

## Supplementary Material S1

### Simplified Reaction Equations Used for Modeling Elementary Acts of Proliferation and Apoptosis

Elementary reaction equations could be derived assuming that regulator molecules are not consumed in the process:

**A cell of certain age + regulator molecule --(division)--> two young cells + regulator molecule;**

**A cell of certain age + regulator molecule --(apoptosis)--> regulator molecule**

This is a typical example of simple catalytic reaction equations, where the regulatory molecule plays the role of the catalyzer.

Regulatory substances, as many of other bioactive molecules, could have limited lifetime. Thus, adequate models should consider the corresponding age of both cells and the regulating molecules. Thus, elementary reaction equations could be written using the following notation:

**$C(t_1) + R(t_3) \rightarrow 2C(0) + R(t_3 + dt)$  {proliferation};  $C(t_2) + R(t_4) \rightarrow R(t_4 + dt)$  {apoptosis}**

where  $C(...)$  relates to a cell,  $R(...)$  relates to a regulating molecule,  $t_1$  and  $t_2$  are the age of the involved cells,  $t_3$  and  $t_4$  are the age of the involved regulatory molecules, and  $dt$  signifies the formal duration of elementary reaction stage. These equations also presume that newly born cells have full life potential of their mothers, and are born with an age equal to zero.

It is not yet considered that efficiency of all elementary processes could be less than 100%. Thus, to support reaction continuity, the number of the regulatory molecules should be growing. To maintain the stable proliferation cascade, and to provide a fast increase in the numbers of cells - as it is in cases of intense tissue regeneration and wound healing - regulator molecules should both trigger elementary acts of the proliferation and apoptosis but are also should be synthesized during proliferation.

So far, there is no proof that the elementary processes are of the 'catalytic type' represented by the above elementary reaction equations or the regulatory molecules are consumed in process and new are synthesized. If we consider the situation that regulator molecules could be consumed and synthesized, the elementary reaction equations should be changed to:

**$C(t_1) + R(t_3) \rightarrow 2C(0) + R^*$  {proliferation};  $C(t_2) + R(t_4) \rightarrow R(t_4 + dt)/\text{nothing}$  {apoptosis}**

where  $R^*$  denotes either aged initial and a newly synthesized regulatory molecules  $2R^* = R(t_3 + dt) + R(0)$ , or two newly synthesized molecules  $2R^* = 2R(0)$ , depending if the elementary process is of a catalytic- or new-synthesis type. Similarly, the elementary reaction for the apoptosis could also be catalytic-type. In such case, a regulatory molecule only triggers the elementary act and ages ( $R(t_4 + dt)$ ). It is possible that in the act of apoptosis regulatory molecule is also consumed. There is no experimental proof which of the elementary reaction scenarios is realized. Thus corresponding simulator should be capable of testing the situations with both synthesis- and catalytic-type processes.

Corresponding simulator should be capable of monitoring coupled populations with quite large numbers of elements: cells with corresponding age distributions and regulatory molecules with corresponding own age distributions. In addition, to handle the models with quantized elementary reaction steps one should turn from time continuity to discrete time steps  $t = n \cdot \Delta t$ . Thus, simulator deals with two sets of integers  $C_n$  and  $R_k$ , where corresponding values ( $C$  and  $R$ ) represent the numbers of cells and regulating molecules with corresponding age ( $n \cdot \Delta t$  and  $k \cdot \Delta t$ ). At each step, simulating program extracts and compares the numbers of available regulating molecules and cells, along with their current age profiles. Depending on the control settings, it decides: which of the cells and how many of them will divide; which cells and how many of them will be removed by the apoptosis, and which regulation molecules and how many of them will be removed due to their limited lifetime. Differences in the 'elementary reaction rates' are taken into account by allowing the elementary act to take place only after chosen software cycle numbers. Flow diagram of the simulator showing more details is presented in Supplementary Material S2. Supplementary material S3 presents the view of the virtual control panel of the simulator, showing windows for the control parameters that can be changed in process of simulation, windows for the output parameters and the graphs illustrating the current state of the system.

The above equations, with the synthesis of regulatory molecules and continuous division of cells (1-2-4-8-16...), are similar to the ones describing chain reactions. Many of the concepts used for successful modeling of such reactions are applicable in our case. In ideal case, when all processes are having 100% efficiency, corresponding cell and regulatory molecule numbers would grow infinitely, and no stationary situation is possible. Introducing coefficients that account for decreased efficiency of elementary reactions is one of the known options allowing reaching steady state. In present case decreased proliferation reaction efficiency means, that although a regulatory molecule reaches the cell capable of division, actual division is happening in less than 100% cases. In the simulator it is accounted for by introducing division probability coefficient less than 1. Similar probability coefficient is introduced for the efficiency of apoptosis act.

There is no experimental data at this time that can be used in determining corresponding probability values and functions, so the proposed simulator uses synthesized functions developed basing on minimal available data and certain reasoning. For example, although it is known that apoptosis favors senescent cells, there is no data how the corresponding probability of cell removal depends on cell age. So far, one can only suggest that probability of cell removal by the apoptosis should be growing with the age of cells. In addition, in the proliferation branch, synthesized probability function for cell division with the probability decreasing with the cell age basing on existing experimental indications. During simulation trials, few different probability functions were tested. Although output parameter values were changing, general system dynamics showing characteristic 'infancy', 'youth', 'maturity' and 'old age' stages was similar.

## Main Statements Used During the Development of Simulator Software Code

Following fundamental postulates were used in the simulation program.

Meaning of the corresponding superscript notation:

**a**- axiom; **\***- proven experimentally; **\*\***- strong experimental evidence exists; **?**- not proven, but certain indications in the literature or in experiments exist; **bold text** - necessary postulates (basic); underlined - accepted in the model, but at the moment it is unclear if this is relevant for the basic version of the simulator. Brief comments are given in *italic*.

Note also, that used terms 'stem cells' and 'multipotent cells' are generalized to define only most generic differences important for the basic model, and do not incorporate fine features commonly assumed in the publications related to cell biology.

### Regulation Mechanisms

**(1<sup>a</sup>) Homeostasis in the proliferation niche (non-pathologic case) is controlled by the concentration of regulating biochemical substance.**

*This is a main hypothesis, taken as an axiom.*

**(2<sup>\*</sup>) Regulating mechanisms supporting cell number maintenance in the proliferation niche (proliferation as a positive and apoptosis- as negative feedback branches) are intrinsic.**

*It based on different experimental results and has a serious reasoning support.*

**(3<sup>\*</sup>) Model accounts for only one apoptosis mechanism, sensitive to the age of the cells.**

*Modern biology separates different apoptosis pathways, but for simplicity basic model accounts for only one.*

### Cell Types and Their Basic Properties

**(4<sup>\*\*</sup>) Start to the proliferation cascade is given by the stem cell, and is further supported by its daughters.**

*It has numerous experimental confirmations and is described in a number of publications.*

**(5<sup>a,\*</sup>) Stem cells are formally immortal, but can become 'dormant'.**

*Immortality of the stem cells was used for simplification of the first versions of the simulator. In the latest version of the model, stem cell death 'with age' is also incorporated as an option, but it is not critical for the main results. Immortality of the stem cells if they have Hayflick limit of divisions does not lead to the immortality of the system.*

**(6<sup>a</sup>) Dormant stem cells cannot divide. They can be woken up by certain concentration of the regulatory molecules. After waking up, they again become receptive for stimulation of proliferation. With the increased dormant time, concentration of the regulator waking up stem cell increases.**

*Experimental evidence exists that dormant stem cells do not express characteristic receptors, and are not taking part in the active metabolism. Wakeup 'by the concentration of the regulator' is a hypothesis, supported mainly by the results of simulations and special experiments. In latest version of the simulator, there is an option: stem cells that are dormant longer than certain time effectively die and are excluded from further activities.*

**(7<sup>a</sup>) Stem cells become dormant, if they do not divide during certain time.**

*There is no direct evidence, but the simulator should be able to reproduce the ability of ordinary tissue to regenerate and significantly increase proliferation for rapid healing; the appearance of a significant number of proliferating cells during intensive healing and a return to a "maintenance" rate of division after healing is completed. In addition, it was impossible to achieve stable conditions without taking into account this provision, and provisions (3)-(6).*

**(8\*) Consecutive generations of the daughter cells have decreasing proliferation potential. The model distinguishes three types of cells, accounting for changes in the proliferation potential and possible difference in the rate of the division.**

*Decreasing proliferation potential in the line from stem cells: totipotent, pluripotent, multipotent and specialized (somatic) daughter cells, is shown experimentally.*

**(9<sup>a</sup>) Basic simulator accounts for only three different types of the cells, disregarding possibility of gradual changes in consecutive generations of daughter cells:**

- 'initial' stem cells (analog of embryonic ones), with full proliferation capacity; elementary act of proliferation: generation of exact multipotent daughter; ability become dormant and to wake up, when demanded; may be immortal, may be not.
- multipotent daughter cells ('2d generation'), with high proliferation capacity; elementary act of proliferation: division, resulting in two specialized cells; they do not have capacity to become dormant; not immortal, removed by apoptosis depending on age.
- specialized cells (somatic cells, '3d generation'), without any capacity for proliferation, removed by apoptosis depending on age. Option: some of them can be induced into dividing state (become multipotent).

*There is optional possibility to revert the specialized cells that have lost their proliferation potential to active proliferation using certain interventions (induction by the so-called Yamanaka factors is incorporated into the latest simulator version).*

**(10\*) There is a limit for the number of divisions for both stem and multipotent cells, and specialized cells do not have potential to divide at all without induction.**

*Although it is not entirely clear if the Heyflick limit suggested by the telomere theory is a 'hard one' (e.g. the limit of the cell divisions is definite), or it is rather soft (in some cases little more divisions than 'threshold' value is permitted), simulator realizes a 'hard limit case'. After reaching limiting division number, such cells are excluded from the corresponding cell pool.*

### **Regulating Substance and Its Properties**

**(11\*) Regulating substance is synthesized during proliferation.**

*There is a direct experimental proof.*

**(12\*) Regulating substance has limited lifetime in water or buffer solutions.**

*Direct experiments point to simple kinetics with close to exponential decay with characteristic time constant of about 36 hours.*

**(13<sup>a</sup>) Regulating substance is consumed in the proliferation and apoptosis**

*Certain versions of the program were using the concept of the regulating factor being a catalyzer. It makes a difference to the 'timing' part only: newly synthesized regulating molecule has zero lifetime counter, after participating in a catalytic reaction regulator continues 'aging'. Choosing one or another option does not bring any significant differences into the simulation results.*

### **External Interventions**

Such inputs are needed to mimic certain external interventions

**(14\*) Simulator has a possibility of 'waking up' chosen number of dormant stem cells at any moment during the simulation.**

*This is needed to account for the possibilities related to the intensification of the proliferation linked for example to the oxidative stress.*

**(15\*) Simulator has a possibility of adding chosen number of stem cells at any moment during the simulation.**

*This is needed to model the results of stem cell therapy- type interventions.*

**(16\*) Simulator has a possibility of forcing cell divisions at any moment during the simulation without synthesizing regulation substance.**

*This is needed to model the results of experiments with the addition of mitogen substances.*

**(17\*) Simulator has a possibility of forcibly removing some cells of certain age at any moment during the simulation.**

*This is needed to model the results of injuries, or results of interventions using chemical substances with senolytic action.*