

Space-time dynamics of Stem Cell Niches: a unified approach for Plants

Maria del Carmen Pérez^{1,*}, Alejandro López², Pablo Padilla³

¹Universidad Autónoma de la Ciudad de México, UACM, México

²Escuela Superior de Computación, ESCOM, México

³Instituto de Investigaciones en Matemáticas Aplicadas y en Sistemas, IIMAS, UNAM, México

Summary

Many complex systems cannot be analyzed using traditional mathematical tools, due to their irreducible nature. This makes it necessary to develop models that can be implemented computationally to simulate their evolution. Examples of these models are cellular automata, evolutionary algorithms, complex networks, agent-based models, symbolic dynamics and dynamical systems techniques.

We review some representative approaches to model the stem cell niche in *Arabidopsis thaliana* and the basic biological mechanisms that underlie its formation and maintenance. We propose a mathematical model based on cellular automata for describing the space-time dynamics of the stem cell niche in the root. By making minimal assumptions on the cell communication process documented in experiments, we classify the basic developmental features of the stem-cell niche, including the basic structural architecture, and suggest that they could be understood as the result of generic mechanisms given by short and long range signals. This could be a first step in understanding why different stem cell niches share similar topologies, not only in plants. Also the fact that this organization is a robust consequence of the way information is being processed by the cells and to some extent independent of the detailed features of the signaling mechanism.

1 Introduction

Studies on stem cell from diverse systems have shown that their behavior is controlled by extracellular cues from the niche (long and short range signals) and by intrinsic genetic programs within the stem cell itself [1].

Although important progress has been made in understanding these signaling processes, it is still open to a large extent what mechanisms are essential for the formation and maintenance of stem cell niches. In particular, whether some of these mechanisms are common and if it is possible to consider a basic or “universal” architecture for the stem cell niche which is conserved throughout a wide range of organisms. The purpose of this paper is twofold. On the one hand, we review some of the most representative approaches to model stem cell niches and their dynamics. On the other hand, we present a unified approach in an attempt to capture the basic cell communication features (both short and long range) that are necessary to explain the appearance, formation and robustness of the niche. We intend to provide a generic minimal framework, i.e., that can in principle account for the features of the niches that are

* To whom correspondence should be addressed. Email: carpeza3@gmail.com

present in a wide variety of organisms and contexts and that will allow for the formulation of a more realistic mathematical model.

In order to do that, we provide a discrete (in time and space) description in which a collection of cells is endowed with a few basic features. The state of the cell is characterized by several variables: its identity (whether it is quiescent, initial, transit amplifying or differentiated) as well as the status of the cell cycle.

The implementation of the model was carried out in a design platform known as Model-View-Controller, also using OOP, allowing us to test this model not only in the dynamics of stem cell niche in *Arabidopsis thaliana* root, but in any phenomenon of cellular dynamics.

2 The root stem cell niche

Most of the development of a plant is post embryonic and is controlled by the meristems, among whose basic functions is to renew and maintain themselves, to generate new cells and to establish developmental patterns. Meristems can be considered as cell factories in which division, expansion and differentiation processes will determine the structure of the plant [2]. A stem cell niche regulates the number of new cells and enables itself to remain in a quiescent undifferentiated state until their constituting cells are “activated” by signals (e.g. auxins as one of the most important hormones that promote the division of undifferentiated cells [3]). These signals indicate the necessity of forming new cells in the organism and we can consider the root meristem as a functional structure one of whose most important tasks will be to regulate the equilibrium between self-renewal and cell differentiation, part of this control being the way in which stem cell symmetric or asymmetric division occurs.

When a stem cell divides symmetrically, the result is a pair of genetically identical cells that keep the identity of the mother cell, thus being able to repeat this process in principle indefinitely (self-renewal); on the other hand, when considering asymmetric division, the result will be two cells with different genetic expression patterns. That is, one of the daughter cells will be identical to the mother cell, whereas the other can start following a differentiation path. During asymmetrical division, each of the resulting cells can have a different developmental potential [4]. It is important to point out that this type of division is necessary in order to maintain homeostasis.

We say that a cell division is periclinal if the division plane is parallel to the surface of the plant; correspondingly, we say it is anticlinal if it is perpendicular and oblique if this division plane has some intermediate direction. Division of stem cells is controlled by a very delicate interchange of signals among the cells that constitute the niche, the neighboring cells and stem cells themselves [5]. The type (symmetric or asymmetric) and form (periclinal or anticlinal) in which division takes place keeps the stock of stem cells in the meristem.

It is also worth mentioning that even when differentiated cells do not belong to the stem cell niche; it is natural to assume that they play an important role in the determination the structure and dynamics of the meristem. Indeed, this has even been incorporated into some mathematical models of the phenotypic evolution of cells in the stem cell niche [6]. Moreover, because of this, they would be taken into account in the approach developed later on.

Cell organization in *Arabidopsis* root is to some extent relatively simple. So we can determine the cells that belong to it and their corresponding differentiation fate. Cells produced in the meristems will be pushed away and will differentiate into a specific cell type during this process, mainly due to short and long range signals. In *Arabidopsis thaliana* the root meristem has the structure shown in figure 1.

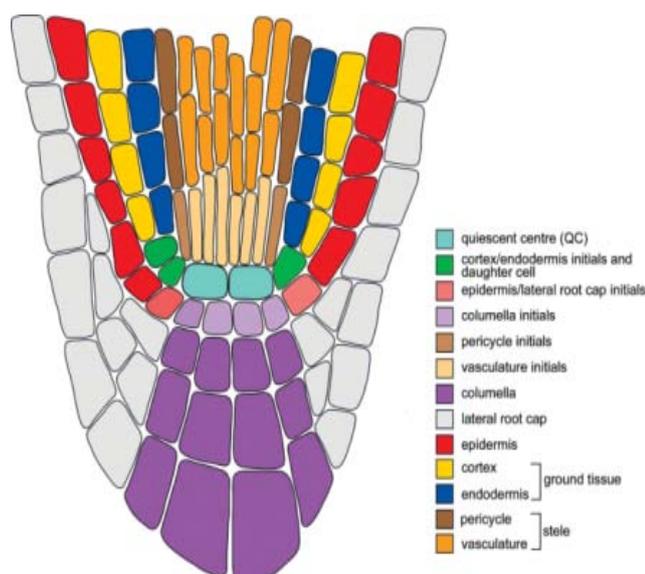


Figure 1: Cell organization of the root meristem in *Arabidopsis thaliana* as presented in [5].

We can summarize it as follows.

- The quiescent center is formed by a group of four cells which generally speaking do not divide after embryogenesis and whose main function is to regulate the cell spatial structure of the plant, that is, to inhibit differentiation of the initial cells with which they are in direct contact and to serve as a cell reservoir in order to replace damaged cells [7].
- Surrounding the quiescent center there is a group of stem cells known as initial cells. According to the type and form in which they divide and to their position, they will give rise to the different tissues in the root. These cells are characterized by their division pattern: a daughter cell keeps its identity as initial, whereas the other will be different known as transit amplifying. In this way, the meristem self-perpetuates and the number of initial cells stays constant. Depending on their position and the tissue they will eventually form they are designated as cortex/endodermis, columella, epidermis, vascular cylinder and pericycle initials [8].
- Transit amplifying cells, TA. They are located in the meristematic zone of the root and their main function is to increase the population of cells generated from a single cell division of stem cells [5]. Transit amplifying cells divide more frequently than the latter, but their proliferative potential is limited. They are considered as the first intermediate stage towards cell differentiation. TA cells are different from stem cells in that, even when they are also able to renew themselves and are multipotent as well, they are in a more advanced stage in the differentiation path. The limited capacity of proliferation that these cells exhibit contributes to amplify each stem cell division and to reduce the need of stem cell divisions, thus also decreasing the probability of introducing errors in the replicated DNA of the stem cell population. They also provide new cells that are closer to their differentiation fate.
- Differentiated cells. The morphological and physiological transformation of meristematic cells into adult tissue cells constitutes the cell differentiation process. This transformation and the subsequent specialization of the cell imply also a division of labor, originating cells with specific functions. Differentiation is produced by the differential activation of some genes and the repression of others. Regarding cell fate determination, experimental data suggest that this fate depends not only on the lineage, but also on positional information [9]. Among the mechanisms used to explain cell differentiation we have the creation of gradients of chemical substances

(Turing morphogenetic proposal), due to the diffusion and chemical interaction of the so called morphogens. Notice that the creation of such gradients would amount to the existence of a long range signal conveyed by the concentration level of the chemical and could give a coupling between neighboring genetic networks in adjacent cells [10].

A complex regulatory system could in principle use the frequency, division type of stem cells, the total number of divisions in TA cells and the length of the cell cycle of all cells in order to keep the global architecture of the niche according to different physiological requirements.

3 The mathematical model: The unified approach

Meristems control the development of the plant due to a balance between cell proliferation and differentiation. In the root of *Arabidopsis*, cells of different types of tissues are originated in the meristems and the whole structure grows in an organized way. In other words, cell divisions are not random; they follow a developmental program that incorporates cues. These cues could in principle be geometric (determined by position and shape) and also chemical or of other nature. The time sequence in which these cues appear is very important and the whole process depends on the current context of the state of development. Some studies indicate that patterning in the root meristem is controlled by different kinds of mechanisms that can be summarized roughly as short range signals inhibiting differentiation and long range signals favoring proliferation [11, 12], see figure 2.

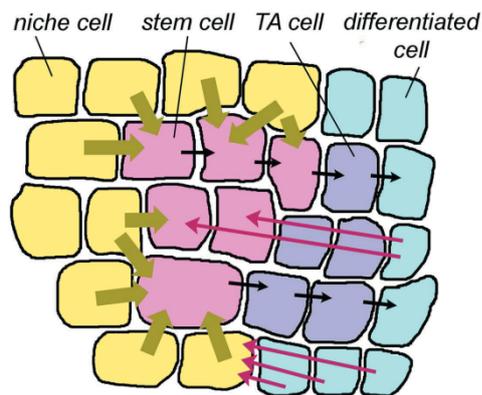


Figure 2: Different types of cell division: symmetric and asymmetric in which short and long range signaling process are shown as depicted in [13].

Regarding the complex interactions among stem cells, other cells in the niche and elsewhere as well as the set of short and long range signals, we have summarized experimental results as follows [14]:

- The niche controls a stem cell by means of certain secreted factors
- A signal originated in specialized cells is received by a stem cell and influences its behavior, although the cascade of subsequent processes is rather complex and still not understood.
- A basal membrane is present in most of the studied niches and it helps structuring them spatially and regulates the concentration of signaling molecules and adhesion properties. Adhesion between stem cells and the basal membrane is mediated by special molecules, being integrines the best characterized. Integrines maintain stem cells in a specific place and loss or modification of these molecules causes stem cells

differentiation or apoptosis. Moreover, integrines can activate receptors of growth factors.

- Stem cell niches can alter their regulatory properties depending on global signals from the organism as a whole, according to the needs of the latter.
- There exists a control mechanism that allows stem cells to divide in synchrony ensuring the population stability (a common clock).
- Stem cells communicate with the neighboring cells in order to maintain the niche active and, in turn, the niche induces and maintains a “working plan” in these cells.

Two signaling paths have been proposed among the cells that constitute the niche and stem cells in particular.

Other experiments have clearly shown that there is a strong correlation between embryonic cell types and their lineage, but that cell fate requires a continuous signaling process and depends strongly on positional information. The signals that are received by the cells are basically of two different types: the ones coming from neighboring cells (i.e. that are in direct contact via the cell wall), those coming from cells not sharing the cell wall and finally signals generated by differentiated cells in different parts of the plant [8].

Here we add some other considerations that we believe are important in understanding the niche space-temporal dynamics and that should be taken into account when constructing a more quantitative model.

It has been found that in the case of the root meristem of *Arabidopsis thaliana*, cell communication is necessary in order to maintain a coordinated cell activity, since some of the important genes during development act in a non-autonomous fashion. A gene or group of genes can have a definite effect on the development of neighboring cells or even on cells of distant tissues via at least three different mechanisms [11]:

1. Signaling induced by a ligand. There are evidences that the components of the cell wall can transmit positional information involved in the process of cell fate determination. Some of these components will give specific cues to a cell whose its neighboring cells are, making cell fate determination to explicitly depend on the geometry of a cell configuration.
2. Signals carried by hormones. There are many plant hormones (auxins, ethylene, cytokinins, among others) that participate in regulating and promoting cell division and other developmental processes.
3. There are most likely other signaling processes yet to be understood. For instance, the existence of global clocks mentioned above enabling a coherent and synchronized activity of cells (e.g. the fact that the elongation region or the differentiation region have definite boundaries) needs to be characterized.

Signaling via auxins is essential for the emergence of polarity in the vascular tissue. During plant growth, auxins controls cell identity, division and expansion [15, 16]. The role of auxins in triggering differentiation processes in the vascular tissue has been known for some time. Some studies have clearly shown that auxin is involved in the way cells will be grouped during embryogenesis and early plant development [17, 18].

Other works have evidenced that genes such as SCARECROW (SCR) or SHORTROOT (SHR) play a very significant role during the development of central cells in root meristems [7, 19].

Additionally, it is also known that stem cells produce transcription factors that interact with other factors produced by cells in the niche. It is likely that these transcription factors help controlling the cell fate of stem cells as well as the self- renewal process. Moreover, each

lineage is controlled by a unique combination of these factors and they can be expressed differently in different lineages.

The balance between cell division and differentiation is important in controlling the size of the root meristem as a result of a delicate regulatory equilibrium between cytokinins and auxins [20].

Recent research points to the fact that there are short and long range signaling processes influencing gene expression patterns. Frequently these mechanisms constitute positive or negative feedback loops in regulatory networks [21].

Auxins are also involved in regulating cell elongation, as well as in the development and maintenance of the root meristem and other processes. There are several works that show the relevance of auxin distribution along the root [5,15,16,17,22]. However, we want to stress the fact that in an abstract way, auxins influence can be summarized as short or long range signals.

The detailed molecular nature of the positional signals is far from being understood and it is not clear how they are generated and distributed. However, further experimental evidence shows that there are signals that lead the maturation process, as well as proliferation and differentiation. Moreover, there exist cell-to-cell interactions mediated by integral membrane proteins and, on the other hand, there are also interactions between each cell and the extracellular matrix via membrane receptors such as integrins. It should be taken into account that the cell fate can be determined by selective sedimentation of signaling molecules on the cell wall. The quiescent center plays a determinant role in several of these processes. Cell ablation experiments clearly establish the fact that the quiescent center inhibits cell differentiation of initial cells with which it has contact. Quiescent center cells are replaced quickly by initial vascular cells in case the four cells forming it are ablated [11].

In the case when only one or two cells from the quiescent center are lost, these are replaced more slowly by initial vascular cells. Depending on the position it has, each cell receives different stimuli in order to carry out certain processes. At present, much effort is being made in order to understand them. It is believed that signaling could also be affected or generate by gradients in the concentration of certain molecules.

In order to summarize the dynamics of all the processes mentioned above in the root of *Arabidopsis*, a series of rules which could constitute the base of a cellular automaton is presented.

State space $Q = \{s, i, a, d, m, v\}$.

Notice that the letters refer to a state variable, i.e. they do not represent the number of cells in a particular state, but denote the possible states that a cell can adopt.

- i. We refer to local neighborhoods or extended neighborhoods in a loose way, so that each cell may react to the different conditions of its environment. The rules also take into account early pattern formed during embryogenesis. More specifically, we consider four important factors:
 - a. Differentiation fate of the meristematic cells depends to a great extent on their position within the meristem.
 - b. Meristem dynamics obeys a well-defined sequence followed from early stages of development in the embryo.
 - c. The state of the quiescent center
 - d. Cell division patterns are well established and also depend on specific cues given mainly by position.

- Stem cells in the quiescent center (*s*):
 - A cell belonging to the quiescent center will not divide unless there is no initial cell that can divide in order to substitute another initial cell or a stem cell also in the quiescent center.
 - In case it divides it will do so symmetrically or asymmetrically depending on the cell they have to replace.
 - They will inhibit cell differentiation of initial stem cells with which they are in contact [11].
 - They will promote the division of neighboring initial cells.
 - In order to describe the dynamics of stem cells in the quiescent center, we will consider both the local and extended neighborhoods. The local neighborhood allows us to take into account the influence of stem cells in the quiescent center and initial cells around, whereas the extended neighborhood will provide long range signals coming from differentiated cells.
- Initial cells (*i*):
 - For these cells, long range signaling is originated in mature cells. These will direct cell fate of the daughter cells, coming from initial cells of the same type (again, position plays a central role).
 - We summarize the behavior of the initial cells belonging to the meristem depending on their position as follows:

Table 1: Rules of transition for initials cells.

| Cell type | Division form | Plane of division | Other considerations |
|------------------------|-------------------------------|--------------------------------|--|
| Columella | Asymmetric | Anticlinal | <ul style="list-style-type: none"> • Only if they are in direct contact with the quiescent center. • The quiescent center controls the differentiation process of these cells if there is no division, so division is not a necessary prerequisite to inhibit differentiation. • A local neighborhood is considered. |
| Cortex/endodermis | 1. Symmetric 2. Asymmetric | 1. Anticlinal 2. Periclinal | <ul style="list-style-type: none"> • These cells divide twice: the first gives rise to two initial daughter cells. • One of these cells after division produces an inner layer of endodermis and an outer layer of cortical cells. • If the quiescent center is eliminated these cells begin to differentiate. • A local neighborhood is considered. |
| Epidermis | Asymmetric | Anticlinal | <ul style="list-style-type: none"> • Their division is conditioned to that of the initial cells of the columella. • If there is no direct contact with initial columella cells, they die. • They replace initial cortex/endodermis cells if they are eliminated or die. • A local neighborhood is considered. |
| Vascular and pericycle | Asymmetric | Anticlinal | <ul style="list-style-type: none"> • They replace the stem cells in the quiescent center when one is missing. • A local neighborhood is considered. |

- Amplifying cells (*a*):
 - About these cells it is known their division takes place anticlinally, also that those located closer to the quiescent center will divide preferentially, even when their division rate is limited. It is estimated that these cells can divide up to 8 times.
 - These cells are the only ones that are pushed away from the meristematic zone entering the elongation zone. Eventually they will differentiate into specific tissues or organs, depending on the signals they receive according to their position.
- Differentiated cells (*d*):
 - The majority of differentiated cells come from the meristems, and so it is natural to consider that a differentiated cell will eventually be able to divide symmetrically and anticlinally.
- Other considerations:
 - It has been shown experimentally that when cells from the root meristem are ablated in *Arabidopsis*, the neighboring cells occupy the empty spaces and when doing it, these cells copy the existing patterns already present in the embryo in order to compensate variations [12].

4 Discussion

We present in what follows some of the most representative simulated scenarios which should be reproduced by any other model.

The first shows the evolution of niche and meristematic organization when the niche is already present (see 3.A). When all the cells in the quiescent center are removed, they are replaced by vascular cells (3.B). Since this replacement is so fast, there is no time for the surrounding cells to differentiate. In C and D we see how the quiescent center is recovered.

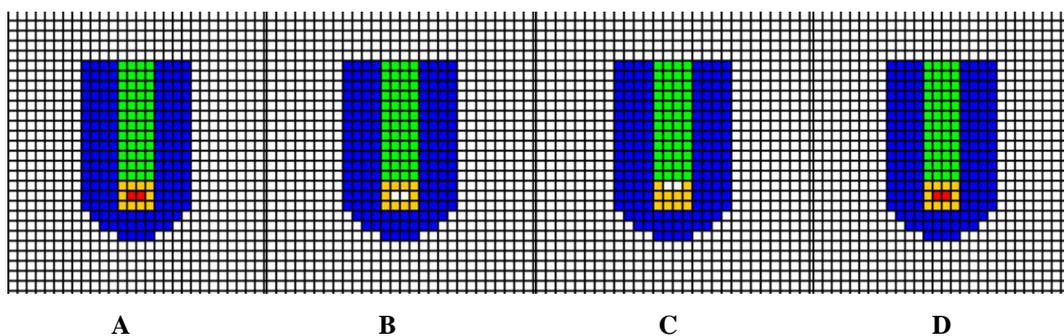


Figure 3: Simulations showing the quiescent center in red, initial stem cells in yellow, transit amplifying cells in green and differentiated cells in blue.

The second example shows how, when only one cell of the quiescent center is ablated, it is replaced by an initial vascular cell, but this process is not as fast as the one described in scenario 1 (see 4.A and 4.B). This is why the columella initial cell (below the ablated cell) that was in direct contact begins to differentiate (see 4.C).

The epidermis initial cell no longer receives a division signal from the columella cell undergo apoptosis. However, given that the cells in the quiescent center are in turn replaced, the usual niche structure is recovered (see 4.D and 4.E).

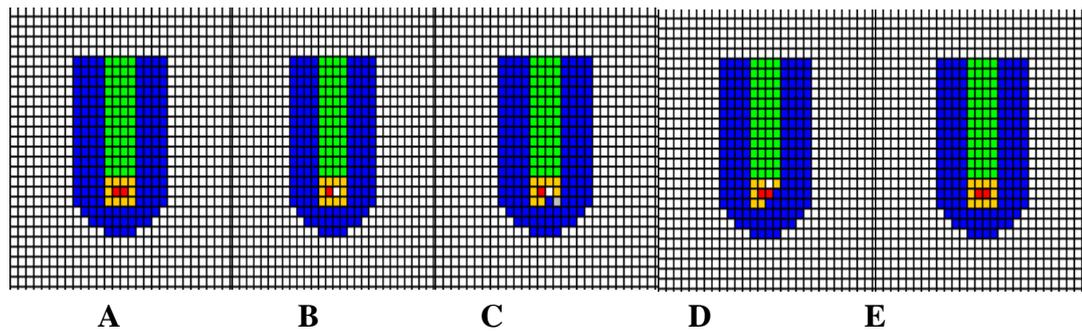


Figure 4: Second simulation.

5 Conclusions

We have provided a mathematical model and its computational implementation for the spatiotemporal dynamics of the root meristem in *Arabidopsis* by summarizing experimental work in a set of rules. Additionally, this setting shows that there is a subtle equilibrium involving short and long range signals in order to maintain the functionality of the meristem.

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