RESILIENCE IN STEM CELL RENEWAL: DEVELOPMENT OF THE AGUR–DANIEL–GINOSAR MODEL

OLEG U. KIRNASOVSKY, YURI KOGAN, ZVIA AGUR

Institute for Medical BioMathematics (IMBM)
10 Hate'ena St.
P.O. Box 282
60991 Bene Ataroth, Israel

ABSTRACT. This work is based on a previous model of Z. Agur, Y. Daniel and Y. Ginosar (2002), retrieving the essential properties of homeostatic tissue development, as reflected by the bone marrow. The original model, represented by cellular automata on a connected, locally finite undirected graph, identifies the minimal basic properties essential for maintaining tissue homeostasis and for guaranteeing the ability of a few stem cells to repopulate the tissue following its depletion. However, this model is too general to ensure a relative "stability" of stem cell numbers in the tissue, a prerequisite for the integrity of biological systems. In the present work, some natural limitations on the model are introduced, under which a formula for the state of a given cell at any given time is obtained, as well as for the proportion of stem cells as a function of model parameters. For tube-like graphs, defined for modeling tissue engineering scaffolds and known tumor geometries, the system obtains a fixed cellular composition, interpreted as homeostasis, thus enabling precise calculation of the necessary conditions for tissue reconstruction. These results also can shed light on conditions for disrupting homeostasis in cancerous tissues.

1. **Introduction.** Homeostasis, that is, the ability of living organisms to maintain their physical integrity even under severe perturbations, is the most fundamental property of complex organisms. For example, it guarantees the ability of a few stem cells to repopulate a depleted bone marrow after extensive therapeutic irradiation [3]. A downside of the same homeostatic property is the persistent tumor growth under intensive chemotherapy. Tumor integrity is still maintained by a relatively small number of cancer stem cells even if the majority of cancer cells are successfully eliminated by the drug.

Stem cells are unspecialized cells that can develop into the many different cell types constituting our body. They can theoretically divide without limit, so as to replenish cells of different organs, such as skin, bone, blood, brain etc. Stem cell research has thus aroused considerable excitement in the medical research community worldwide, due to the potential applications in fighting serious medical conditions, such as cancer or birth defects, which are due to problems that occur during the process of stem cell development. If researchers better understand the process of stem cell development, they can perhaps fix disease-causing errors. Moreover, stem cell research has potential applications in replacing damaged organs and tissues in

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patients, currently possible only by donation from a healthy donor — a difficult and often impossible task. Stem cells are thus a potentially renewable replacement source which might be used to treat numerous diseases including Parkinson's and Alzheimer's diseases, spinal cord injuries, stroke, burn injuries, heart disease and many others [6].

To address such medical challenges, we need to better understand the mechanisms by which the balance between stem cell self-renewal and differentiation is controlled. This understanding will enable medical manipulation of tissue development so as to maintain tissue homeostasis under disruptive medical interventions, or to upset it, for example, in treating oncologic diseases. The model presented here is simulated elsewhere for analyzing this problem in the context of tissue damage in the intestine, caused by Helicobacter pylori [5]. Results show that the proliferation-differentiation balance is primarily determined by the magnitude of cell-to-cell signalling [2]. For differential drug treatment and for optimizing tissue engineering it is imperative to evaluate the cellular composition of the developing tissue at any given moment, as well as the time until cellular structure stability of the replenished, or reconstructed, tissue is attained. These latter tasks are taken up in the present work, where the general model is restricted to describe developing tissues such as bone marrow, intestines crypts and some solid tumor geometries.

In [1], a simple discrete model was introduced, based on a minimal number of basic cell properties. This model suffices for showing how a few stem cells can repopulate a depleted tissue and system's homeostasis can be maintained. The reader is cross-referred to the work [1] for the history and biological background of the theme.

However, although the ability to maintain a minimal lower bound on cell numbers is shown in the aforesaid work, so that the system is never extinguished, the model is too general to guarantee a certain "stability" of the stem cell number in the tissue. In other words, it does not exclude the possibility that large fluctuations exist in the system so that tissue's resilience is at risk.

2. **ADG-models.** In [1], $G = \langle V, E \rangle$ is a connected, locally finite undirected graph. The set V of its vertices describes the set of all places for bone marrow cells. The set E of graph edges describes neighbourhoods of influence in the bone marrow. A *state of a vertex* is a pair. The first coordinate denotes the cell type: S, D or N. Here, S is the designation for a stem cell, D for differentiated cell, and N represents a vacant site in the tissue for a new cell to seed in. The second coordinate is a non-negative integer which denotes an internal counter of the corresponding vertex. A state of the graph is understood as states of all vertices of the graph. More formally, a *state of the graph* is a mapping from the set of all its vertices into the set of all possible vertex states.

An iterative operator on the set of all states of the graph is constructed in [1]. The operator depends on three non-negative integers Φ, Ψ and Θ, which represent life span of differentiated cells, minimal time for differentiation of stem cell and cell cycle length, respectively, the time units being the model steps. The state of the graph at time t is denoted by x^t. Then $x^t(v)$ means the state of a vertex v at time t, so that there exists the initial state x^0 at time 0. The iterative operator is

determined by the following conditions:

$$x^t(v) = \langle D, \tau \rangle \Longrightarrow x^{t+1}(v) = \begin{cases} \langle N, 0 \rangle & \text{if } \tau = \Phi, \\ \langle D, \tau + 1 \rangle & \text{otherwise}; \end{cases}$$

$$x^t(v) = \langle S, \tau \rangle \Longrightarrow$$
$$\Longrightarrow x^{t+1}(v) = \begin{cases} \langle D, 0 \rangle & \text{if } \tau = \Psi \text{ and each } v\text{'s neighbour is a stem cell}, \\ \langle S, \tau \rangle & \text{if } \tau = \Psi \text{ and there exists a non-stem neighbour of } v, \\ \langle S, \tau + 1 \rangle & \text{otherwise}; \end{cases}$$

$$x^t(v) = \langle N, \tau \rangle \Longrightarrow x^{t+1}(v) = \begin{cases} \langle S, 0 \rangle & \text{if } \tau = \Theta \text{ and } v \text{ has a stem neighbour}, \\ \langle N, \tau + 1 \rangle & \text{if } \tau < \Theta \text{ and } v \text{ has a stem neighbour}, \\ \langle N, 0 \rangle & \text{otherwise}. \end{cases}$$

Thus, the above iterative operator represents a single time step in the modelled tissue:

- a differentiated cell at the stage τ (state $\langle D, \tau \rangle$), with $\tau < \Phi$, increases its life-time counter to state $\langle D, \tau + 1 \rangle$;
- a differentiated cell at the last stage of its life time (state $\langle D, \Phi \rangle$) dies and its state becomes $\langle N, 0 \rangle$;
- a stem cell at the stage τ (state $\langle S, \tau \rangle$), with $\tau < \Psi$, increases its differentiation counter to state $\langle S, \tau + 1 \rangle$;
- a stem cell at the last stage (state $\langle S, \Psi \rangle$) either remains in the same state (when it has a non-stem neighbour, including vacant site), or differentiates to state $\langle D, 0 \rangle$ (when all its neighbours are stem cells);
- a vacant cell at the stage τ (state $\langle N, \tau \rangle$), with $\tau < \Theta$, which has a stem neighbour, increases its cell-cycle counter to state $\langle N, \tau + 1 \rangle$;
- a vacant cell at the last stage (state $\langle N, \Theta \rangle$), which has a stem neighbour, becomes a stem cell with the state $\langle S, 0 \rangle$;
- a vacant cell at the stage τ (state $\langle N, \tau \rangle$), which has no stem neighbour, accepts the state $\langle N, 0 \rangle$ (see Remark in the third paragraph of section 4).

We see that for every connected, locally finite graph G, every initial state and every triple of parameters Φ, Ψ and Θ, a separate model is constructed. Let us call it *the Agur–Daniel–Ginosar model (shortly, ADG-model) of the type* $\langle \Phi, \Psi, \Theta \rangle$ *on the graph G with the initial state x^0.*

In the ADG-model the number of daughters of a stem cell depends on the number of N-vertices neighboring the S-vertex considered as the dividing stem cell. This property of the model agrees with the empirical decreasing S-shaped curve which describes the fraction of proliferating cells as a function of cell density [7, 9]. At low density, cells proliferate at a maximal rate, and at higher densities, when cells are confined by space and nutrition is limited, the percent of dividing cells will diminish. At a certain limit, proliferation rate will tend to zero and a further elevation of cell density will not considerably affect cell proliferation. This sigmoid-like curve characterizing natural cell proliferation is also portrayed in the ADG-model. Let proliferation rate at time t be the ratio between the number of N-vertices adjacent to at least one S-vertex (and becoming S-vertices at some later moment, so that we consider all the neighbors of S-vertex that are in the state $\langle N, \tau \rangle$ with $0 \leqslant \tau \leqslant \Theta$) to the number of all S-vertices. This number actually is proportional to the fraction of new S-vertices within the total number of S-vertices at the moment t. Cell density is described as the sum of S- and D-vertex numbers in the graph. When considering the

relation between proliferation rate and cell density, the ADG-model also describes a low-density range, in which all S-vertices are adjacent merely to N-vertices. At this range, the rate of proliferation, i.e. N to S transition, is high, and is slightly reduced when elevating cell density. There comes a point at which N-vertices are simultaneously adjacent to more than one S-vertex and that is when proliferation rate decreases considerably with the elevation of cell density. This effect of "mutual" N-vertices increases when cell density is high, and, correspondingly, the effect of "free" S-vertices (meaning S-vertices surrounded merely by N-vertices) decreases. Above a certain density the changes in proliferation rate are negligible, and at the maximal cell density it will be equal to zero. These results are a property of the model, reflecting the behavior of tissue cell populations and serving as an additional support for the model validity.

3. **Regular subsets.** In [1] some general results on ADG-models are obtained. Here we limit the heterogenous class of all ADG-models in order to obtain more detailed results, which may prove useful in the future. This description can be viewed as defining properties of a stem cell signaling and proliferation network, which is highly dependent on the available settling sites in the developing tissue (bone marrow, intestine, etc.), as well as on the distance between stem cells and the boundaries of the tissue. To follow the spread of the stem cells in the developing tissue we define "culs-de-sac" as those stem cell sites, from which no further spread is possible.

Let us denote by $\rho(u,v)$ the distance between vertices u and v of a considered connected graph in the shortest-path metric induced by it. And, similarly, let us denote by $\rho(u,U)$ the distance between a vertex u and a non-empty subset U (when it is clear from the context that it is a set of graph vertices) of the graph base set:

$$\rho(u,U) = \min_{v \in U} \rho(u,v).$$

It is obvious that any couple of adjacent sites vary in distance from any subset of the graph by no more than 1. Therefore in the following analysis we take for granted that

$$(\forall u, v \in V)(\forall U \subseteq V)\left(U \neq \varnothing \ \& \ \rho(u,v) = 1 \longrightarrow\right.$$
$$\left.\longrightarrow \rho(u,U) - 1 \leqslant \rho(v,U) \leqslant \rho(u,U) + 1\right).$$

A vertex u of a connected graph will be called a *cul-de-sac for a non-empty set U of graph vertices in the graph*, iff there is no vertex $v \neq u$ of the graph such that the equality

$$\rho(v,U) = \rho(u,U) + \rho(u,v)$$

holds. It is clear that u is a cul-de-sac for U in the graph if and only if there is no vertex v such that the following equalities hold:

$$\rho(u,v) = 1, \qquad \rho(v,U) = \rho(u,U) + 1. \tag{1}$$

A cul-de-sac u for a set U in the graph will be called *strong*, iff there is no vertex v such that the equalities

$$\rho(u,v) = 2, \qquad \rho(v,U) = \rho(u,U) + 1$$

hold, and *weak* otherwise. It is clear that the cul-de-sac u for U is strong if and only if for every vertex v, for which

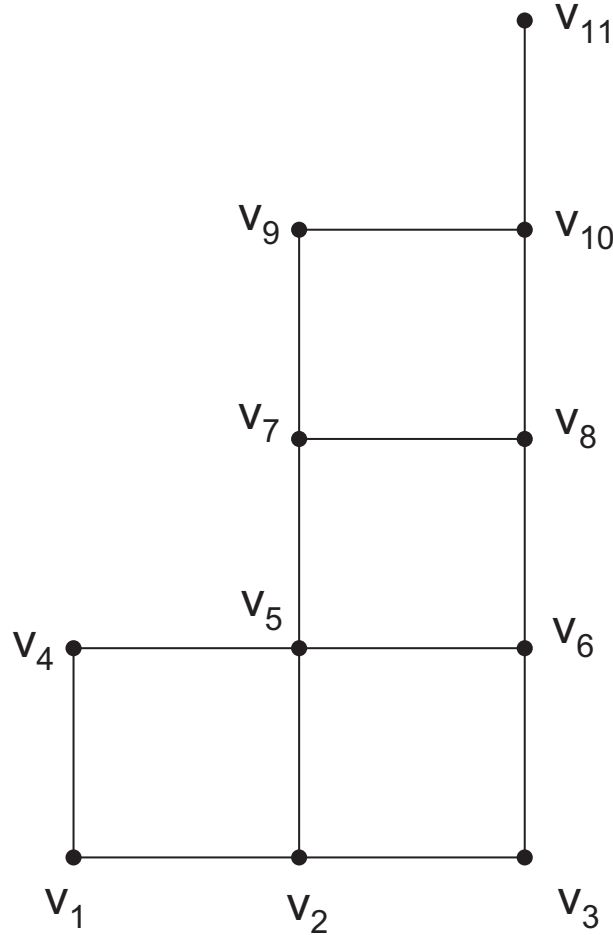$$\rho(u,v) = 1, \qquad \rho(u,U) = \rho(v,U),$$

FIGURE 1. An example of a graph as presented by its diagram. In this example, the vertex v_{11} is a strong cul-de-sac for the set $\{v_1; \dots; v_6\}$, while the vertex v_9 is a weak cul-de-sac for the same set.

v is a cul-de-sac for U in the graph. For example, in the graph, presented by its diagram in Figure 1, the vertex v_{11} is a strong cul-de-sac for the set $\{v_1; \dots; v_6\}$, while the vertex v_9 is a weak cul-de-sac for the same set.

Let the value C_u for every vertex u of a connected graph be determined by the following condition (where the graph and the non-empty set U of graph vertices are clear from the context):

$$C_u = \begin{cases} 0 & \text{if the vertex } u \text{ is a cul-de-sac for a set } U \text{ in the graph,} \\ 1 & \text{otherwise.} \end{cases}$$

A non-empty set U of vertices of a connected graph $\langle V, E \rangle$ will be called a *regular subset of the graph*, iff each vertex from U is connected by an edge with a vertex from $V \setminus U$, and for every cul-de-sac u for U in the graph the integer $\rho(u, U)$ is even and the cul-de-sac is strong.

Example 1. *Let k and n be non-negative integers, a_1, $\ldots$, a_k be positive even integers, V be the following subset of the $(k+n)$-dimensional space[1]:*

$$V = \{\langle x_1, \ldots, x_{k+n}\rangle \in \mathbb{Z}^{k+n} \,|\, (\forall i \leqslant k)\, 0 \leqslant x_i \leqslant a_i\},$$

E be a set of all pairs of neighbour points (i.e. connected by an edge) of V satisfying the following formula:

$$\langle \langle x_1, \ldots, x_{k+n}\rangle, \langle y_1, \ldots, y_{k+n}\rangle \rangle \in E \longleftrightarrow \sum_{i=1}^{k+n} |x_i - y_i| = 1. \qquad (2)$$

Then each subset of the form $M_1 \times \cdots \times M_{k+n} \subseteq V$, where for all i and for all $x, y \in M_i$ the conditions

$$x \equiv 0 \pmod 2, \qquad x \equiv y \pmod 4$$

are valid, is a regular subset of the graph $\langle V, E\rangle$.

Example 2. *Same as in Example 1, but replacing the definition (2) of E with the following formula:*

$$\langle \langle x_1, \ldots, x_{k+n}\rangle, \langle y_1, \ldots, y_{k+n}\rangle \rangle \in E \longleftrightarrow \Big((\forall i)\, |x_i - y_i| \leqslant 1\Big) \,\&\, \Big((\exists i)\, x_i \neq y_i\Big).$$

4. **Regular ADG-models.** Transplantation of donor stem cells to a depleted bone marrow justifies the consideration of initial states in which there are no differentiated cells and only a few stem cells, because, due to [1], even one initial stem cell can well repopulate the entire tissue. It is biologically realistic to add to the condition that all internal counters are equal to 0, that the life span of differentiated cells is much longer than the stem cell-cycle time and that the process of differentiation is relatively short. To consider such cases we define regular ADG-models as follows hereafter. Note that the justifications for making various simplifications are discussed in details in [2].

An ADG-model of a type $\langle \Phi, \Psi, \Theta\rangle$ with an initial state x^0 will be called *regular*, iff there exists a regular subset U of the graph such that the following conditions hold:

$$\Phi \geqslant \Theta, \qquad \Psi \leqslant 2\Theta + 1, \qquad x^0(u) = \begin{cases} \langle S, 0\rangle & \text{if } u \in U, \\ \langle N, 0\rangle & \text{otherwise.} \end{cases}$$

Then the set U will be called the *corresponding regular subset*.

Remark 1. Note that if the property

$$(\forall i, u)\, \Big(x^0(u) = \langle N, i\rangle \longrightarrow i = 0\Big)$$

of the initial state is fulfilled (this takes place always in the case of regular ADG-models), then the following formula[2] is true:

$$(\forall k, u)\Big(\forall i \in \{1; \ldots; \Theta - 1\}\Big)\Big(x^k(u) = \langle N, i\rangle \longrightarrow x^{k+1}(u) = \langle N, i+1\rangle\Big).$$

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[1]It is natural to consider the case $k + n = 3$ only. But the other cases can be represented naturally as well, because every graph has a suitable representation (diagram) in the three-dimensional space.

[2]It is evident that in [1] this formula is taken for granted, without explicitly mentioning.

Indeed, the equality $x^k(u) = \langle N, i \rangle$ for positive integers i and k implies the equality $x^{k-1}(u) = \langle N, i-1 \rangle$ and existence of a stem neighbor s of the vertex u at time $k-1$; however the stem neighbor does not become a differentiated cell at time k, due to the fact that u is a non-stem neighbor of s at time $k-1$.

Let $(\dot{-})$ denote the following binary operation on the set $\mathbb{Z}$:

$$x \dot{-} y = \begin{cases} x - y & \text{if } x \geqslant y, \\ 0 & \text{otherwise.} \end{cases}$$

In the following we use the symbol "{" to denote a system (a conjunction) of few formulae, while the symbol "[" is used, in an analogous way, to denote a disjunction.

The proof of the following statement can be found in the Appendix.

Lemma 4.1. *Let a tissue be described by a regular ADG-model of a type $\langle \Phi, \Psi, \Theta \rangle$ and U be the corresponding regular subset. At time $t \geqslant 0$ a vertex u of the graph is in the following state $x^t(u)$:*

- $\langle N, 0 \rangle$ *if*

$$\left[\begin{array}{ll} t \leqslant (\Theta+1)(\rho(u,U)-1), & \text{(a)} \\ \left\{ \begin{array}{ll} \rho(u,U) \equiv 0 \pmod 2, & \text{(b)} \\ t > \Psi + C_u \cdot ((\Theta+1)\dot{-}\Psi) + (\Theta+1) \cdot \rho(u,U), & \text{(c)} \\ t \equiv \Phi + \Psi + 2 + C_u \cdot ((\Theta+1)\dot{-}\Psi) + \\ \quad + (\Theta+1) \cdot \rho(u,U) \pmod{\Phi+\Psi+\Theta+3}; & \text{(d)} \end{array} \right. \end{array} \right. \qquad (3)$$

- $\langle N, \tau \rangle$ *(where $\tau \in \{1; \ldots; \Theta\}$) if*

$$\left[\begin{array}{ll} t = \tau + (\Theta+1) \cdot (\rho(u,U)-1), & \text{(a)} \\ \left\{ \begin{array}{l} (\textit{3}b), \\ (\textit{3}c), \\ t \equiv \Phi + \Psi + \tau + 2 + C_u \cdot ((\Theta+1)\dot{-}\Psi) + \\ \quad + (\Theta+1) \cdot \rho(u,U) \pmod{\Phi+\Psi+\Theta+3}; \end{array} \right. & \text{(b)} \end{array} \right. \qquad (4)$$

- $\langle S, \tau \rangle$ *(where $\tau \in \{0; \ldots; \Psi-1\}$) if*

$$\left[\begin{array}{ll} t = \tau + (\Theta+1) \cdot \rho(u,U), & \text{(a)} \\ \left\{ \begin{array}{l} (\textit{3}b), \\ (\textit{3}c), \\ t \equiv \tau + C_u \cdot ((\Theta+1)\dot{-}\Psi) + \\ \quad + (\Theta+1) \cdot \rho(u,U) \pmod{\Phi+\Psi+\Theta+3}; \end{array} \right. & \text{(b)} \end{array} \right. \qquad (5)$$

- $\langle S, \Psi \rangle$ *if*

$$\left\{ \begin{array}{ll} t \geqslant \Psi + (\Theta+1) \cdot \rho(u,U), & \text{(a)} \\ \left[\begin{array}{ll} \rho(u,U) \equiv 1 \pmod 2, & \text{(b)} \\ t < \Psi + C_u \cdot ((\Theta+1)\dot{-}\Psi) + (\Theta+1) \cdot \rho(u,U), & \text{(c)} \\ t \equiv \Psi + C_u \cdot ((\Theta+1)\dot{-}\Psi) + \\ \quad + (\Theta+1) \cdot \rho(u,U) \pmod{\Phi+\Psi+\Theta+3}; & \text{(d)} \end{array} \right. \end{array} \right. \qquad (6)$$

- $\langle D, \tau \rangle$ *(where $\tau \in \{0; \ldots; \Phi\}$) if*

$$\left\{ \begin{array}{ll} (\textit{3}b), \\ (\textit{3}c), \\ t \equiv \Psi + \tau + 1 + C_u \cdot ((\Theta+1)\dot{-}\Psi) + \\ \quad + (\Theta+1) \cdot \rho(u,U) \pmod{\Phi+\Psi+\Theta+3}. & \text{(a)} \end{array} \right. \qquad (7)$$

The enumerated cases are all possible ones.

Remark 2. The above lemma allows to determine the state of any vertex of the graph at time t, depending on its distance from the initial set. To facilitate the later use of the results of this lemma we also formulate its statement for different positions of u, relatively to U, and for sequential time periods.

Consider ADG-model of a type $\langle \Phi, \Psi, \Theta \rangle$ with the corresponding regular subset U. Let u be a vertex.

Define $P = \Phi + \Psi + \Theta + 3$, $t_1 = (\Theta + 1)(\rho(u, U) - 1)$, $t_2 = (\Theta + 1)\rho(u, U)$.

- If $u \in U$, then:
  - for $0 \leqslant t \leqslant \Psi$, the state of u is $\langle S, t \rangle$,
  - for $\Psi + 1 \leqslant t \leqslant \max\{\Psi, \Theta + 1\}$, the state of u is $\langle S, \Psi \rangle$
  - for $t > \max\{\Psi, \Theta + 1\}$ the states of u are periodical with period P. Let τ be the remainder of $t - \max\{\Psi, \Theta + 1\} - 1$ modulo P. Then the state of u is:
    * $\langle D, \tau \rangle$, if $0 \leqslant \tau \leqslant \Phi$,
    * $\langle N, \tau - \Phi - 1 \rangle$, if $\Phi + 1 \leqslant \tau \leqslant \Phi + 1 + \Theta$,
    * $\langle S, \tau - \Phi - \Theta - 2 \rangle$, if $\Phi + \Theta + 2 \leqslant \tau \leqslant \Phi + \Theta + 2 + \Psi$.
- If $\rho(u, U) \equiv 1 \pmod 2$, then:
  - for $t \leqslant t_1$, the state of u is $\langle N, 0 \rangle$,
  - for $t_1 + 1 \leqslant t \leqslant t_2 - 1$, the state of u is $\langle N, t - t_1 \rangle$,
  - for $t_2 \leqslant t \leqslant t_2 + \Psi - 1$, the state of u is $\langle S, t - t_2 \rangle$,
  - for $t \geqslant t_2 + \Psi$, the state of u is $\langle S, \Psi \rangle$.
- If $\rho(u, U) \equiv 0 \pmod 2$, $\rho(u, U) > 0$ and u is cul-de-sac:
  - for $t \leqslant t_1$, the state of u is $\langle N, 0 \rangle$,
  - for $t_1 + 1 \leqslant t \leqslant t_2 - 1$, the state of u is $\langle N, t - t_1 \rangle$,
  - for $t_2 \leqslant t \leqslant t_2 + \Psi$, the state of u is $\langle S, t - t_2 \rangle$,
  - for $t > t_2 + \Psi$ the states of u are periodical with period P. Let τ be the remainder of $t - t_2 - \Psi - 1$ modulo P. Then the state of u is:
    * $\langle D, \tau \rangle$, if $0 \leqslant \tau \leqslant \Phi$,
    * $\langle N, \tau - \Phi - 1 \rangle$, if $\Phi + 1 \leqslant \tau \leqslant \Phi + 1 + \Theta$,
    * $\langle S, \tau - \Phi - \Theta - 2 \rangle$, if $\Phi + \Theta + 2 \leqslant \tau \leqslant \Phi + \Theta + 2 + \Psi$.
- If $\rho(u, U) \equiv 0 \pmod 2$, $\rho(u, U) > 0$ and u is not cul-de-sac:
  - for $t \leqslant t_1$, the state of u is $\langle N, 0 \rangle$,
  - for $t_1 + 1 \leqslant t \leqslant t_2 - 1$, the state of u is $\langle N, t - t_1 \rangle$,
  - for $t_2 \leqslant t \leqslant t_2 + \Psi - 1$, the state of u is $\langle S, t - t_2 \rangle$,
  - for $t_2 + \Psi \leqslant t \leqslant t_2 + \max\{\Psi, \Theta + 1)\}$, the state of u is $\langle S, \Psi \rangle$,
  - for $t > t_2 + \max\{\Psi, \Theta + 1)\}$ the states of u are periodical with period P. Let τ be the remainder of $t - t_2 - \max\{\Psi, \Theta + 1\} - 1$ modulo P. Then the state of u is:
    * $\langle D, \tau \rangle$, if $0 \leqslant \tau \leqslant \Phi$,
    * $\langle N, \tau - \Phi - 1 \rangle$, if $\Phi + 1 \leqslant \tau \leqslant \Phi + 1 + \Theta$,
    * $\langle S, \tau - \Phi - \Theta - 2 \rangle$, if $\Phi + \Theta + 2 \leqslant \tau \leqslant \Phi + \Theta + 2 + \Psi$.

Let us define for each connected finite graph the function χ, which maps every subset U of graph vertices to the real number, equal to the fraction of those vertices u of the graph for which the integer $\rho(u, U)$ is odd.

Theorem 4.2. *Let a tissue be described by a regular ADG-model of a type $\langle \Phi, \Psi, \Theta \rangle$ on a finite graph having n vertices and U be the corresponding regular subset. Then starting with the time $(\Theta + 1) \max_u \rho(u, U)$ the average numbers of all stem cells and of all differentiated ones in the tissue over any time period of $\Phi + \Psi + \Theta + 3$ steps*

are equal correspondingly to

$$\bar{S} = n\left(\chi(U) + \left(1 - \chi(U)\right) \cdot \frac{\Psi + 1}{\Phi + \Psi + \Theta + 3}\right),$$

$$\bar{D} = n\left(1 - \chi(U)\right) \cdot \frac{\Phi + 1}{\Phi + \Psi + \Theta + 3}.$$

Proof. From Lemma 4.1 it follows that over each such time period $n(1 - \chi(U))$ vertices will be $\Psi + 1$ moments in the state of stem cells and $\Phi + 1$ moments in the state of differentiated cells, while the rest $n\chi(U)$ vertices will be $\Phi + \Psi + \Theta + 3$ moments in the state of stem cells. Thus, the average numbers of stem cells and differentiated ones are equal to $\bar{S}$ and $\bar{D}$ correspondingly. $\square$

Due to the Theorem 4.2 the following definition is natural. Define the *fraction of stem cells in the population* as the ratio between the average number of all stem cells over a time period of $\Phi + \Psi + \Theta + 3$ steps to the average number of all cells over the same period[3].

Theorem 4.3. *Let a tissue be described by a regular ADG-model of a type $\langle \Phi, \Psi, \Theta \rangle$ on a finite graph and U be the corresponding regular subset. Then starting with the time $(\Theta + 1)\max_u \rho(u, U)$ the fraction of stem cells in the population in the tissue is*

$$\frac{(\Phi + \Theta + 2)\chi(U) + \Psi + 1}{(\Theta + 1)\chi(U) + \Phi + \Psi + 2}.$$

Proof. The desired fraction is given by the expression

$$\frac{\bar{S}}{\bar{S} + \bar{D}},$$

using the notation of Theorem 4.2. Substituting the expressions given by Theorem 4.2 and simplifying, we obtain the result. $\square$

5. **Tube-like regular ADG-models.** Here we would like to focus on specific tissue shapes, in particular, on the cylindrical ones. Those are of interest especially in tissue engineering, where an artificial scaffold of a certain shape and dimensions is constructed and mounted with stem cells at specific sites [8]. The evolved system is then transplanted at the desired organ. This shape is also relatively common in tumor cooption forming cylindrical arrangements of tumor cells growing around a blood vessel [4]. It is natural to restrict our attention by considering ADG-models that are cylindrical, as is the shape of the bones in the body and intestinal crypts, for example. To describe a cylindrical tissue in our model, we demand an equal texture of different layers in the sections of such cylinder and direct connections between closed layers. Let us formulate the concept precisely.

For a set B and a natural number h, a graph $(B \times \{0; \dots; h\}; \delta)$, where the set δ of edges satisfies the conditions

$$(\forall x, y \in B)(\forall i, j \in \{0; \dots; h\})\left(\delta(\langle x, i \rangle, \langle y, i \rangle) \longleftrightarrow \delta(\langle x, j \rangle, \langle y, j \rangle)\right),$$

$$(\forall x, y \in B)(\forall i \in \{0; \dots; h\})(\forall j \neq i)\left(\delta(\langle x, i \rangle, \langle y, j \rangle) \longleftrightarrow x = y \,\&\, |i - j| = 1\right),$$

will be called a *simple tube with the layers* $B \times \{0\}$, $\dots$, $B \times \{h\}$ or a *simple tube*, for short. Then the subsets $B \times \{0\}$ and $B \times \{h\}$ will be called the *bases of the simple*

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[3]It should not be assumed that the ratio must be equal to the time average of the fraction of stem cells in the set of all cells.

tube with these layers, h will be called the *altitude of the simple tube with these layers* and the longest distance inside one layer in the shortest-path metric induced by the graph will be called the *diameter of the simple tube with these layers*. The set B in this definition represents a projection of all layers in the sections of the cylinder. A regular ADG-model on a graph will be called *tube-like* or in other words *cylindric-like* or *prism-like*, iff the graph is isomorphic to a simple tube with some layers, which will be called a *corresponding tube with these layers*, and the isomorphic image of the corresponding regular subset under this isomorphism contains only vertices of one layer. Note, that in this case for such regular subset U we have the following inequalities:

$$\frac{h}{2h+2} \leqslant \chi(U) \leqslant \frac{h+2}{2h+2}.$$

This follows from the fact that if some vertex in the layer $B \times \{i\}$ is at distance ρ from U, then the vertices connected to it in the layers $B \times \{i \pm 1\}$ (i.e. the vertices "above" an "below" it) are at distances of either $\rho + 1$ or $\rho - 1$ from U. That means that for sufficiently large values of h the value of $\chi(U)$ is close to 0.5. It follows that in the statements of Theorem 4.2 and Theorem 4.3 the calculated values practically almost do not depend of the choice of the regular subset. Note also that in tube-like ADG-model, the set of all culs-de-sac is confined within the bases of the tube, $B \times \{0\}$ and $B \times \{h\}$.

Theorem 5.1. *Let a tissue be described by a tube-like regular ADG-model of a type $\langle \Phi, \Psi, \Theta \rangle$ with layers $L_0, \ldots, L_h$, and integers h and d be correspondingly the altitude and the diameter of the corresponding simple tube with these layers. Assume that the integers $2(\Theta + 1)$ and $\Phi + \Psi + \Theta + 3$ are relatively prime. Then there exist integers s and u, such that at each moment starting from $(d+h)(\Theta+1)$, the numbers of all stem cells and of all differentiated cells in the tissue do not differ from s and u, correspondingly, more than by $\gamma\%$, where*

$$\gamma = \frac{400(\Phi + \Psi + \Theta + 3)}{h+1} < \frac{1600(\Phi + 1)}{h}.$$

Proof. Without loss of generality we can assume that the graph is just this simple tube with these layers and the corresponding regular subset is included in the layer $B \times \{m\}$ with the condition $m \leqslant \dfrac{h}{2}$. Let m_1 be the minimal non-negative integer satisfying the condition

$$m_1 \equiv m \pmod{2(\Phi + \Psi + \Theta + 3)},$$

m_2 be the maximal integer satisfying the conditions

$$m_2 \leqslant h, \qquad m_2 \equiv m \pmod{2(\Phi + \Psi + \Theta + 3)}$$

and k, s and u be the following integers:

$$k = 2 + \frac{m_2 - m_1}{2(\Phi + \Psi + \Theta + 3)}, \qquad s = k(\Phi + 2\Psi + \Theta + 4) \cdot |B|, \qquad u = k(\Phi + 1) \cdot |B|.$$

By Lemma 4.1, the numbers of all stem cells at an arbitrary moment, starting from $(d+h)(\Theta+1)$, in the sets

$$B \times \{m_1 + 1; \ldots; m_2\}, \qquad T, \qquad (B \times \{0; \ldots; m_1; m_2 + 1; \ldots; h\}) \setminus T,$$

where T is the set of all culs-de-sac for the regular subset, are as follows: the first one is equal to

$$\frac{m_2 - m_1}{2(\Phi + \Psi + \Theta + 3)} \cdot (\Phi + 2\Psi + \Theta + 4) \cdot |B| = \frac{k-2}{k} \cdot s,$$

and each one of the two others is less than or equal to

$$2(\Phi + 2\Psi + \Theta + 4) \cdot |B| = \frac{2}{k} \cdot s.$$

Then the sum of these three integers is between $\dfrac{k-2}{k} \cdot s$ and $\dfrac{k+2}{k} \cdot s$, whence the sum does not differ from s more than $\dfrac{200}{k}\%$. The analogous assertion for differentiated cells holds, replacing $\Phi + 2\Psi + \Theta + 4$ with $\Phi + 1$ and s with u. Finally,

$$\gamma \leqslant \frac{200}{k} \leqslant \frac{400(\Phi + \Psi + \Theta + 3)}{h+1} \leqslant \frac{400(\Phi + 2\Theta + 1 + \Theta + 3)}{h+1} \leqslant$$
$$\leqslant \frac{400(\Phi + 2\Phi + 1 + \Phi + 3)}{h+1} < \frac{1600(\Phi + 1)}{h},$$

as required. $\square$

Corollary 1. *Let a tissue be a tube-like regular ADG-model of a type $\langle \Phi, \Psi, \Theta \rangle$ with layers L_0, ..., L_h, integers h and d be correspondingly the altitude and the diameter of corresponding simple tube with these layers, and the integers $2(\Theta + 1)$ and $\Phi + \Psi + \Theta + 3$ be relatively prime. And let γ be an arbitrary given real number from the interval $(0; 100)$. Then a sufficient condition for the existence of integers s and u, such that at each moment, beginning at the time $(d+h)(\Theta+1)$, the numbers of all stem cells and of all differentiated cells in the tissue do not differ from s and u correspondingly more than $\gamma\%$, is the inequality*

$$h \geqslant \frac{1600(\Phi + 1)}{\gamma}.$$

One can see from the proof of Theorem 5.1 (and this is corroborated by computer simulations[4]) that the estimations from Theorem 5.1 and consequently from Corollary 1 are rough and can be improved under further constraints. In contrast, attempts to narrow the estimated interval impede generality of the results or lead to considerably more bulky wordings and computations.

Nevertheless, we consider a result, which is special by its absolute stationarity.

Theorem 5.2. *Let a tissue be a tube-like regular ADG-model of a type $\langle \Phi, \Psi, \Theta \rangle$ with layers L_0, ..., L_h, where the regular subset is included in one of the bases L_0 or L_h, and integers h and d be correspondingly the altitude and the diameter of corresponding simple tube with these layers. And let the integers $2(\Theta + 1)$ and $\Phi + \Psi + \Theta + 3$ be relatively prime and the conditions*

$$\Psi > \Theta, \qquad (h+1) \equiv 0 \pmod{2(\Phi + \Psi + \Theta + 3)} \tag{8}$$

hold. Then starting with the time $(d + h)(\Theta + 1)$ the numbers of all stem cells and of all differentiated cells in the tissue do not change at all.

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[4]Z. Agur, O. U. Kirnasovsky and L. Levi; in preparation.

Proof. Without loss of generality we can assume that the graph is just this simple tube with these layers and the corresponding regular subset is included in the layer $B \times \{0\}$. By Lemma 4.1, at each moment t, starting from $(d + h)(\Theta + 1)$, the numbers of all stem cells and of all differentiated cells in every set of the form

$$B \times \{i \cdot 2(\Phi + \Psi + \Theta + 3); \ldots ; (i + 1) \cdot 2(\Phi + \Psi + \Theta + 3) - 1\}$$

do not depend on t and on i, when

$$0 < i \cdot 2(\Phi + \Psi + \Theta + 3), \qquad (i + 1) \cdot 2(\Phi + \Psi + \Theta + 3) - 1 < h$$

or when

$$\Psi > \Theta, \qquad 0 \leqslant i \cdot 2(\Phi + \Psi + \Theta + 3), \qquad (i + 1) \cdot 2(\Phi + \Psi + \Theta + 3) - 1 \leqslant h.$$

Thus, the conditions (8) are sufficient for such steady state of the numbers of stem cells and differentiated cells. $\qquad \square$

6. **Discussion.** Recall that the model constructed in [1] determines the common sense ground rules governing individual stem cell behavior, allowing factors which influence the "decision" of individual stem cells to proliferate, differentiate or remain quiescent, to be modulated. Even though the representation of the developing system is very simple, the properties that emerge are general and hold for more complex descriptions.

It is clear that all properties of the general model are conserved for the model presented here. Moreover, our regular model guarantees (almost-) stability of stem cell numbers in the considered system. For the general case, the exact average numbers of cells of all types are computed and are shown to be constant. In addition, for the special case of tube-like shape, it is shown that all cell numbers at each time step are close to that average. Based on these results we can add to the discussion of the work [1] the statement that even a simple non-stochastic model enables to maintain practically stable number of stem cells. This means, that there is no necessity to assume existence of more elaborate mechanisms to explain the basic properties of such homeostasis. Moreover, this is an important result as it enables to *a priori* determine the initial tissue structure which, eventually, will become resilient.

The average fraction of stem cells in a steady state of the system is expressed via the kinetic parameters of the model and its spatial organization. This formula enables to evaluate the fraction of stem cells at any moment. Note that this may be important in all in vivo situations, since the available experimental methods to evaluate these proportions may be detrimental. Moreover, this information is extremely valuable when therapy of solid tumor is considered. As treatment should target only the malignant stem cells, their fraction at the moment of therapy initiation should determine the drug schedule. Moreover, the formula for the time elapsing from process initiation to the achievement of a fully resilient tissue may be important for bone marrow transplantation procedures and tissue engineering, which rely on a small number of stem cells replenishing empty scaffolds.

In summary, our work provides further support to the previously developed notion that homeostasis in developing tissues is obtained by a negative feedback of stem cells on their proliferation. Furthermore, our work provides analytical tools for designing efficient medical interventions.

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E-mail address: O.U. Kirnasovsky — `oleg@imbm.org`

E-mail address: Z. Agur — `agur@imbm.org`

E-mail address: Y. Kogan — `yuri@imbm.org`

7. **Appendix: Proof of Lemma 4.1.** Let us show, first, that there are no conditions of the theorem for different states of the vertex u that can take place simultaneously. Then we will be able, obtaining the condition for a state, to conclude automatically that conditions for all other states are false. Let us consider all possible cases.

If the inequality (3a) is true then the inequalities (3c) and (6a), for $1 \leqslant \tau \leqslant \Theta$ the equality (4a) and for $0 \leqslant \tau < \Psi$ the equality (5a) are false. If the conjunction of the relationships (3b), (3c) and (3d) is true then the relationship (6b) is false due to (3b), the inequality (6c), for $1 \leqslant \tau \leqslant \Theta$ the equality (4a) and for $0 \leqslant \tau < \Psi$ the equality (5a) are false due to (3c), and the relationship (6d), for $1 \leqslant \tau \leqslant \Theta$ the relationship (4b), for $0 \leqslant \tau < \Psi$ the relationship (5b) and for $0 \leqslant \tau \leqslant \Phi$ the relationship (7a) are false due to (3d). Thus, the condition for the state $\langle N, 0 \rangle$ is possible simultaneously with the condition of no another state.

Let $\mu \in \{1; \ldots; \Theta\}$. If for $\tau = \mu$ the equality (4a) is true then the inequalities (3c), (6a), for $\tau \neq \mu$ where $1 \leqslant \tau \leqslant \Theta$ the equality (4a) and for $0 \leqslant \tau < \Psi$ the equality (5a) are false. If the conjunction of the relationships (3b), (3c) and (4b) with $\tau = \mu$ is true then the relationship (6b) is false due to (3b), the inequality (6c), for $1 \leqslant \tau \leqslant \Theta$ the equality (4a) and for $0 \leqslant \tau < \Psi$ the equality (5a) are false

due to (3c), and the relationship (6d), for $\tau \neq \mu$ where $1 \leqslant \tau \leqslant \Theta$ the relationship (4b), for $0 \leqslant \tau < \Psi$ the relationship (5b) and for $0 \leqslant \tau \leqslant \Phi$ the relationship (7a) are false due to (4b) with $\tau = \mu$. Thus, the condition for the state $\langle N, \mu \rangle$ is possible simultaneously with the condition of no another state.

Now, let $\mu \in \{0; \ldots; \Psi - 1\}$. If for $\tau = \mu$ the equality (5a) is true then the inequalities (3c), (6a) and for $\tau \neq \mu$ where $0 \leqslant \tau < \Psi$ the equality (5a) are false. If the conjunction of the relationships (3b), (3c) and (5b) with $\tau = \mu$ is true then the relationship (6b) is false due to (3b), the inequality (6c) and for $0 \leqslant \tau < \Psi$ the equality (5a) are false due to (3c), and the relationship (6d), for $\tau \neq \mu$ where $0 \leqslant \tau < \Psi$ the relationship (5b) and for $0 \leqslant \tau \leqslant \Phi$ the relationship (7a) are false due to (5b) with $\tau = \mu$. Thus, the condition for the state $\langle S, \mu \rangle$ is possible simultaneously with the condition of no another state.

Now, let $\mu \in \{0; \ldots; \Phi\}$. If for $\tau = \mu$ the conjunction (7) is true then the relationship (6b) is false due to (3b), the inequality (6c) is false due to (3c), and the relationship (6d) and for $\tau \neq \mu$ where $0 \leqslant \tau \leqslant \Phi$ the relationship (7a) are false due to (7a) with $\tau = \mu$. Thus, the condition for the state $\langle D, \mu \rangle$ is possible simultaneously with the condition of no another state.

So, for no pair of different states the corresponding conditions of the theorem for the states hold simultaneously. Therefore, it remains to prove that for every t and u the corresponding condition of the theorem for the state $x^t(u)$ is true.

Note that the condition (6) is equivalent to the same condition when replacing the strict inequality (6c) with the corresponding not strict one:

$$t \leqslant \Psi + C_u \cdot ((\Theta + 1) \dot{-} \Psi) + (\Theta + 1) \cdot \rho(u, U), \tag{9}$$

because in the case, when (9) is true but (6c) does not, the relationship (6d) holds.

Fix a vertex u. Let us prove the statement of the theorem by mathematical induction on the variable t. Let $t = 0$. If $u \notin U$ then

$$x^t(u) = \langle N, 0 \rangle.$$

On the other hand, the condition (3) is true, because (3a) is true. If $u \in U$ then

$$x^t(u) = \langle S, 0 \rangle.$$

On the other hand, the corresponding condition for $\langle S, 0 \rangle$ is true. Indeed, if $\Psi = 0$ then this condition is (6) which follows from the true inequalities (6a) and (9), and if $\Psi \neq 0$ then this condition is (5) with $\tau = 0$ which follows from the true equality (5a). The base case $t = 0$ is proved. Now, let us fix an arbitrary integer $k \geqslant 0$. Assume that with $t = k$ the statement is true and prove that one is true with $t = k + 1$ as well. Firstly note that there exists a vertex v such that the equalities

$$\rho(u, v) = 1, \qquad \rho(u, U) = \rho(v, U) + 1 \tag{10}$$

are true if and only if $u \notin U$. Secondly, recall remarks to the definitions of a cul-de-sac and of a strong cul-de-sac. Let us consider now all possible cases.

Case 1: the state $x^k(u)$ is either $\langle N, \mu \rangle$ with $1 \leqslant \mu < \Theta$ or $\langle S, \mu \rangle$ with $\mu \leqslant \Psi - 2$ or $\langle D, \mu \rangle$ with $\mu < \Phi$. Then the corresponding equality from the following ones is true:

$$x^{k+1}(u) = \langle N, \mu + 1 \rangle, \qquad x^{k+1}(u) = \langle S, \mu + 1 \rangle, \qquad x^{k+1}(u) = \langle D, \mu + 1 \rangle.$$

On the other hand, it is clear that if one of three conditions (4), (5) or (7) is true when $t = k$ and $\tau = \mu$ then the same condition of them is true when $t = k + 1$ and $\tau = \mu + 1$ as well.

Case 2: $x^k(u) = \langle D, \Phi \rangle$. Then $x^{k+1}(u) = \langle N, 0 \rangle$. On the other hand, by inductive assumption, the condition (7) is true when $t = k$ and $\tau = \Phi$. Thence the relationships (3b), (3c) and (3d) are true when $t = k + 1$, and therefore (3) is true when $t = k + 1$.

Case 3: $x^k(u) = \langle N, 0 \rangle$. By inductive assumption, we have (3) for $t = k$.

Subcase 3.1: $k < (\Theta + 1)(\rho(u, U) - 1)$. It is clear that for each neighbor vertex v of u either the inequality (3a) or for some $\tau \in \{1; \ldots; \Theta\}$ the equality (4a) holds substituting $t = k$ and $u = v$. By inductive assumption, we have that all neighbors of u are in states of the form $\langle N, \alpha \rangle$ at time k. So,

$$x^{k+1}(u) = \langle N, 0 \rangle.$$

On the other hand, for $t = k + 1$ the inequality (3a) holds.

Subcase 3.2: $k = (\Theta + 1)(\rho(u, U) - 1)$. Then $u \notin U$, and therefore there exists a vertex v satisfying the equalities (10). This means that for $\tau = 0$ the equality (5a) holds substituting $t = k$ and $u = v$ (if $\Psi = 0$, then the inequalities (6a) and (9) hold with the same substitutions). By inductive assumption, we have that

$$x^k(v) = \langle S, 0 \rangle.$$

Thence,

$$x^{k+1}(u) = \begin{cases} \langle S, 0 \rangle & \text{if } \Theta = 0, \\ \langle N, 1 \rangle & \text{if } \Theta \neq 0. \end{cases} \tag{11}$$

On the other hand, the equality (4a), and hence the condition (4), is true when $t = k + 1$ and $\tau = 1$, whence for the case $\Theta = 0$ the condition (5) is true when $t = k + 1$ and $\tau = 0$ (and thence for the case $\Theta = \Psi = 0$ the condition (6) is true when $t = k + 1$, because the inequalities (6a) and (9) are true).

Subcase 3.3: the relationships (3b), (3c) and (3d) for $t = k$ are fulfilled simultaneously (note that then the first and the second ones are true for $t = k + 1$ as well). Because U is a regular subset of the graph, there exists a neighbor vertex v of u such that either (1) or (10). From (3c) and (3d) for $t = k$ we have that

$$k \geqslant \Phi + \Psi + 2 + C_u \cdot ((\Theta + 1) \dot{-} \Psi) + (\Theta + 1) \cdot \rho(u, U).$$

However, $\Phi \geqslant \Theta$, therefore

$$k \geqslant \Psi + \Theta + 2 + C_u \cdot ((\Theta + 1) \dot{-} \Psi) + (\Theta + 1) \cdot \rho(u, U),$$

whence for $t = k$ the inequality (6a) is true when substituting $u = v$. It is clear that the relationship (6b) substituting $u = v$ is true as well. Then by inductive assumption, we have that

$$x^k(v) = \langle S, \Psi \rangle.$$

Thence we obtain (11). On the other hand, the relationship (4b), and hence the condition (4), is true when $t = k + 1$ and $\tau = 1$, whence for the case $\Theta = 0$ the condition (5) is true when $t = k + 1$ and $\tau = 0$ (and thence for the case $\Theta = \Psi = 0$ the condition (6) is true when $t = k + 1$, because the inequalities (6a) and (9) are true).

Case 4: $x^k(u) = \langle N, \Theta \rangle$ with $\Theta \neq 0$. Then $x^{k+1}(u) = \langle S, 0 \rangle$. On the other hand, by inductive assumption, the condition (4) is true when $t = k$ and $\tau = \Theta$. Thence, the condition (5) is true when $t = k + 1$ and $\tau = 0$, and for the case $\Psi = 0$ the condition (6) is true when $t = k + 1$, because the inequalities (6a) and (9) are true.

Case 5: $x^k(u) = \langle S, \Psi - 1 \rangle$. Then $x^{k+1}(u) = \langle S, \Psi \rangle$. On the other hand, by inductive assumption, the condition (5) is true when $t = k$ and $\tau = \Psi - 1$. Then

the condition (6) is true when $t = k+1$. Indeed, if the equality (5a) is true for $t = k$ and $\tau = \Psi - 1$ then the inequalities (6a) and (9) are true for $t = k + 1$, and if the relationships (3c) and (5b) are true for $t = k$ and $\tau = \Psi - 1$ then the relationships (6a) and (6d) are true for $t = k + 1$.

Case 6: $x^k(u) = \langle S, \Psi \rangle$. By inductive assumption, we have (6) for $t = k$. In particular, the inequality (6a) is true with $t = k$.

Subcase 6.1: the integer $\rho(u, U)$ is odd. Then u is not a cul-de-sac for U and $u \notin U$. This implies that there exist a vertex v with the conditions (1) and a vertex w with the conditions (10) substituting $v = w$. Note that the vertex w is not a cul-de-sac for the set U. Let us assume that the vertices v and w are in states of the form $\langle S, \alpha \rangle$ and $\langle S, \beta \rangle$ correspondingly at time k. Then by inductive assumption, we have the following facts: if $\alpha = \Psi$, then the inequality

$$k \geqslant \Psi + (\Theta + 1)(\rho(u, U) + 1) \tag{12}$$

and at least one of the conditions

$$k < \Psi + C_v \cdot ((\Theta + 1) \dot{-} \Psi) + (\Theta + 1)(\rho(u, U) + 1), \tag{13}$$

$$
\begin{aligned}
k \equiv \quad & \Psi + C_v \cdot ((\Theta + 1) \dot{-} \Psi) + \\
& + (\Theta + 1)(\rho(u, U) + 1) \quad (\text{mod } \Phi + \Psi + \Theta + 3)
\end{aligned} \tag{14}
$$

are true; if $\alpha \in \{0; \ldots; \Psi - 1\}$, then the equality

$$k = \alpha + (\Theta + 1)(\rho(u, U) + 1) \tag{15}$$

or both of the conditions

$$k > \Psi + C_v \cdot ((\Theta + 1) \dot{-} \Psi) + (\Theta + 1)(\rho(u, U) + 1), \tag{16}$$

$$
\begin{aligned}
k \equiv \quad & \alpha + C_v \cdot ((\Theta + 1) \dot{-} \Psi) + \\
& + (\Theta + 1)(\rho(u, U) + 1) \quad (\text{mod } \Phi + \Psi + \Theta + 3)
\end{aligned} \tag{17}
$$

is (are) true; if $\beta = \Psi$, then at least one of the conditions

$$k < \Psi + ((\Theta + 1) \dot{-} \Psi) + (\Theta + 1)(\rho(u, U) - 1), \tag{18}$$

$$
\begin{aligned}
k \equiv \quad & \Psi + ((\Theta + 1) \dot{-} \Psi) + \\
& + (\Theta + 1)(\rho(u, U) - 1) \quad (\text{mod } \Phi + \Psi + \Theta + 3)
\end{aligned} \tag{19}
$$

is true; and if $\beta \in \{0; \ldots; \Psi - 1\}$, then the congruence

$$
\begin{aligned}
k \equiv \quad & \beta + ((\Theta + 1) \dot{-} \Psi) + \\
& + (\Theta + 1)(\rho(u, U) - 1) \quad (\text{mod } \Phi + \Psi + \Theta + 3)
\end{aligned} \tag{20}
$$

is true, because the equality

$$k = \beta + (\Theta + 1)(\rho(u, U) - 1)$$

contradicts the inequality (6a).

Let $\alpha = \beta = \Psi$. Then we have

$$(12) \,\&\, \Big((13) \lor (14)\Big) \,\&\, \Big((18) \lor (19)\Big).$$

If the inequalities (12) and (18) hold both, then

$$\Psi + (\Theta + 1)(\rho(u, U) + 1) < \Psi + ((\Theta + 1) \dot{-} \Psi) + (\Theta + 1)(\rho(u, U) - 1),$$

whence $2(\Theta + 1) < (\Theta + 1) \dot{-} \Psi \leqslant \Theta + 1$. A contradiction. If the congruences (14) and (19) hold both, then

$$(1 - C_v) \cdot ((\Theta + 1) \dot{-} \Psi) \equiv 2(\Theta + 1) \quad (\text{mod } \Phi + \Psi + \Theta + 3).$$

But this contradicts the chain of the following evident inequalities:

$$0 \leqslant (1 - C_v) \cdot ((\Theta + 1) \dot{-} \Psi) \leqslant \Theta + 1 < 2(\Theta + 1) \leqslant \Phi + \Theta + 2 < \Phi + \Psi + \Theta + 3.$$

Hence, $(12) \,\&\, (13) \,\&\, (19)$. Accounting the evident inequalities

$$
\begin{aligned}
& ((\Theta + 1) \dot{-} \Psi) + (\Theta + 1)(\rho(u, U) - 1) \leqslant \\
\leqslant \;& (\Theta + 1) + (\Theta + 1)(\rho(u, U) - 1) < \\
< \;& (\Theta + 1)(\rho(u, U) + 1),
\end{aligned}
\tag{21}
$$

we have from the conditions (12) and (19) the inequality

$$k \geqslant \Phi + \Psi + \Theta + 3 + ((\Theta + 1) \dot{-} \Psi) + (\Theta + 1)(\rho(u, U) - 1). \tag{22}$$

Combining it with (13), we obtain the inequality

$$
\begin{aligned}
& \Phi + \Psi + \Theta + 3 + ((\Theta + 1) \dot{-} \Psi) + (\Theta + 1)(\rho(u, U) - 1) < \\
< \;& \Psi + C_v \cdot ((\Theta + 1) \dot{-} \Psi) + (\Theta + 1)(\rho(u, U) + 1),
\end{aligned}
\tag{23}
$$

which contradicts the inequality $\Phi \geqslant \Theta$.

Let now $\alpha < \beta = \Psi$. Then we have

$$\Big((15) \;\vee\; \big((16) \,\&\, (17) \big) \Big) \,\&\, \Big((18) \;\vee\; (19) \Big).$$

If the conditions (15) and (18) hold both, then

$$\alpha + 2(\Theta + 1) < \Psi + ((\Theta + 1) \dot{-} \Psi),$$

whence we obtain the inequality

$$2(\Theta + 1) - ((\Theta + 1) \dot{-} \Psi) \leqslant \Psi, \tag{24}$$

which contradicts the inequality $\Psi \leqslant 2\Theta + 1$. Indeed, if two these inequalities are true both, then

$$(\Theta + 1) \dot{-} \Psi \geqslant 2(\Theta + 1) - \Psi \geqslant 2(\Theta + 1) - (2\Theta + 1) = 1 > 0,$$

whence $\Theta + 1 > \Psi$, and consequently we get a contradiction in the following way:

$$2(\Theta + 1) - ((\Theta + 1) \dot{-} \Psi) = 2(\Theta + 1) - (\Theta + 1 - \Psi) = \Psi + \Theta + 1 > \Psi.$$

If the conditions (15) and (19) hold both, then

$$\alpha + 2(\Theta + 1) \equiv \Psi + ((\Theta + 1) \dot{-} \Psi) \pmod{\Phi + \Psi + \Theta + 3},$$

whence $\Psi - \alpha \equiv 2(\Theta + 1) - ((\Theta + 1) \dot{-} \Psi) \pmod{\Phi + \Psi + \Theta + 3}$. Then, accounting the evident inequalities

$$
\begin{aligned}
0 \;&<\; \Psi - (\Psi - 1) \leqslant \Psi - \alpha \leqslant \Psi < \Phi + \Psi + \Theta + 2, \\
0 \;&<\; 2(\Theta + 1) - (\Theta + 1) \leqslant 2(\Theta + 1) - ((\Theta + 1) \dot{-} \Psi) \leqslant \\
&\leqslant\; 2(\Theta + 1) \leqslant \Phi + \Theta + 2 \leqslant \Phi + \Psi + \Theta + 2,
\end{aligned}
\tag{25}
$$

we obtain that $\Psi - \alpha = 2(\Theta + 1) - ((\Theta + 1) \dot{-} \Psi)$, whence we have the inequality (24), which contradicts the inequality $\Psi \leqslant 2\Theta + 1$. Thus, the conditions (16) and (17) are true both. If the inequality (18) is true as well, then

$$\Psi + C_v \cdot ((\Theta+1) \dot{-} \Psi) + (\Theta+1)(\rho(u, U) + 1) < \Psi + ((\Theta+1) \dot{-} \Psi) + (\Theta+1)(\rho(u, U) - 1),$$

whence $2(\Theta + 1) < (1 - C_v) \cdot ((\Theta + 1) \dot{-} \Psi) \leqslant (\Theta + 1) \dot{-} \Psi \leqslant \Theta + 1$. A contradiction. Hence, (19) is true. Then from (17) we obtain the congruence

$$\alpha + C_v \cdot ((\Theta + 1) \dot{-} \Psi) + 2(\Theta + 1) \equiv \Psi + ((\Theta + 1) \dot{-} \Psi) \pmod{\Phi + \Psi + \Theta + 3},$$

whence $\Psi - \alpha \equiv 2(\Theta + 1) - (1 - C_v) \cdot ((\Theta + 1) \dot{-} \Psi) \pmod{\Phi + \Psi + \Theta + 3}$. Then, accounting the evident inequalities (25) and

$$0 < 2(\Theta + 1) - (\Theta + 1) \leqslant 2(\Theta + 1) - (1 - C_v) \cdot ((\Theta + 1) \dot{-} \Psi) \leqslant$$
$$\leqslant 2(\Theta + 1) \leqslant \Phi + \Theta + 2 \leqslant \Phi + \Psi + \Theta + 2,$$

we obtain that $\Psi - \alpha = 2(\Theta + 1) - (1 - C_v) \cdot ((\Theta + 1) \dot{-} \Psi)$, whence we have the inequality (24), which contradicts the inequality $\Psi \leqslant 2\Theta + 1$.

Let now $\beta < \alpha = \Psi$. Then we have

$$(12) \,\&\, \Big((13) \,\vee\, (14)\Big) \,\&\, (20).$$

If (14) is true then from (14) and (20) we obtain the congruence

$$\Psi + C_v \cdot ((\Theta + 1) \dot{-} \Psi) + 2(\Theta + 1) \equiv \beta + ((\Theta + 1) \dot{-} \Psi) \pmod{\Phi + \Psi + \Theta + 3},$$

whence

$$\Psi - \beta + 2(\Theta + 1) \equiv (1 - C_v) \cdot ((\Theta + 1) \dot{-} \Psi) \pmod{\Phi + \Psi + \Theta + 3},$$

that contradicts the chain of the following evident inequalities

$$0 \leqslant (1 - C_v) \cdot ((\Theta + 1) \dot{-} \Psi) \leqslant \Theta + 1 < \Psi - \beta + 2(\Theta + 1) \leqslant \Psi + 2(\Theta + 1) \leqslant \Phi + \Psi + \Theta + 2.$$

Hence, (13) is true. Accounting the inequalities (21), we have from the conditions (12) and (20) the inequality (22). Combining it with (13), we obtain the inequality (23), which contradicts the inequality $\Phi \geqslant \Theta$.

At last, let now $\alpha < \Psi$ and $\beta < \Psi$. Then we have

$$\Big((15) \,\vee\, \big((16) \,\&\, (17)\big)\Big) \,\&\, (20).$$

If the conditions (15) and (20) hold both, then

$$\alpha + 2(\Theta + 1) \equiv \beta + ((\Theta + 1) \dot{-} \Psi) \pmod{\Phi + \Psi + \Theta + 3}, \tag{26}$$

whence $\beta - \alpha \equiv 2(\Theta + 1) - ((\Theta + 1) \dot{-} \Psi) \pmod{\Phi + \Psi + \Theta + 3}$. Accounting the evident inequalities

$$|\beta - \alpha| \leqslant (\Psi - 1) - 0 < \Psi, \tag{27}$$

we have that either the inequality (24) is true or the inequality

$$2(\Theta + 1) - ((\Theta + 1) \dot{-} \Psi) > (\Phi + \Psi + \Theta + 3) - \Psi$$

is true. But the first one contradicts the inequality $\Psi \leqslant 2\Theta + 1$, and the second one implies the inequality

$$2(\Theta + 1) > \Phi + \Theta + 3,$$

which contradicts the inequality $\Phi \geqslant \Theta$. Hence, the congruences (17) and (20) hold both, whence

$$\alpha + 2(\Theta + 1) \equiv \beta + (1 - C_v) \cdot ((\Theta + 1) \dot{-} \Psi) \pmod{\Phi + \Psi + \Theta + 3}.$$

When $C_v = 0$ we get the congruence (26), which is false as it is proved above. Then $C_v = 1$, and consequently

$$\alpha + 2(\Theta + 1) \equiv \beta \pmod{\Phi + \Psi + \Theta + 3}.$$

Accounting the inequalities (27), we have that at least one of two following inequalities is true:

$$\begin{aligned} 2(\Theta + 1) &< \Psi, \\ 2(\Theta + 1) &> (\Phi + \Psi + \Theta + 3) - \Psi. \end{aligned}$$

But the inequalities contradict to the inequalities $\Psi \leqslant 2\Theta + 1$ and $\Phi \geqslant \Theta$ correspondingly.

Thus, the assumption, that the vertices v and w are in states of the form $\langle S, \alpha \rangle$ and $\langle S, \beta \rangle$ correspondingly at time k, is false. Then

$$x^{k+1}(u) = \langle S, \Psi \rangle. \tag{28}$$

In contrast, the conditions (6a) and (6b) are true for $t = k + 1$. So, (6) is true for $t = k + 1$.

Subcase 6.2: the relationship (6c) is true when $t = k$. Accounting (6a) for $t = k$, we have the inequality

$$C_u \cdot ((\Theta + 1) \dot{-} \Psi) > 0,$$

whence $C_u = 1$ and $\Theta \geqslant \Psi$. Then there exists a vertex v with (1). For this vertex v the equality (4a) holds substituting $u = v$ when $t = k$ and

$$\tau = k - (\Theta + 1) \cdot \rho(u, U).$$

For this integer τ the inequalities (6a) and (6c) with $t = k$ imply that

$$\begin{aligned} \Psi + (\Theta + 1) \cdot \rho(u, U) &\leqslant \tau + (\Theta + 1) \cdot \rho(u, U) < \\ < \quad \Psi + C_u \cdot ((\Theta + 1) \dot{-} \Psi) + (\Theta + 1) \cdot \rho(u, U) &= \\ = \quad \Psi + 1 \cdot ((\Theta + 1) - \Psi) + (\Theta + 1) \cdot \rho(u, U) &= \\ = \quad (\Theta + 1)(\rho(u, U) + 1), \end{aligned}$$

whence $\Psi \leqslant \tau < \Theta + 1$, and hence, $0 \leqslant \tau \leqslant \Theta$. This means that, substituting $u = v$, either (3a) or (4a) with $\tau \in \{1; \ldots; \Theta\}$ holds when $t = k$. By inductive assumption, this implies that

$$x^k(v) = \langle N, \tau \rangle.$$

Then we have (28). In contrast, the inequalities (6a) and (9) are true for $t = k + 1$. So, (6) is true for $t = k + 1$.

Subcase 6.3: the relationship (6d) is true when $t = k$ and the integer $\rho(u, U)$ is even. Because

$$0 \leqslant C_u \cdot ((\Theta + 1) \dot{-} \Psi) \leqslant \Theta + 1 < \Phi + \Psi + \Theta + 3,$$

the conditions (6a) and (6d) with $t = k$ imply the inequality

$$k \geqslant \Psi + C_u \cdot ((\Theta + 1) \dot{-} \Psi) + (\Theta + 1) \cdot \rho(u, U). \tag{29}$$

Let v be an arbitrary neighbour vertex of the vertex u.

If the equalities (1) are true, then $C_u = 1$, and consequently from the inequality (29) we obtain the inequality

$$k \geqslant (\Theta + 1)(\rho(u, U) + 1).$$

Therefore, substituting $t = k$ and $u = v$, either the equality (5a) for some non-negative integer $\tau < \Psi$ or the conditions (6a) and (6b) is (are) true. Then by inductive assumption, we get

$$(\exists \alpha) \, x^k(v) = \langle S, \alpha \rangle. \tag{30}$$

Let now the equalities (10) be true. Then the conditions (6a) and (6b) substituting $t = k$ and $u = v$ are true, whence by inductive assumption, we have (30).

At last, let the equality

$$\rho(u, U) = \rho(v, U)$$

be true. Because all culs-de-sac for U are strong, the vertices u and v either are culs-de-sac for U both or do not both. Then the conditions (6a) and (6d) substituting $t = k$ and $u = v$ are true, whence by inductive assumption, we have (30).

Thus, for every neighbour vertex v of u the condition (30) is true, whence
$$x^{k+1}(u) = \langle D, 0 \rangle.$$
In contrast, accounting the inequality (29), we have that the condition (7) is true when $t = k + 1$ and $\tau = 0$.

By the principle of mathematical induction, the statement of the Lemma is true. $\blacksquare$

E-mail address: `agur@imbm.org`