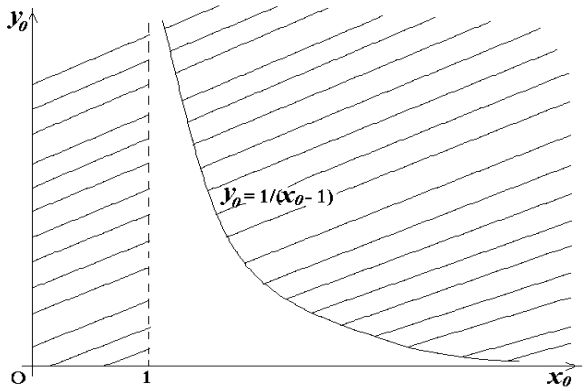


Figure 6: The existence area for positive root (7) (the shading area).

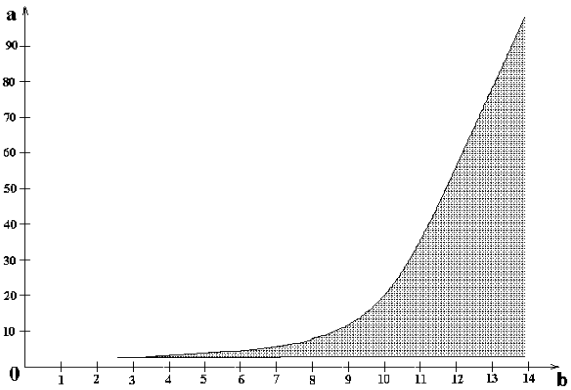


Figure 7: Instability area of equilibrium points (3) according to (9).

When considered equilibria of system (3) loses stability, then we have Poincaré type limit cycles (i.e. it is the attractor). If the Poincaré type limit cycles situated in the area of $y_0 < x_0$ or $y_0 > x_0$ (Figure 8, a) then we have pro-apoptotic (or anti-apoptotic) cell

development. If the limit cycles situated in both areas $y_0 < x_0$ and $y_0 > x_0$ (Figure 8, b), then there exist asymptotic uncertainties (anomalies) for the cell activity: the cell partly executes initial functional activity with the incomplete activation the pro-apoptotic and anti-apoptotic systems. Here probability the cell entering in apoptosis is decreased and the apoptotic index of cell population falls. Thereby, if cellular anti-apoptotic potential is high (above determined level) then the apoptotic signal can lead to the anomalous type of the cell development.

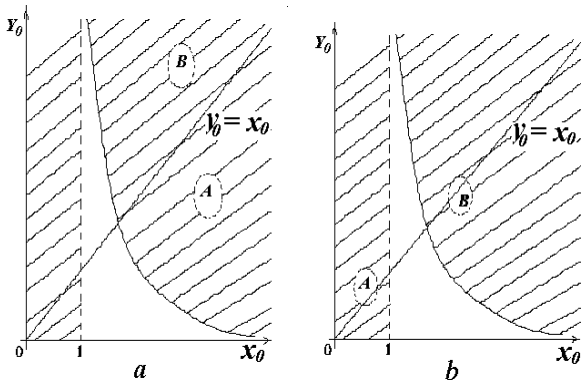


Figure 8: Possible types of the cell developments in apoptosis effector phase according to (9) (a - deterministic development by pro-apoptotic (A), anti-apoptotic (B) ways; b - abnormal behaviour).

Existence of two positive equilibrium points (3) is low-probably and for small changes of the values of parameters there exist one or three equilibrium points. At existence of three positive $(\alpha, \beta, \gamma)$ equilibrium points (Figure 2) there are two attractors - $\alpha, \gamma$, and one anti-attractor $\beta$ (Figure 9).

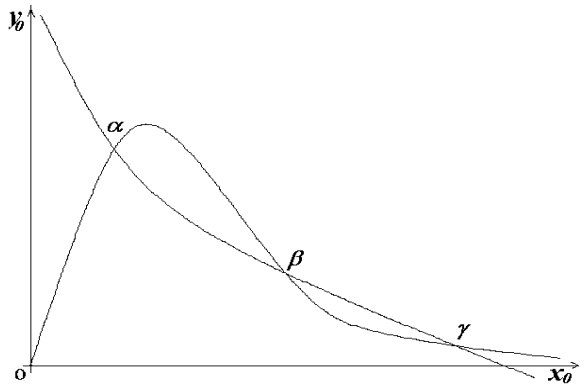


Figure 9: Existence of three equilibrium points (3) in the first quadrant.

Depending on initial conditions, the solutions (3) tends to $\alpha$ or $\gamma$. Comparison of existence area for three equilibrium points (Figure 2) and instability area (Figure 7) shows possibility of stability loss for $\alpha$ and $\gamma$ . Using computer (at $\epsilon_1 = \epsilon_2 = 1$) studies we observed auto-oscillations (Figure 10a ($a = 17, b = 25$)) and irregular oscillations (Figure 10b ($a = 10, b = 14$), Figure 11 ($a = 16, b = 15$)).

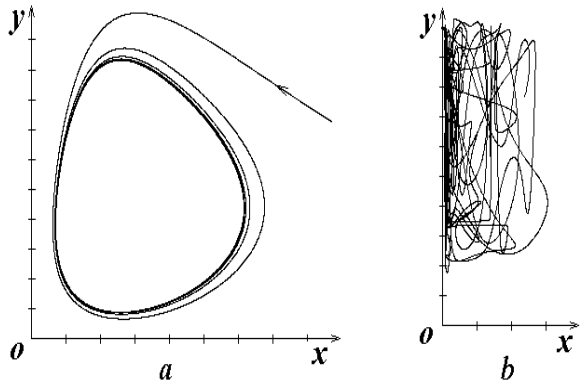


Figure 10: The regular (a) and irregular (b) solutions of (3).

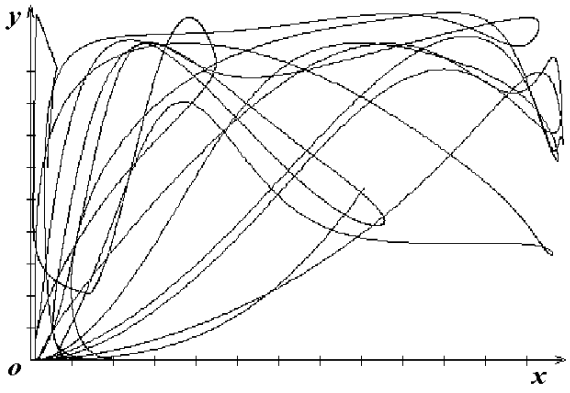


Figure 11: The chaotic nature of the equations (3) solutions.

In the last case model behaviour has the dynamic chaos nature. In some cases the oscillatory regimes envelop both sides of bisector $\angle XOY$. Here domination is passed to the pro-apoptotic and anti-apoptotic systems consecutively (but in unpredictable regime).

**4 Conclusion** Thus, results of the qualitative studies and the computing experiments have shown that there are several regimes of interconnected activity between pro-apoptotic and anti-apoptotic systems: predominance one of them, periodic activation and unpredictable, chaotic predominance. Consequently, if there is apoptotic signal, then cells with the pro-apoptotic and anti-apoptotic development, cells with asymptotically unpredictable (abnormal) activity and cells with chaotic behaviour can be observed in multi-cellular organisms. In the last case the normal functioning is disturbed in cell's molecular-genetic system (activation of silent genes, intensification of genes mutations, failure of cellular regulatorika etc.).

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